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Editorial

Diagnostic challenge in periodontics: The need for clarity when multiple periodontal and peri-implant diseases coexist – what is the course of action?

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Keywords: periodontics, diagnosis, bitewing radiography, periodontal attachment loss

Improving diagnostic precision in periodontology and implantology is essential for better patient care, scientific advancement and study consistency, while always considering the worst-case scenario for diagnosis.

Introduction

An accurate diagnosis is the foundation of effective periodontal and peri-implant treatment planning.[1,2] The concurrent presentation of two distinct yet related pathologies pose a significant and often underestimated diagnostic challenge in periodontology. An important and underdiscussed problem therefore persists in both the literature and clinical practice – the difficulty of establishing an accurate diagnosis when two pathologies occur simultaneously.[3,4] The current classification systems,[5,6] while attempting to be comprehensive, struggle to provide clear guidance for clinicians when these entities coexist, leading to potential diagnostic inaccuracies, compromised treatment planning and inconsistencies in epidemiological reporting.

Although these conditions may represent points along a biological continuum, they remain distinct entities with different prognostic implications, treatment priorities and documentation standards.[1,7,8] Misclassification can lead to inappropriate treatment decisions, erroneous prevalence data and inconsistency in clinical research. A recurring diagnostic error arises when clinicians report the less severe disease simply because it is visually obvious (e.g., erythema and bleeding on probing (BoP) suggesting gingivitis), while the presence of clinical attachment loss (CAL), radiographic bone loss (RBL) or deep periodontal pockets is overlooked.[7] The same occurs in implant dentistry[9]: a case with soft-tissue inflammation around an implant may be labeled "peri-implant mucositis", even when radiographs reveal progressive bone loss, indicating clear peri-implantitis. This confusion is reinforced by ambiguous terminology or insufficient diagnostic criteria in publications, causing inexperienced or inattentive clinicians to misinterpret cases and hindering standardization across studies.

Problem of coexisting pathologies

The core of the problem lies in the hierarchical relationship and symptomatic overlap between these diseases. In natural dentition,[5,6] gingivitis (Fig. 1, Fig. 2A,B) is defined as the reversible inflammation of the gingiva without CAL, and periodontitis is characterized by inflammation with CAL and RBL. Gingivitis is fundamentally, but not necessarily, the precursor or co-manifestation of periodontitis. In implant dentistry, peri-implant mucositis is the reversible inflammation of the peri-implant soft tissues without bone loss, while peri-implantitis is characterized by inflammation and progressive peri-implant bone loss (CAL and RBL).

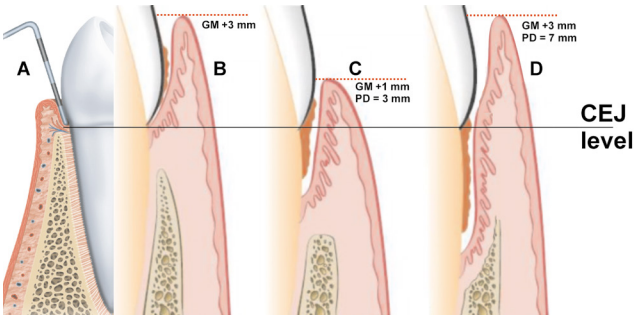

Fig. 1. A – probing the gingival sulcus in a healthy condition; B – gingivitis – the presence of bleeding on probing (BoP) in a case scenario without clinical attachment loss (CAL); C – periodontitis without gingival inflammation – the migration of the junctional epithelium apically with BoP and CAL; D – periodontitis with gingival inflammation (pseudopocket) – the migration of the junctional epithelium apically with BoP and CAL, associated with the presence of a pseudopocket

GM – gingival margin; PD – probing depth, CEJ – cementoenamel junction.

When a patient presents with established periodontitis (Fig. 2C) – defined by the presence of CAL and RBL – alongside active, site-specific gingivitis, the most severe diagnosis should be considered periodontitis. Similarly, the presence of peri-implantitis (bone loss present) supersedes and includes the inflammatory component of peri-implant mucositis.

Thus, to ensure diagnostic accuracy, the principle of "worst diagnosis prevails" must be emphasized. When signs of both gingivitis and periodontitis are present (Fig. 2D), the correct diagnosis is periodontitis, characterized by a high level of gingival inflammation and, typically, a high percentage of BoP. Similarly, when peri-implant mucositis and peri-implantitis coexist, the peri-implantitis diagnosis should take precedence. The "worst diagnosis prevails" rule is a safety-first approach to dental health. It means that if a patient shows signs of both a minor, treatable/reversible condition (such as gingivitis) and a serious, permanent/irreversible condition (such as bone loss (CAL and RBL) – periodontitis), the dentist must treat the case as the most serious condition. This ensures that the most damaging aspect of the disease is never overlooked.

Diagnostic confusion for clinicians (specialists, dentists and dental students) arises, because the less severe, reversible condition (gingivitis/peri-implant mucositis) is often the most visually apparent manifestation of active inflammation. This can lead to the underestimation of the severity of the underlying, irreversible tissue destruction – namely CAL and RBL – associated with periodontitis/peri-implantitis. Alternatively, clinicians may correctly diagnose periodontitis, yet fail to account for the influence of gingival inflammation on the probing depth (PD). This can lead to the overestimation of CAL, based on the simplified calculation: CAL = PD – GM (gingival margin). When GM is coronal to the cementoenamel junction (CEJ), it is assigned a positive value; when apical to CEJ, a negative value.[1] This potential inaccuracy can be mitigated by radiographic assessment, particularly with bitewing radiographs.

The literature's reliance on separate definitions can inadvertently promote the notion that clinicians must choose between diagnoses, rather than recognize the more severe, irreversible disease as the definitive diagnosis that encompasses the active inflammatory component. For instance, a site with established periodontitis that has recently become inflamed is not merely gingivitis; it is periodontitis with active inflammation (or a high level of gingival inflammation/BoP). This ambiguity has direct implications for treatment planning. An exception arises when a patient is observed over an extended period and the clinical parameters of periodontitis remain stable throughout follow-up; in such cases, the diagnosis may be revised to periodontal health or gingivitis in a reduced periodontium.

Recognizing the more severe condition is not merely a semantic distinction; it directly determines appropriate therapy, recall intervals, risk communication with patients, and the ability to monitor disease progression. Misdiagnosing an active case of periodontitis as gingivitis, for example, may result in inadequate therapy, failing to address the need for root surface debridement and the management of attachment loss.

Critical role of the probing depth (PD) and inflammation: Misdiagnosis arising from PD interpretation

The probing depth alone does not equate to CAL, RBL, or overall disease severity[1,6,10]; it must be interpreted within the context of gingival inflammation and tissue swelling. Inflammatory enlargement commonly produces pseudopockets, which may mimic increased PD in the absence of true attachment loss. Therefore, clinicians must distinguish between the increased PD resulting from edematous gingiva and the increased PD associated with the apical migration of the junctional

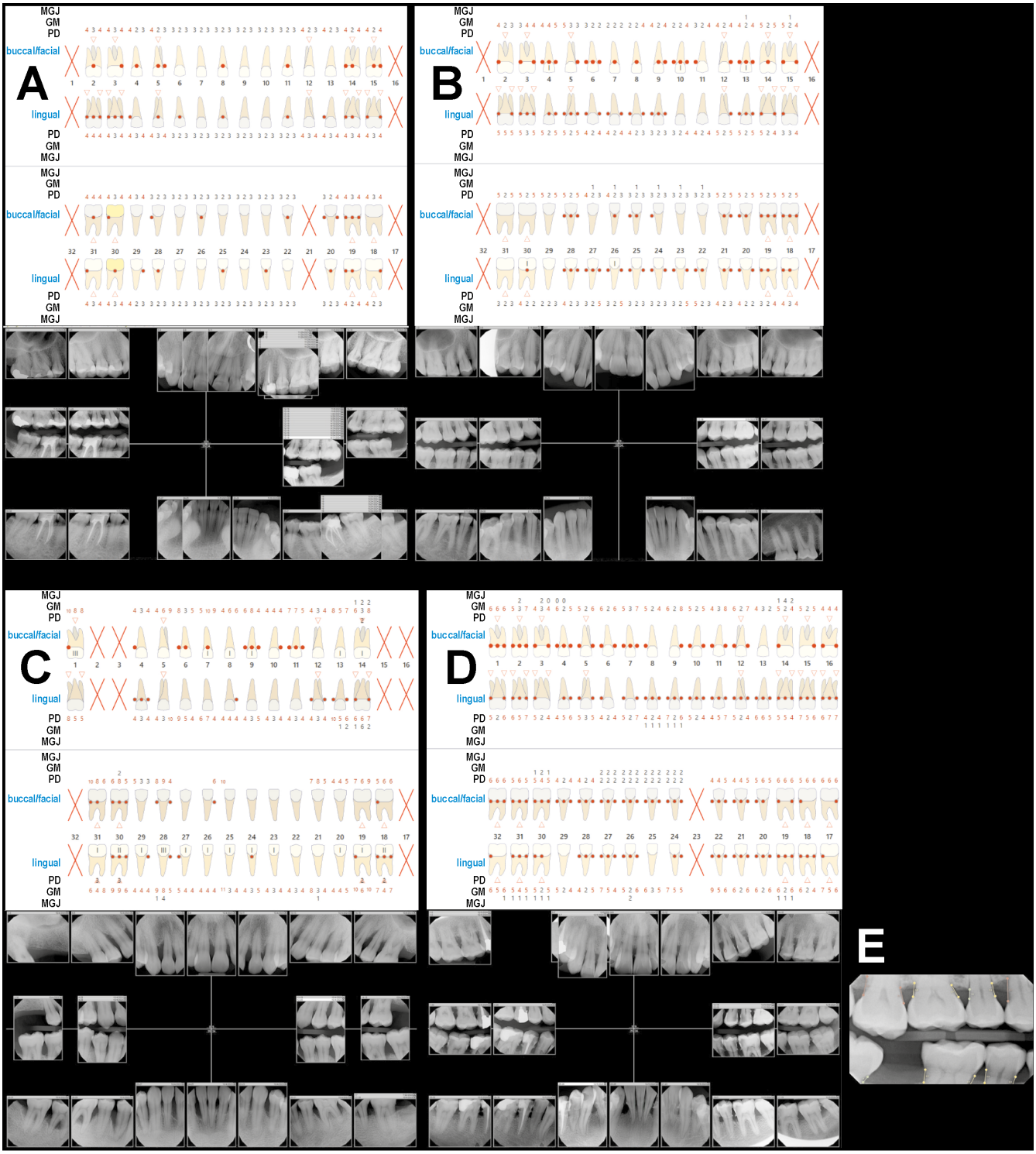

Fig. 2. A – plaque-induced localized gingivitis case, with 29.0% of bleeding on probing (BoP), no clinical attachment loss (CAL) and radiographic bone loss (RBL) due to periodontitis; B – plaque-induced generalized gingivitis case, with 55.3% of BoP, with no CAL and RBL; C – generalized molar–incisor periodontitis Stage III, Grade C (former aggressive periodontitis); D – generalized periodontitis Stage III, Grade C, with 75.8% of BoP; E – bitewing of the right side, showing the measure of the standard average dimension of supracrestal tissue attachment (STA) per tooth

MGJ – mucogingival junction.

epithelium, which reflects true periodontal breakdown. Failure to account for the inflammatory component may lead to the overdiagnosis of periodontitis in cases of gingivitis or underdiagnosis when inflammation masks the existing bone loss.

To accurately differentiate and identify the more severe condition, the clinical measurement of PD must be interpreted within the essential context of gingival inflammation/BoP and the radiographic bone levels. The probing depth is defined as the distance from GM to

the base of the gingival sulcus/periodontal pocket, and its clinical significance changes substantially in the presence of inflammation:

1. Increased PD without BoP: a PD of 4–5 mm without BoP usually indicates a stable, treated, or fibrotic periodontal pocket ("pocket of residual depth") that may not require immediate invasive intervention, particularly in cases of resolved/stable periodontitis (a reduced periodontium). If PD exceeds 5 mm, a "red flag" should be raised to investigate the underlying cause at that specific site. Possible explanations include a smoker with deep PD, the presence of a pseudopocket (which is uncommon without BoP, but can occur), or measurement errors. Radiographic assessment, preferably with bitewing radiographs, is essential to confirm true CAL or RBL. If radiographs do not show CAL/RBL, the site likely represents a pseudopocket, which is generally associated with a high level of BoP.
2. Increased PD with BoP: a PD over 3 mm accompanied by BoP indicates an active pathological site. This active inflammation signals that the measured PD reflects an ongoing disease process, such as active gingivitis/peri-implant mucositis or periodontitis/peri-implantitis. In cases of coexisting pathologies, clinicians must prioritize the measurements that indicate irreversible damage. This involves assessing CAL, using an accurate reference point – typically CEJ for the teeth – and confirming the findings with RBL assessment. Radiographic bone loss is measured from CEJ to the crestal bone, and should not exceed approx. 2.0 mm, which corresponds to the average supracrestal tissue attachment (STA), formerly known as the biological width.

The measurement of CAL, combined with the assessment of BoP, distinguishes the two conditions: (A) gingivitis/peri-implant mucositis – an increased PD is primarily due to gingival swelling (a pseudopocket), with no CAL present; (B) periodontitis/peri-implantitis – an increased PD results from both inflammation and the apical migration of the junctional epithelium – or from migration alone – leading to irreversible CAL and RBL.

In summary, differentiating transient inflammation from permanent tissue destruction requires careful distinction between PD and CAL/RBL. The probing depth is a purely topographic measure, representing the distance from GM to the base of the periodontal pocket. As such, it is highly sensitive to the gingival phenotype and the inflammatory edema, which can produce pseudopockets without actual structural loss. In contrast, CAL and RBL are definitive clinical indicators of periodontal stability, reflecting the apical migration of the junctional epithelium relative to a fixed anatomical landmark – CEJ. While a deep PD signals the presence of a clinical niche, only CAL, corroborated by RBL, confirms a diagnosis of periodontitis. This distinction is essential: PD reflects the 'depth' of the current clinical state, often associated with BoP, whereas CAL represents the cumulative history of irreversible attachment loss.

Radiographic assessment – the decisive factor

The definitive, non-negotiable criterion for identifying the "worst" pathology is the radiographic evidence of bone loss. Bitewing radiographs (Fig. 2E) are essential for the accurate assessment of the alveolar bone, offering a clear, undistorted view of the interproximal bone crest relative to CEJ or the implant–abutment interface for implants.[1] Properly taken bitewings are crucial for determining whether an increased PD corresponds to true bone loss. In comparison with periapical radiographs, which are often affected by variable angulation, bitewings provide more precise visualization and measurement of the interproximal crestal bone levels.

For diagnosing periodontitis,[1,5,6] bitewing radiographs are necessary to confirm that the measured CAL corresponds to RBL, the two most critical parameters in periodontal diagnosis. In a patient with deep pockets and BoP, radiographic evidence confirms that the condition represents true periodontitis rather than merely gingival inflammation. The interdental bone level provides a definitive proof of the irreversible nature of the disease, taking precedence over superficial inflammatory signs.

However, while bitewing radiographs are the primary tool for assessing the crestal bone, other imaging modalities are valuable in specific clinical situations. Periapical radiographs are required to evaluate the full root length and the periapical status, whereas cone-beam computed tomography (CBCT) may be indicated for assessing complex three-dimensional (3D) defects, furcation involvement or anatomical relationships prior to advanced surgical interventions.

Final comments

Diagnostic accuracy depends on the careful evaluation of both clinical parameters and radiographic evidence. Without appropriate imaging, clinicians may misclassify gingivitis as periodontitis or fail to detect early peri-implantitis beneath apparently healthy soft tissue. The literature remains inconsistent in defining periodontal and peri-implant diseases when multiple signs coexist. Some studies consider increases in PD and inflammation sufficient to indicate disease progression, whereas others prioritize radiographic confirmation.

Until clearer diagnostic hierarchies and standardized operational definitions are widely adopted, variability in diagnosis will persist. This inconsistency affects not only clinical communication, but also the interpretation of epidemiological data, meta-analyses and evidence-based treatment recommendations. The irreversible condition – periodontitis or peri-implantitis – must always be considered the primary diagnosis when there is evidence of CAL/RBL, regardless of the severity of the reversible inflammatory component.

Clinical decision-making should be based on a tripartite assessment: (1) CAL/RBL (bitewing radiographs); (2) PD; and (3) BoP. This structured approach is fundamental for moving beyond the current diagnostic dilemma and ensuring that patients receive appropriate, definitive care targeting the most destructive aspect of their disease.

Conclusions

In light of these challenges, this editorial urges the periodontal and implantology communities to place greater emphasis on:

– assigning the most severe diagnosis when multiple conditions coexist;
– interpreting PD in the context of gingival inflammation;
– utilizing appropriate, standardized bitewing radiographs to confirm bone loss; and
– clarifying diagnostic criteria in publications and educational materials.

Enhancing diagnostic precision is essential not only for delivering high-quality patient care, but also for advancing the scientific literature and ensuring consistency across clinical studies.

Ethics approval and consent to participate

Not applicable.

Data availability

All data for this study are included within the article.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Fernandes GVdO, Fernandes JCH. Revisiting and rethinking on staging (severity and complexity) periodontitis from the new classification system: A critical review with suggestions for adjustments and a proposal of a new flowchart. *Dent Med Probl*. 2025;62(2):371–391. doi:10.17219/dmp/192121
2. Fernandes GVO, Fernandes JCH. Call to review how staging periodontitis using the 2017 Classification of Periodontal and Peri-Implant Diseases and Conditions: Suggestions for modifications and improvements to help clinicians in the decision-making diagnosis. *Env Dent J*. 2025;6(1–2):1–5. https://www.dentaljournal.org/pdf/volno1-2/EDJ_Vol6_No1-2_p_01-05.pdf. Accessed November 26, 2025.
3. Fernandes GVdO, Mosley G, Cañizares AC, Fernandes JCH, Muller R. Standardizing clinical evaluations of periodontal condition to guide assessments and diagnoses using the Periodontal Assessment Protocol (GF-PAPro). *Adv Clin Exp Med*. 2025;34(7):1069–1077. doi:10.17219/acem/207502
4. Heitz-Mayfield LJ. Conventional diagnostic criteria for periodontal diseases (plaque-induced gingivitis and periodontitis). *Periodontol 2000*. 2024;95(1):10–19. doi:10.1111/prd.12579
5. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89(Suppl 1):S173–S182. doi:10.1002/JPER.17-0721
6. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – introduction and key changes from the 1999 classification. *J Periodontol*. 2018;89(Suppl 1):S1–S8. doi:10.1002/JPER.18-0157
7. Fernandes GVdO, Muller R. Back to the basics: Mastering probing techniques for comprehensive periodontal assessment. *J Basic Clin Dent*. 2024;1(1):1–8. https://j-bcd.com/index.php/j-bcd/article/view/18. Accessed November 26, 2025.
8. Muller R, Fernandes GVdO. Navigating furcation: A guide for an accurate assessment. *J Basic Clin Dent*. 2025;2(1):1–12. https://j-bcd.com/index.php/j-bcd/article/view/23. Accessed November 26, 2025.
9. Fernandes GVdO, Martins BGdS, Fraile JF. Revisiting peri-implant diseases in order to rethink the future of compromised dental implants: Considerations, perspectives, treatment, and prognosis. *Dent Med Prob*. 2024;61(5):637–640. doi:10.17219/dmp/187215
10. Fernandes GVdO, Fernandes JCH. New algorithm/tool used to achieve the periodontal risk assessment, diagnosis and prognosis (GF-PeDRA©): A clinical study with 221 patients. *Dent Med Probl*. 2025;62(5):801–813. doi:10.17219/dmp/199583

Original paper

Comparative analysis of postoperative pain following bioceramic sealer application with three obturation methods: A randomized controlled trial

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Abstract

Background. One of the most important aspects of root canal therapy is pain control after treatment. Postoperative pain is an unwanted but common sensation following endodontic procedures. Recently, bioceramic sealers have gained attention in endodontics; however, clinical evidence supporting their effectiveness in pain reduction is still limited.

Objectives. This randomized controlled trial aimed to evaluate the efficacy of 3 obturation techniques using a bioceramic sealer (Cerafill RCS®) on postoperative pain in patients with asymptomatic irreversible pulpitis.

Material and methods. Ninety-three patients diagnosed with asymptomatic irreversible pulpitis were randomly allocated to one of three groups according to the obturation technique: cold lateral compaction; single cone obturation; or a hot modified technique. The severity of pain was assessed using a visual analogue scale (VAS) at 6, 12, 24, and 48 h after treatment. The study utilized Kruskal–Wallis and Mann–Whitney *U* tests to analyze pain scores across groups. Linear regression analysis was performed to identify predictors of postoperative pain.

Results. The sealer-based and hot modified techniques were associated with significantly lower post-operative pain levels compared to cold lateral compaction at all measured time points ($p < 0.05$). The regression analysis confirmed the obturation technique as a significant predictor of postoperative pain. The hot modified method, in particular, provided the most consistent pain relief, underscoring the potential of advanced obturation methods in enhancing patient recovery and comfort.

Conclusions. The sealer-based and hot modified methods significantly reduced postoperative pain compared to traditional techniques. These findings support the clinical adoption of innovative bioceramic sealer techniques to optimize pain management and patient satisfaction.

Keywords: postoperative pain, randomized controlled trial, root canal therapy, endodontics, bioceramics

Highlights

- A randomized controlled trial compared 3 bioceramic-based obturation techniques using Cerafill RCS® in patients with asymptomatic irreversible pulpitis.
- Single cone obturation and hot-modified techniques were associated with significantly lower postoperative pain compared with cold lateral compaction.
- The hot modified technique provided the most consistent pain reduction across all evaluated time intervals.
- Linear regression analysis identified the obturation technique as the primary predictor of postoperative pain outcomes.
- The use of bioceramic sealers may improve patient comfort and support the adoption of modern endodontic protocols.

Introduction

Endodontic success is generally defined as the resolution or sustained absence of clinical signs and symptoms associated with pulpal or periapical disease following treatment.[1]

Clinician-reported outcomes, such as tenderness to percussion or palpation, the presence of a sinus tract, increased tooth mobility, periodontal pockets, and radiographic findings, remain central to defining treatment success. Yet, patient-reported outcomes, particularly those concerning pain and overall quality of life, are equally important for a comprehensive evaluation of endodontic effectiveness.[2]

A comprehensive approach to pain management should consider factors influencing each stage of treatment.[3] Several clinical variables have been identified as contributors to intraoperative and postoperative pain, including the choice of irrigating solutions,[4] irrigation activation techniques,[5,6] apical extrusion of irrigants,[7,8] flare-ups,[9] instrumentation protocols,[10,11] extrusion of debris,[12,13] and the use or omission of occlusal reduction.[14] Furthermore, mechanical and procedural factors, such as over-enlargement of the apical foramen,[15] lack of apical patency,[16] over-instrumentation, instrument kinematics,[17,18] and the physicochemical properties of root canal sealers,[19,20] may significantly influence the onset and severity of postoperative pain.

Postoperative pain remains a prevalent concern in endodontic therapy. It typically decreases substantially within 24 h and rarely persists for more than a week.[21,22] This multifactorial phenomenon arises from mechanical trauma, such as over-instrumentation or extrusion of filling materials, as well as chemical irritation and microbial extrusion of periradicular tissues.[23–26] While systematic reviews have established a robust correlation between root canal instrumentation and postoperative pain,[27,28] limited evidence is available regarding the influence of bioceramic-based obturation techniques on this outcome.[29–32]

Achieving a three-dimensional seal of the root canal system is critical, as it prevents the leakage of microorganisms and their byproducts, thereby reducing the likelihood of reinfection and mitigating postoperative discomfort.[33,34] Procedures such as cold lateral compaction, single cone obturation and the hot modified technique differ in methodology, sealing efficacy and potential impact on patient comfort. Conflicting evidence underscores the need for further investigation into the influence of these techniques on postoperative outcomes, particularly when used in conjunction with bioceramic sealers.

Calcium silicate-based sealers (CSBSs), also referred to as bioceramic sealers, including Cerafill RCS® (Prevest DenPro Ltd., Bari Brahmana, India), have garnered significant attention due to the bioactive properties such as hydroxyapatite formation and the ability to promote tissue healing.[35–37] These materials exhibit a high alkaline pH, improved apical sealing properties, and the potential to enhance periapical healing through odontoblastic differentiation and dentin mineralization.[36,37] Although previous studies have reported comparable postoperative pain levels between resin-based and bioceramic sealers,[36,38–40] further exploration is necessary to elucidate the unique benefits of individual bioceramic sealers, including Cerafill RCS®.

The potential of Cerafill RCS® as a bioceramic root canal sealer supports its inclusion in the present randomized controlled trial. To date, no clinical studies have rigorously evaluated its effectiveness, leaving a significant gap in the literature. Addressing this gap is critical to provide clinicians with robust evidence for optimizing endodontic outcomes. Therefore, the present randomized controlled trial aimed to evaluate postoperative pain associated with the use of Cerafill RCS® in combination with 3 bioceramic-based obturation techniques: cold lateral compaction; single cone obturation; and the hot modified technique. By generating evidence-based recommendations, this research would seek to advance clinical practice in single-visit root canal treatments. The null hypothesis posits that there are no significant differences in postoperative pain scores between the 3 obturation techniques using Cerafill RCS® in patients diagnosed with asymptomatic irreversible pulpitis and undergoing single-visit root canal treatment.

Material and methods

Study protocol

The present study was designed as a prospective, multicenter, randomized, single-blind controlled clinical trial. Ethical approval for the study was obtained from the Institutional Human Ethics Committee of Saveetha Dental College and Hospitals, Chennai, India (approval No. IHEC/SDC/FACULTY/21/ENDO/134), prior to study initiation. Additionally, the study was prospectively registered in the Clinical Trials Registry – India (CTRI) (http://ctri.nic.in; CTRI/2024/10/074776.) Research was conducted at the Mamata Institute of Dental Sciences, Kaloji Narayana Rao University of Health Sciences, Hyderabad, India. The conducted procedures posed minimal risk to participants and were carried out following the principles outlined in the Declaration of Helsinki. The study adhered to the Consolidated Standards of Reporting Trials (CONSORT) 2025 guidelines (Fig. 1),[41] as well as the Preferred Reporting Items for Randomized Trials in Endodontics (PRIRATE) 2020 guidelines.[42]

Sample size

The sample size was calculated a priori using the G*Power software, v. 3.0.10, based on data from a previous study.[28] Using a one-way analysis of variance (ANOVA) model for 3 independent groups, with an effect size of 1.73 (Cohen's *f*), a significance level of 0.05, and a statistical power of 95% ($1 - \beta = 95\%$, $\alpha = 0.05$), the required total sample size was determined to be 93 participants ($n = 31$/group). No adjustments for potential dropout were made.

Inclusion and exclusion criteria

Patients aged between 18 and 60 years with asymptomatic irreversible pulpitis in mandibular first or second molars, without associated periapical pathology, and classified as systemically healthy according to the American Society of Anesthesiologists (ASA) were included in the study. Individuals who were medically compromised or unable to communicate symptoms due to cognitive or psychological impairment, pregnant women, or those receiving antibiotics or analgesics for preoperative pain, as well as patients with incomplete apex formation, calcified canals, periapical abscess, tooth affected by cracks or sinus openings were excluded from the study.

Preoperative assessment

Two calibrated endodontists with comparable clinical experience and proficiency in all obturation techniques performed the entire experimental protocol under an operating microscope (Prima DNT; Labomed Europe, Capelle aan den Ijssel, the Netherlands). Before allocating participants to the operators, an examiner who was not involved in the study charted the preoperative pulpal and periapical status. All patients were preoperatively diagnosed with asymptomatic irreversible pulpitis without associated periapical pathosis, according to the criteria of the American Association of Endodontists (AAE).[43] Pulpal diagnosis was confirmed using a cold test (Endo-Ice™ Green; Hygenic Corp., Akron, USA) and electrical pulp stimulation (Vitality Scanner; Kerr Analytic Technology Corp., Redmond, USA). Patient history, including past and present symptoms, was considered prior

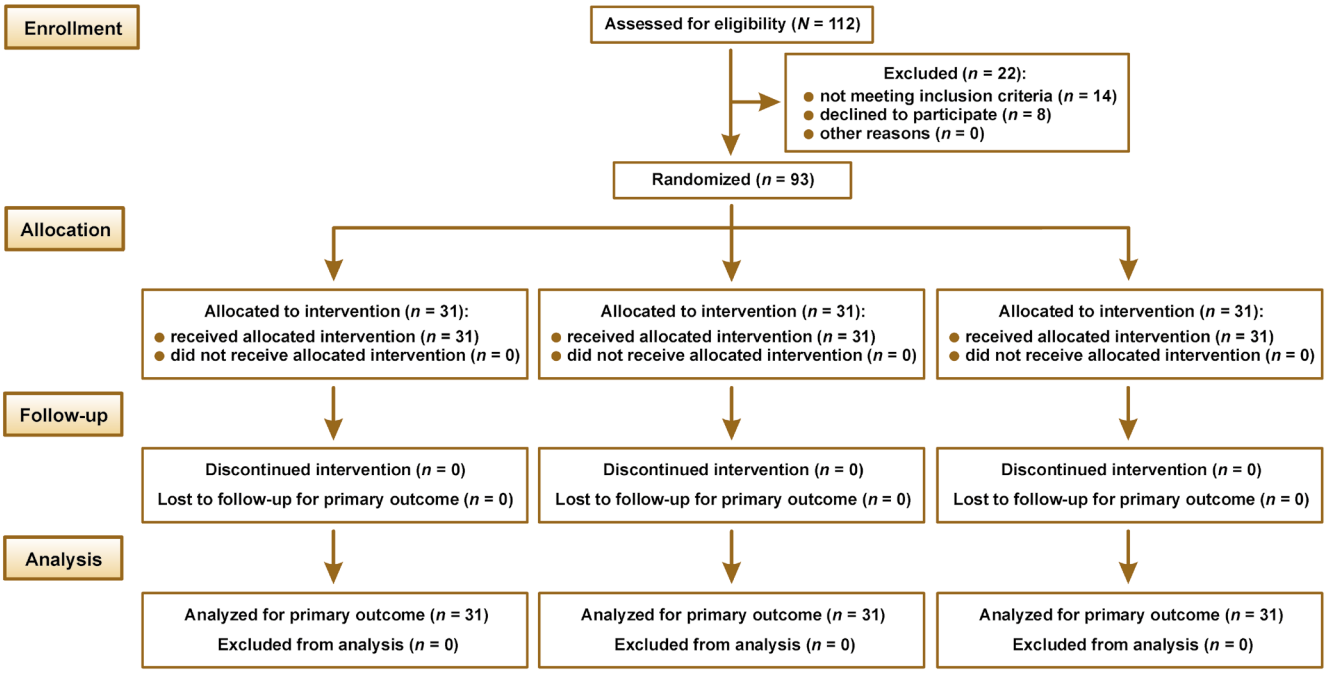

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) 2025 flow diagram of the study

to inclusion. Patients who exhibited tenderness upon vertical or lateral percussion of teeth or showed periapical pathosis on radiographic examination were excluded from the study. Additionally, individuals whose postoperative radiographs revealed underfilling or overfilling of thermoplasticized gutta-percha of the root canal obturation were excluded from the final analysis.

Participant allocation and randomization

The study followed a single-blind protocol. A computer-generated randomization sequence (https://www.random.org) was used for allocation. The SNOSE (sequentially numbered, opaque, sealed envelopes) technique was implemented for allocation concealment, which concealed the sequence until interventions were assigned. A piece of paper containing a randomized group number was sealed in a dark-colored envelope prepared by a third party. Patients were assigned study numbers as they enrolled in the study. Each envelope was opened after participant enrollment and immediately prior to intervention. Participants were randomly divided into 3 groups (n = 31/group) based on the bioceramic-based obturation technique: group I – cold lateral compaction; group II – single cone obturation; and group III – hot modified technique.

Treatment protocol

After allocation, all participants received a standardized inferior alveolar nerve block with 1.8 mL of 2% lidocaine and 1:200,000 epinephrine (LOX 2%; Neon Laboratories Ltd., Mumbai, India) administered with a 27-gauge long needle. Supplemental doses of the same anesthetic were provided as needed. After achieving profound numbness, the tooth was isolated with a rubber dam, and the access cavity was prepared using sterile carbide burs (Cavity Access Set; Dentsply Maillefer, Ballaigues, Switzerland) and the Endo-Access Burs (size 2; Dentsply Maillefer). Access refinement was performed using the Endo-Z Bur (Dentsply Maillefer) under the operating microscope. Initial canal patency was established with the use of stainless steel K-files (size #10; MANI Inc., Tochigi, Japan), and initial orifice enlargement was carried out using rotary files (ProTaper Gold Shaper X; Dentsply Maillefer). Working length was determined with the use of a specific initial binding file with an apex locator (Root ZX Mini; J. Morita USA, Inc., Irvine, USA) and confirmed radiographically. Subsequently, standardized instrumentation procedures were performed with rotary files (ProTaper Gold Shaper X) using a crown-down technique. Apical enlargement was standardized to three file sizes larger than the initial binding file. Apical patency was maintained throughout the shaping procedure using stainless steel K-files between successive instruments. Each canal was irrigated with 10 mL of 5% NaOCl (HYPOSOL®; Prevest DenPro Ltd.) between each instrument. Irigants were delivered with continuous oscillations using a conventional syringe attached to a flexible root canal irrigation needle (IrriFlex®; Produits Dentaires SA, Vevey, Switzerland), positioned 1 mm short of the working length. After complete instrumentation, canals were flushed with 5 mL of normal saline. Then, 1 mL of 5% NaOCl was activated for 20 s over 3 cycles using an ultrasonic device (VDW Ultra; VDW GmbH, Munich, Germany) with an attached ultrasonic tip (IRRI S; VDW GmbH) at a power setting of 30, placed 1 mm short of the working length. Final irrigation consisted of 4 mL of 5% NaOCl, 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (EDTA Solution; Prevest DenPro Ltd.) and 5 mL of normal saline. Canals were then dried with paper points. After experimental irrigation, obturation was conducted during a single visit, according to the assigned intervention.

Group I: cold lateral compaction

After root canal debridement, the master cone was verified with an intraoral periapical radiograph. Once the working length was confirmed, the premixed injectable Cerafill RCS® sealer (Prevest DenPro Ltd.) was dispensed onto a paper mixing pad. The master cone (Dentsply Maillefer) was adequately coated with the sealer and placed into the canal at working length. Spreaders of an appropriate size were introduced 1 mm short of the working length to create lateral space for accessory cones. Accessory cones (Dentsply Maillefer) were then coated with the sealer and packed until the spreader penetrated no more than 1–2 mm into the root canal orifice. Before the excision of the accessory cones, an intraoral periapical radiograph was taken to confirm the quality of the seal. If any voids or spaces were detected, additional accessory cones were placed until an adequate seal was obtained. Finally, excess gutta-percha was removed using a heated plugger to 1 mm below the orifice level and condensed (Fig. 2).

Group II: single cone obturation

Following master cone verification, the premixed Cerafill RCS® sealer was injected into the root canal using flexible syringe injection tips provided by the manufacturer. Before injection, the applicator tip was adjusted to 5–6 mm short of the working length using a rubber stop and calibrated



Fig. 2. Representative preoperative (A) and postoperative (B) radiographs following obturation using the cold lateral compaction technique

with the Endo-Block (Dentsply Maillefer). The flexible syringe injection tip was then placed into the canal until the rubber stop was reached. The Cerafill RCS® sealer was injected by gently pressing the syringe plunger while withdrawing the tip until the sealer was visible at the root canal orifice. The tip of the master cone was then coated with the bioceramic sealer and inserted carefully to the desired working length. Excess gutta-percha was removed using a heat carrier tip set at 180°C, and the material was condensed to 1 mm below the canal orifice level (Fig. 3).

Group III: hot modified technique

The hot modified technique was performed following the protocol described in a previous report.[44] After verification of the master cone, the premixed bioceramic sealer (Cerafill RCS®) was injected into the canal using a disposable tip. The master gutta-percha cone was lightly coated with the sealer and inserted to full working length.

A heated plugger set at 180°C was used for down-packing. The plugger was inserted to a depth of 6–10 mm short of the working length and activated for 4 s to thermosoften and compact the gutta-percha in the middle and coronal thirds. Care was taken to avoid disturbing the apical 3–4 mm to preserve the apical seal and prevent sealer extrusion.

The softened gutta-percha was vertically compacted using pre-fitted pluggers. Accessory cones were placed in wide canals to ensure adequate filling. Additional thermoplasticized gutta-percha was added as necessary to fill voids, and the quality of obturation was verified radiographically. Excess gutta-percha was removed to 1 mm below the canal orifice using a heated plugger and vertically compacted to complete the obturation (Fig. 4).

All obturation procedures were standardized in duration to approx. 45–50 min per tooth, regardless of group allocation. Following obturation, in all groups, excess sealer was removed using an ultrasonic scaler tip under water irrigation, and the chamber was air-dried. The access cavity was examined under an operating microscope to ensure complete removal of the sealer contents. Radiographs confirmed that no under- or overfilling of thermoplasticized gutta-percha occurred in any treated canal and that all canals had been properly treated. An intraorifice seal was placed using a flowable core build-up composite (Fusion Core DC Flo™; Prevest DenPro Ltd.), and the final core buildup was completed with packable composite restoration (Magma NT™; Prevest DenPro Ltd.) for all the groups.

Evaluation of postoperative pain

All participants received a visual analogue scale (VAS) sheet after the procedure to record pain intensity. No medications were prescribed immediately after treatment. Patients were advised to contact the investigator by telephone if they experienced discomfort and were instructed not to take any medication without prior consultation. If patients reported pain, they were prescribed Aceclofenac. To ensure compliance and accurate data collection, the participants were contacted by telephone at 6, 12, 24, and 48 h after treatment. These follow-up calls served to assess patient status, reinforce completion of the VAS form at each time point, and document pain scores accordingly.

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA). The analyses were designed to evaluate both inter- and intragroup variations in VAS pain scores. Data normality was assessed using the Shapiro–Wilk test, which indicated a non-normal distribution of VAS scores; therefore, non-parametric tests were applied. The homogeneity of categorical variables such as sex and tooth type across groups was evaluated using the Pearson's χ^2 test. The Kruskal–Wallis test was used to compare pain scores across the 3 groups at each time point (6, 12, 24, and 48 h post-treatment). Wilcoxon signed-rank test was applied for intergroup comparisons of pain scores across successive time points, while the Friedman test was used to assess overall changes over time within each group. Pairwise comparisons between the groups at each time interval were performed using the Mann–Whitney *U* test. Pain scores were reported as medians and interquartile ranges (*IQR*s). Linear regression analysis was performed to explore the relationships between patient characteristics, treatment modalities and postoperative pain scores, providing insights into the factors that significantly affected pain outcomes. A p-value <0.05 was considered statistically significant.

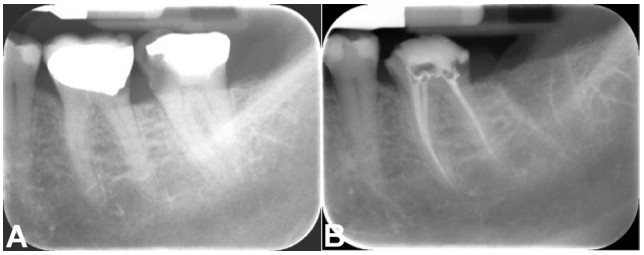

Fig. 3. Representative preoperative (A) and postoperative (B) radiographs following obturation using the single cone obturation technique

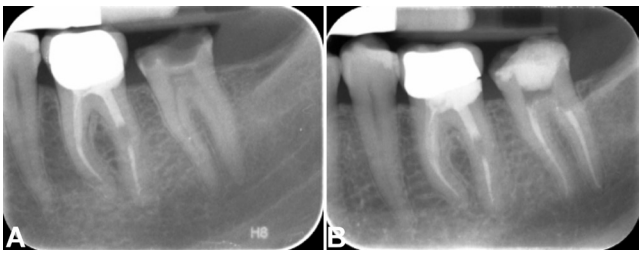

Fig. 4. Representative preoperative (A) and postoperative (B) radiographs following obturation using the hot modified technique

Results

Demographic and baseline characteristics

A total of 115 patients were enrolled between January and February 2025. Of these, 14 did not meet the inclusion criteria, and 8 individuals declined to participate, resulting in the exclusion of 22 patients. Finally, 93 patients were randomly allocated into 3 study groups (n = 31/group), all of whom received the intended intervention and were included in the final analysis. No losses to follow-up, exclusions after randomization, or deviations from the prespecified protocol occurred. All outcomes were analyzed in accordance with the registered trial protocol. The mean age of participants was 32.43 ±6.32 years (median: 32; *IQR*: 28–37). The demographic characteristics of the study population are presented in Table 1. All procedures were performed by 2 calibrated endodontists under standardized conditions using an operating microscope. Radiographic evaluation confirmed obturation fidelity in all cases. No adjunctive medications were administered unless patients reported postoperative pain during follow-up.

VAS scores

No missing data was noted; all randomized participants completed the follow-up and were included in the primary analysis. VAS scores were assessed at 6, 12, 24, and 48 h post-treatment across all groups. When all groups were considered collectively, the Friedman test demonstrated a significant reduction in VAS scores over time ($p < 0.001$). A significant decrease in mean VAS scores was also observed within each group across all evaluated time points ($p < 0.001$) (Table 2). The Kruskal–Wallis test revealed statistically significant differences is VAS scores among the 3 obturation techniques at 6, 12, 24, and 48 h ($p < 0.001$) (Fig. 5).

Intergroup comparisons indicated that groups II and III exhibited significantly lower VAS scores compared to group I at all time points ($p < 0.001$) (Table 3).

The Wilcoxon signed-rank test demonstrated a significant reduction in pain between successive time intervals (6 h vs. 12 h, 12 h vs. 24 h, and 24 h vs. 48 h) for the overall sample. Intragroup evaluation showed that groups II and III demonstrated significant reductions in VAS scores at each follow-up interval. Group I exhibited a substantial reduction in pain 24 h after treatment ($p < 0.001$), while no significant differences were observed between 6 h and 12 h ($p = 0.131$).

Table 4 presents the pairwise comparisons of VAS scores at each time interval. Statistically significant differences were observed at 6, 12, 24, and 48 h, with groups II and III reporting significantly lower pain levels than group I at each time point ($p < 0.001$). Notably, at 12 h and 24 h, the differences between groups II and III were not significant ($p = 0.312$ and $p = 0.703$, respectively), while at 48 h, group II exhibited significantly lower VAS scores compared to group III ($p = 0.035$).

Table 2. Intragroup comparison of visual analogue scale (VAS) scores across time points

Group	VAS				*p*-value
	6 h	12 h	24 h	48 h	
Group I	7.54 ±0.49	7.00 ±1.15	2.84 ±0.68	2.07 ±0.30	<0.001*
Group II	4.79 ±0.52	2.78 ±0.85	1.52 ±0.90	1.03 ±0.30	<0.001*
Group III	5.14 ±0.38	2.78 ±0.91	1.63 ±0.59	1.20 ±0.26	<0.001*

* statistically significant ($p < 0.05$, Friedman test). Data presented as mean ± standard deviation ($M \pm SD$).

Table 3. Intergroup comparison of visual analogue scale (VAS) scores across time points

Time interval	VAS			*p*-value
	group I	group II	group III	
6 h	7.54 ±0.49	4.79 ±0.52	5.14 ±0.38	<0.001*
12 h	7.00 ±1.15	2.78 ±0.85	2.78 ±0.91	<0.001*
24 h	2.84 ±0.68	1.52 ±0.90	1.63 ±0.59	<0.001*
48 h	2.07 ±0.30	1.03 ±0.30	1.20 ±0.26	<0.001*

* statistically significant ($p < 0.05$, Wilcoxon signed-rank test). Data presented as $M \pm SD$.

Table 1. Demographic and baseline characteristics of the study participants

Variable		Total (N = 93)	Group I (n = 31)	Group II (n = 31)	Group III (n = 31)	χ^2	*p*-value
Age	≤30 years	43 (46.2)	17 (54.8)	14 (45.2)	12 (38.7)	0.663	0.718
	>30 years	50 (53.8)	14 (45.2)	17 (54.8)	19 (61.3)		
Sex	male	38 (40.9)	14 (45.2)	13 (41.9)	11 (35.5)	0.169	0.919
	female	55 (59.1)	17 (54.8)	18 (58.1)	20 (64.5)		
Tooth	first molar	46 (49.5)	15 (48.4)	16 (51.6)	15 (48.4)	0.022	0.989
	second molar	47 (50.5)	16 (51.6)	15 (48.4)	16 (51.6)		
Sealer extrusion	yes	11 (11.8)	4 (12.9)	2 (6.5)	5 (16.1)	1.552	0.460
	no	82 (88.2)	27 (87.1)	29 (93.5)	26 (83.9)		

Data presented as frequency (percentage) (n (%)).

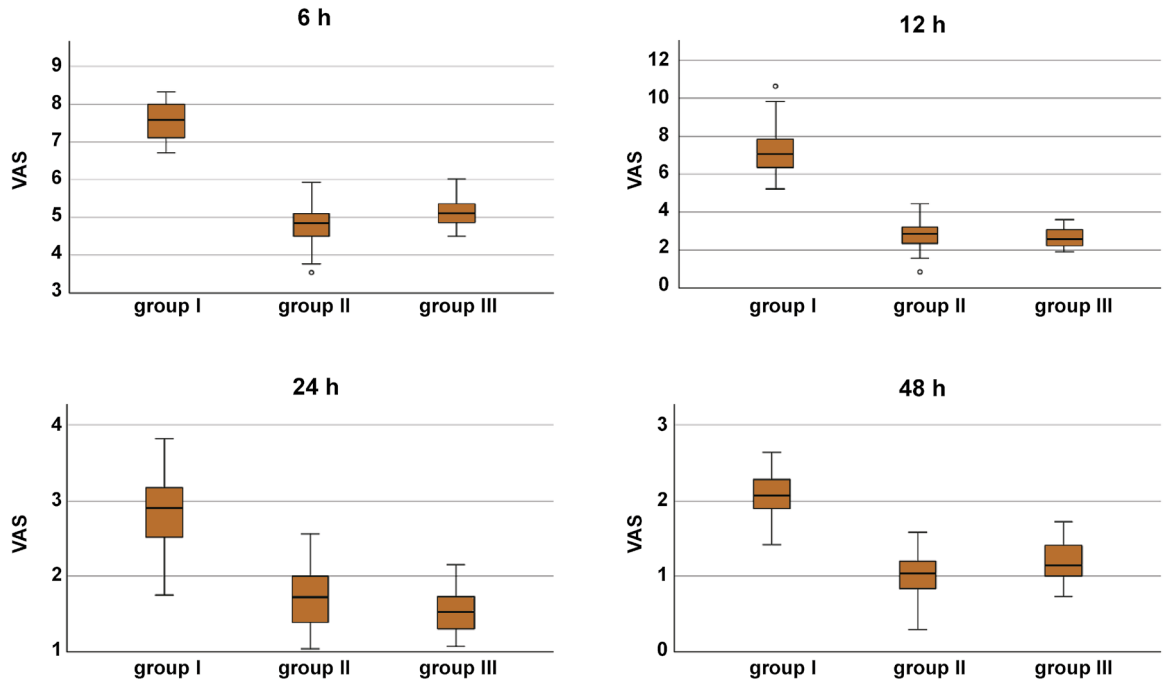

Fig. 5. Kruskal–Wallis analysis of mean visual analogue scale (VAS) scores across treatment groups at 6, 12, 24, and 48 h post-treatment

Table 4. Pairwise comparisons of visual analogue scale (VAS) scores at each time point

Time interval	Group	VAS *Me* (*IQR*)	*p*-value
6 h	group I	7.58 (7.11–8.00)	<0.001*
	group II	4.85 (4.51–5.10)	
	group I	7.58 (7.11–8.00)	<0.001*
	group III	5.11 (4.87–5.34)	
	group II	4.85 (4.51–5.10)	0.005*
	group III	5.11 (4.87–5.34)	
12 h	group I	7.21 (6.36–7.82)	<0.001*
	group II	2.23 (2.36–3.19)	
	group I	7.21 (6.36–7.82)	<0.001*
	group III	2.61 (2.24–3.05)	
	group II	2.23 (2.36–3.19)	0.312
	group III	2.61 (2.24–3.05)	
24 h	group I	2.69 (2.52–3.17)	<0.001*
	group II	1.72 (1.41–1.99)	
	group I	2.69 (2.52–3.17)	<0.001*
	group III	1.41 (1.31–1.74)	
	group II	1.72 (1.41–1.99)	0.703
	group III	1.41 (1.31–1.74)	
48 h	group I	2.20 (1.89–2.28)	<0.001*
	group II	1.14 (0.83–1.20)	
	group I	2.20 (1.89–2.28)	<0.001*
	group III	1.20 (1.00–1.40)	
	group II	1.14 (0.83–1.20)	0.035*
	group III	1.20 (1.00–1.40)	

* statistically significant ($p < 0.05$, Mann–Whitney *U* test).

Influence of sex

Analysis of postoperative pain according to sex revealed no significant differences at 6 h ($p = 0.713$), 12 h ($p = 0.495$), 24 h ($p = 0.942$), or 48 h ($p = 0.969$).

Predictors of postoperative pain

Linear regression analysis indicated that the bioceramic-based obturation technique and sealer extrusion had a significantly negative impact on VAS scores at 6 h (Table 5, Fig. 6). At subsequent time points, only the obturation technique remained a significant predictor of postoperative pain, while no other variables showed a statistically significant effect.

Discussion

The current randomized controlled trial evaluated the impact of 3 different bioceramic-based obturation techniques using Cerafill RCS® on postoperative pain in patients with asymptomatic irreversible pulpitis.

The findings demonstrated significant differences in pain reduction among the bioceramic-based obturation techniques, highlighting their clinical relevance in optimizing endodontic outcomes. Moreover, these results align with the current shift toward patient-centered care in endodontics, where minimizing post-treatment discomfort is a key objective. Statistically significant differences in VAS scores were observed between the groups at 6, 12, 24, and 48 h post-treatment ($p < 0.05$), suggesting that the choice of obturation technique directly influences

Table 5. Linear regression analysis of predictors of postoperative pain at 6 h

Coefficients[a]								
model		unstandardized coefficients		standardized coefficients	*t*	sig.	correlations	
		B	std. error	beta			zero-order	partial
1	(constant)	8.347	0.479	–	17.413	<0.001	–	–
	sex	−0.068	0.178	−0.026	−0.383	0.703	0.016	−0.041
	sealer	0.889	0.274	0.220	3.241	0.002	0.185	0.327
	group	−1.200	0.111	−0.748	−10.792	<0.001	−0.746	−0.755
	tooth type	−0.010	0.176	−0.004	−0.056	0.955	0.015	−0.006
	age	−0.006	0.014	−0.031	−0.443	0.659	−0.209	−0.047

[a] dependent variable: postoperative pain (VAS scores at 6 h).

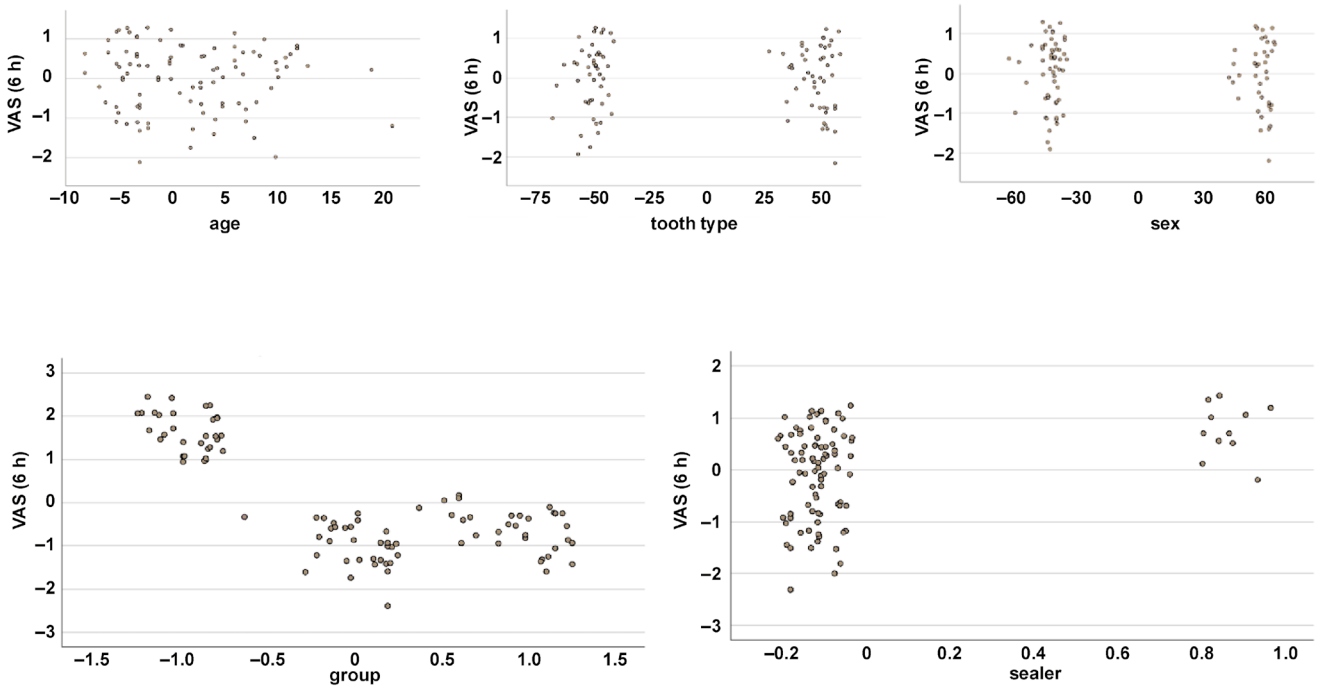

Fig. 6. Partial regression plots illustrating the relationship between visual analogue scale (VAS) scores at 6 h and significant predictors

early postoperative pain. These findings are consistent with previous reports showing a wide range of postoperative pain incidence (3.0–69.3%) following non-surgical root canal treatment.[23] Moreover, symptomatic teeth have been reported to exhibit a higher incidence of postoperative pain compared to asymptomatic cases (15.9% vs. 7.1%).[24] Therefore, postoperative pain might be considered an important indicator of treatment outcomes.[45]

Cerafill RCS® has demonstrated superior in vitro properties, including enhanced bioactivity, crystallinity and solubility,[46,47] which may contribute to improved healing and reduced postoperative pain.[48] Its handling characteristics and low solubility support the formation of a durable and stable apical seal, thereby minimizing the risk of reinfection. Comparative studies evaluating its performance across obturation techniques have suggested that warm vertical compaction methods may provide superior sealing results when compared to lateral compaction or single cone techniques.[46,47,49] Furthermore, Cerafill RCS® outperforms other sealers in maintaining excellent flow characteristics and a consistently high alkaline pH, essential for periapical healing.[48]

In the present study, the intragroup analysis indicated a significant reduction in pain over time within each group ($p < 0.001$), validating the effectiveness of bioceramic sealers in pain alleviation, probably due to their bioactive properties.[35–37,42,46,47,50] Moreover, groups II (single cone obturation) and III (hot modified technique) demonstrated significantly lower pain levels compared

to group I (cold lateral compaction). These findings are consistent with the study by Martín-González et al. who reported higher pain levels following traditional compaction techniques.[32] The authors reported a 55.3% incidence of pain after obturation in subjects treated with cold lateral compaction compared to 44.7% in those treated with thermoplasticized gutta-percha.[32] The higher pain levels reported in group I might be attributed to the undue lateral forces exerted on the periodontium.[32] Although interappointment flare-ups are uncommon, postoperative pain is frequent even when appropriate treatment is performed.[51] Findings of the present study suggest that the hot modified technique (group III) consistently outperformed cold lateral compaction (group I) and sealer-based obturation (group II), not only at early postoperative time points but throughout all follow-up intervals. This was further corroborated by the Mann–Whitney *U* test results, which emphasized superior outcomes in group III, indicating a more effective management of pain compared to the traditional methods. Although some studies report higher pain levels with carrier-based thermoplastic obturation and lateral compaction compared to back-fill thermoplastic methods,[30,32] others show no significant differences between cold lateral compaction and continuous wave technique.[29]

The superior efficacy of the hot modified technique in reducing postoperative discomfort is consistent with existing literature, which established that bioceramic sealers paired with warm obturation techniques yield notably lower postoperative pain compared to traditional cold lateral compaction.[20] Bioceramic sealers have favorable physical properties, such as reduced porosity and enhanced material adaptability, which are critical factors in minimizing periapical inflammation and associated postoperative symptoms.[52] The reduced pain associated with the hot modified technique may be explained by the superior thermoplastic characteristics of gutta-percha employed in this method. By promoting closer adaptation of gutta-percha and sealer to the dentinal walls, this approach minimizes void spaces and reduces the risk of apical microleakage. Consequently, the inflammatory response triggered by extruded materials is significantly mitigated.

Conversely, the increased postoperative pain observed with lateral compaction techniques may be attributed to mechanical stress applied to the root to achieve the compaction and less optimal material adaptation, increasing the risk of sealer extrusion and periapical inflammation.[20]

Previous studies have shown that factors such as age, sex, pulpal status, tooth type, site, and preoperative pain play a significant role in postoperative pain outcomes.[23] In the present study, no significant differences were observed among groups with respect to these variables. Pain perception is a highly subjective experience, modulated by various physical and psychological factors. Furthermore, measuring pain is challenging due to a high potential for error.[53] The VAS is a validated and sensitive tool for measuring acute pain,[54,55] therefore, this method was adopted in the present study. The time intervals chosen for pain assessment ranged from 6 h to 48 h, as maximum post-treatment pain typically occurs within this period.[21] Comorbidities such as pain before obturation were not reported among all 3 groups.

Linear regression analysis further confirmed that the bioceramic-based obturation technique was a significant predictor of postoperative pain, with the hot modified technique showing the most favorable outcomes. This statistical insight highlights the clinical efficacy of the method, aligning with recent advancements in endodontic materials and techniques that prioritize patient outcomes and procedural innovations.

According to the present study, it is recommended that clinicians consider adopting sealer-based and hot modified techniques over traditional cold lateral compaction. The demonstrated efficacy of these methods in reducing postoperative pain can significantly improve patient satisfaction and treatment acceptance, which are crucial in clinical settings. This recommendation is evidenced by superior clinical and radiographic success rates with bioceramic sealers used in single cone techniques.[33,56] This is the first trial focusing on assessing the real-time clinical benefits of the hot modified technique and investigating postoperative pain in patients undergoing single-visit root canal treatment using different bioceramic-based obturation methods. However, this study focused exclusively on asymptomatic irreversible pulpitis, which limited its applicability to symptomatic patients, who often represent a substantial portion of endodontic cases. Future research should consider subjects with varying degrees of symptomatic pulpitis and apical periodontitis. Additionally, exploring other bioceramic sealers could provide broader insights and validate the generalizability of current findings across different materials.

Conclusions

Single cone obturation and the hot modified technique were associated with significantly lower postoperative pain compared to conventional cold lateral compaction. These findings suggest that the adoption of these techniques may improve patient outcomes, such as pain control and long-term treatment results. Future research involving symptomatic cases and a broader range of materials is recommended to strengthen the generalizability of these results.

Trial registration

The study was prospectively registered in the Clinical Trials Registry – India (CTRI) (http://ctri.nic.in; CTRI/2024/10/074776).

Ethics approval and consent to participate

The conducted procedures posed minimal risk to participants and were carried out following the principles outlined in the Declaration of Helsinki. All personal data was treated with strict anonymity and confidentiality. Ethical approval for the study was obtained from the Institutional Human Ethics Committee of Saveetha Dental College and Hospitals, Chennai, India (approval No. IHEC/SDC/FACULTY/21/ENDO/134), prior to study initiation.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. European Society of Endodontology. Quality guidelines for endodontic treatment: Consensus report of the European Society of Endodontology. *Int Endod J*. 2006;39(12):921–930. doi:10.1111/j.1365-2591.2006.01180.x
2. Duncan HF, Nagendrababu V, El-Karim IA, Dummer PMH. Outcome measures to assess the effectiveness of endodontic treatment for pulpitis and apical periodontitis for use in the development of European Society of Endodontology (ESE) S3 level clinical practice guidelines: A protocol. *Int Endod J*. 2021;54(5):646–654. doi:10.1111/iej.13501
3. Arias A, de la Macorra JC, Hidalgo JJ, Azabal M. Predictive models of pain following root canal treatment: A prospective clinical study. *Int Endod J*. 2013;46(8):784–793. doi:10.1111/iej.12059
4. da Silva EJNL, Monteiro MR, Belladonna FG, Almeida JF, De-Deus G, de Almeida Neves A. Postoperative pain after foraminal instrumentation with a reciprocating system and different irrigating solutions. *Braz Dent J*. 2015;26(3):216–221. doi:10.1590/0103-6440201300311
5. Liapis D, De Bruyne MAA, De Moor RJG, Meire MA. Postoperative pain after ultrasonically and laser-activated irrigation during root canal treatment: A randomized clinical trial. *Int Endod J*. 2021;54(7):1037–1050. doi:10.1111/iej.13500
6. Penukonda R, Teja KV, Kacharaju KR, et al. Comparative evaluation of smear layer removal with Ultra-X device and XP-Endo Finisher file system: An ex-vivo study. *G Ital Endod*. 2023;37(2). doi:10.32067/GIE.2023.37.01.18
7. Karatas E, Ozsu Kirici D, Arslan H. Postoperative pain after the use of sodium hypochlorite gel and solution forms: A randomized clinical study. *Eur Endod J*. 2021;6(1):34–37. doi:10.14744/eej.2020.08370
8. Lakshmanan L, Gurunathan D, Shanmugam R. Effectiveness of white tea-mediated silver nanoparticles as an intracanal irrigant against *Enterococcus faecalis*: An in vitro study. *Dent Med Probl*. 2024;61(4):593–598. doi:10.17219/dmp/143545
9. Bassam S, El-Ahmar R, Salloum S, Ayoub S. Endodontic postoperative flare-up: An update. *Saudi Dent J*. 2021;33(7):386–394. doi:10.1016/j.sdentj.2021.05.005
10. Western JS, Dicksit DD. Apical extrusion of debris in four different endodontic instrumentation systems: A meta-analysis. *J Conserv Dent*. 2017;20(1):30–36. doi:10.4103/0972-0707.209066
11. Kolberg-Babrzyńska I, Grzech-Leśniak K, Kiryk J, Dominiak M, Matys J. Effects of endodontic retreatment by conventional therapy compared to combined therapy with an Er:YAG laser and photobiomodulation: A randomized clinical trial. *Dent Med Probl*. 2025;62(6):1079–1087. doi:10.17219/dmp/188864
12. Eliasz W, Czarnecka B, Surdacka A. Apical extrusion of debris during root canal preparation with ProTaper Next, WaveOne Gold and Twisted Files. *Materials (Basel)*. 2021;14(21):6254. doi:10.3390/ma14216254
13. Teja KV, Mustafa M, Esposito L, Iaculli F, Cernera M. Novel non-obturation based concept of regeneration. *G Ital Endod*. 2024;38(2). doi:10.32067/GIE.2024.38.01.12
14. Nguyen-Nhon D, Nagendrababu V, Pulikkotil SJ, Rossi-Fedele G. Effect of occlusal reduction on postendodontic pain: A systematic review and meta-analysis of randomised clinical trials. *Aust Endod J*. 2020;46(2):282–294. doi:10.1111/aej.12380
15. Silva EJNL, Menaged K, Ajuz N, Monteiro MRFP, Coutinho-Filho TS. Postoperative pain after foraminal enlargement in anterior teeth with necrosis and apical periodontitis: A prospective and randomized clinical trial. *J Endod*. 2013;39(2):173–176. doi:10.1016/j.joen.2012.11.013
16. Yaylali IE, Demirci GK, Kurnaz S, Celik G, Kaya BU, Tunca YM. Does maintaining apical patency during instrumentation increase postoperative pain or flare-up rate after nonsurgical root canal treatment? A systematic review of randomized controlled trials. *J Endod*. 2018;44(8):1228–1236. doi:10.1016/j.joen.2018.05.002
17. Adiguzel M, Tufenkci P, Pamukcu II. Comparison of postoperative pain intensity following the use of three different instrumentation techniques: A randomized clinical trial. *J Dent Res Dent Clin Dent Prospects*. 2019;13(2):133–140. doi:10.15171/joddd.2019.021
18. Yeşildal Yeter K, Gunes B, Bal EZ. Effect of glide path preparation on the apical debris extrusion for three single-file systems. *Dent Med Probl*. 2024;61(4):599–604. doi:10.17219/dmp/136464
19. de Souza Ferreira N, Gollo EKF, Boscato N, Arias A, da Silva EJNL. Postoperative pain after root canal filling with different endodontic sealers: A randomized clinical trial. *Braz Oral Res*. 2020;34:e069. doi:10.1590/1807-3107bor-2020.vol34.0069
20. Mittal N, Thangamuthu T, Gupta S, Gupta S, Aggarwal H, Kharat S. Comparative evaluation of resin-based sealers and bioceramic sealers for postoperative pain after endodontic treatment: A systematic review. *Dent Med Probl*. 2024;61(2):293–300. doi:10.17219/dmp/155885
21. Pak JG, White SN. Pain prevalence and severity before, during, and after root canal treatment: A systematic review. *J Endod*. 2011;37(4):429–438. doi:10.1016/j.joen.2010.12.016
22. Shamszadeh S, Shirvani A, Asgary S. Does occlusal reduction reduce post-endodontic pain? A systematic review and meta-analysis. *J Oral Rehabil*. 2020;47(4):528–535. doi:10.1111/joor.12929
23. AlRahabi MK. Predictors, prevention, and management of post-operative pain associated with nonsurgical root canal treatment: A systematic review. *J Taibah Univ Med Sci*. 2017;12(5):376–384. doi:10.1016/j.jtumed.2017.03.004
24. Siqueira Jr JF, Rôças IN, Favieri A, et al. Incidence of postoperative pain after intracanal procedures based on an antimicrobial strategy. *J Endod*. 2002;28(6):457–460. doi:10.1097/00004770-200206000-00010
25. de Oliveira Alves V. Endodontic flare-ups: A prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110(5):e68–e72. doi:10.1016/j.tripleo.2010.05.014

26. Rajamanickam K, Teja KV, Ramesh S, et al. Evaluation of root canal cleanliness on using a novel irrigation device with an ultrasonic activation technique: An ex vivo study. *Appl Sci*. 2023;13(2):796. doi:10.3390/app13020796
27. Sun C, Sun J, Tan M, Hu B, Gao X, Song J. Pain after root canal treatment with different instruments: A systematic review and meta-analysis. *Oral Dis*. 2018;24(6):908–919. doi:10.1111/odi.12854
28. Martins CM, De Souza Batista VE, Andolfatto Souza AC, Andrada AC, Mori GG, Gomes Filho JE. Reciprocating kinematics leads to lower incidences of postoperative pain than rotary kinematics after endodontic treatment: A systematic review and meta-analysis of randomized controlled trial. *J Conserv Dent*. 2019;22(4):320–331. doi:10.4103/JCD.JCD_439_18
29. Alonso-Ezpeleta LO, Gasco-Garcia C, Castellanos-Cosano L, Martín-González J, López-Frías FJ, Segura-Egea JJ. Postoperative pain after one-visit root-canal treatment on teeth with vital pulps: Comparison of three different obturation techniques. *Med Oral Patol Oral Cir Bucal*. 2012;17(4):e721–e727. doi:10.4317/medoral.17898
30. Koçer A, Dönmez Özkan H, Turk T. Postoperative pain intensity and incidence following single visit root canal treatment with different obturation techniques: A randomized clinical trial. *PeerJ*. 2022;10:e13756. doi:10.7717/peerj.13756
31. Yu YH, Kushnir L, Kohli M, Karabucak B. Comparing the incidence of postoperative pain after root canal filling with warm vertical obturation with resin-based sealer and sealer-based obturation with calcium silicate-based sealer: A prospective clinical trial. *Clin Oral Investig*. 2021;25(8):5033–5042. doi:10.1007/s00784-021-03814-x
32. Martín-González J, Echevarría-Pérez M, Sánchez-Domínguez B, et al. Influence of root canal instrumentation and obturation techniques on intra-operative pain during endodontic therapy. *Med Oral Patol Oral Cir Bucal*. 2012;17(5):e912–e918. doi:10.4317/medoral.18234
33. Zamparini F, Lenzi J, Duncan HF, Spinelli A, Gandolfi MG, Prati C. The efficacy of premixed bioceramic sealers versus standard sealers on root canal treatment outcome, extrusion rate and post-obturation pain: A systematic review and meta-analysis. *Int Endod J*. 2024;57(8):1021–1042. doi:10.1111/iej.14069
34. Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: Systematic review of the literature – Part 2. Influence of clinical factors. *Int Endod J*. 2008;41(1):6–31. doi:10.1111/j.1365-2591.2007.01323.x
35. Guivarc'h M, Jeanneau C, Giraud T, et al. An international survey on the use of calcium silicate-based sealers in non-surgical endodontic treatment. *Clin Oral Investig*. 2020;24(1):417–424. doi:10.1007/s00784-019-02920-1
36. Coşar M, Kandemir Demirci G, Çalışkan MK. The effect of two different root canal sealers on treatment outcome and post-obturation pain in single-visit root canal treatment: A prospective randomized clinical trial. *Int Endod J*. 2023;56(3):318–330. doi:10.1111/iej.13870
37. Lee BN, Hong JU, Kim SM, et al. Anti-inflammatory and osteogenic effects of calcium silicate-based root canal sealers. *J Endod*. 2019;45(1):73–78. doi:10.1016/j.joen.2018.09.006
38. Aslan T, Dönmez Özkan H. The effect of two calcium silicate-based and one epoxy resin-based root canal sealer on postoperative pain: A randomized controlled trial. *Int Endod J*. 2021;54(2):190–197. doi:10.1111/iej.13411
39. Nathani TI, Olivieri JG, Tomás J, Elmsmari F, Abella F, Durán-Sindreu F. Post-operative pain after single-visit root canal treatment using resin-based and bioceramic sealers in teeth with apical periodontitis: A randomised controlled-trial. *Aust Endod J*. 2024;50(3):538–546. doi:10.1111/aej.12864
40. Atav Ates A, Dumani A, Yoldas O, Unal I. Post-obturation pain following the use of carrier-based system with AH Plus or iRoot SP sealers: A randomized controlled clinical trial. *Clin Oral Investig*. 2019;23(7):3053–3061. doi:10.1007/s00784-018-2721-6
41. Hopewell S, Chan AW, Collins GS, et al. CONSORT 2025 statement: Updated guideline for reporting randomised trials. *Lancet*. 2025:S0140-6736(25)00672-5. doi:10.1016/S0140-6736(25)00672-5
42. Nagendrababu V, Duncan HF, Bjørndal L, et al. PRIRATE 2020 guidelines for reporting randomized trials in Endodontics: Explanation and elaboration. *Int Endod J*. 2020;53(6):774–803. doi:10.1111/iej.13304
43. American Association of Endodontists. *Glossary of Endodontic Terms*. 10th ed. Chicago, IL: American Association of Endodontists; 2020. https://www.aae.org/specialty/clinical-resources/glossary-endodontic-terms. Accessed June 7, 2025.
44. Abdellatif D, Amato A, Calapaj M, Pisano M, Iandolo A. A novel modified obturation technique using biosealers: An ex vivo study. *J Conserv Dent*. 2021;24(4):369–373. doi:10.4103/jcd.jcd_142_21
45. Kalkman JC, Visser K, Moen J, Bonsel JG, Grobbee ED, Moons MKG. Preoperative prediction of severe postoperative pain. *Pain*. 2003;105(3):415–423. doi:10.1016/S0304-3959(03)00252-5
46. Abu Zeid ST, Alnoury A. Characterisation of the bioactivity and the solubility of a new root canal sealer. *Int Dent J*. 2023;73(5):760–769. doi:10.1016/j.identj.2023.04.003
47. Abu-Zeid ST, Saif RE, Mostafa HA, Edrees HY. Characterization and crystallinity of two bioactive sealers: Qualitative and quantitative analysis. *Appl Sci*. 2024;14(3):1285. doi:10.3390/app14031285
48. Seron MA, Nunes GP, Ferrisse TM, et al. Postoperative pain after root canal filling with bioceramic sealers: A systematic review and meta-analysis of randomized clinical trials. *Odontology*. 2023;111(4):793–812. doi:10.1007/s10266-023-00830-z
49. Agrawal TS, Singh S, Podar RS, Kulkarni G, Gadkari A, Agarwal N. In vitro comparative evaluation of apical leakage using a bioceramic sealer with three different obturating techniques: A glucose leakage model. *J Conserv Dent Endod*. 2024;27(1):76–81. doi:10.4103/jcd.jcd_303_23
50. Mandlecha D, SR S. Comparative evaluation of the effect of XP-Endo Finisher, passive ultrasonic irrigation and sonic activation on the push-out bond strength of bioceramic sealer to root dentin. *Saudi J Oral Dent Res*. 2023;8(5):162–166. doi:10.36348/sjodr.2023.v08i05.005
51. Siqueira Jr JF. Microbial causes of endodontic flare-ups. *Int Endod J*. 2003;36(7):453–463. doi:10.1046/j.1365-2591.2003.00671.x
52. Yalniz H, Ziraman FG, Oncu A, Celikten B. Porosity analysis of four bioceramic materials used for the repair of furcation perforations via micro-computed tomography. *Dent Med Probl*. 2024;61(1):71–76. doi:10.17219/dmp/146663
53. DiRenzo A, Gresla T, Johnson BR, Rogers M, Tucker D, BeGole EA. Postoperative pain after 1- and 2-visit root canal therapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;93(5):605–610. doi:10.1067/moe.2002.121900
54. Bijur PE, Silver W, Gallagher EJ. Reliability of the visual analog scale for measurement of acute pain. *Acad Emerg Med*. 2001;8(12):1153–1157. doi:10.1111/j.1553-2712.2001.tb01132.x
55. Sirintawat N, Sawang K, Chaiyasamut T, Wongsirichat N. Pain measurement in oral and maxillofacial surgery. *J Dent Anesth Pain Med*. 2017;17(4):253–263. doi:10.17245/jdapm.2017.17.4.253
56. Sabeti MA, Karimpourtalebi N, Shahravan A, Dianat O. Clinical and radiographic failure of nonsurgical endodontic treatment and retreatment using single-cone technique with calcium silicate-based sealers: A systematic review and meta-analysis. *J Endod*. 2024;50(6):735–746.e1. doi:10.1016/j.joen.2024.03.007

Original paper

Osteonecrosis of the jaw: A 5-year analysis of surgical outcomes and risk factors for complications

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Abstract

Background. Radiation-induced and drug-induced bone necrosis present significant challenges for maxillofacial surgery departments and dental clinics. While the incidence of osteoradionecrosis (ORN) has decreased, the number of cases of medication-related osteonecrosis of the jaw (MRONJ) has markedly increased. This trend is associated with higher life expectancy and more frequent use of medications linked to MRONJ. To date, no uniform, scientifically validated treatment standards have been established for these conditions.

Objectives. The aim of the study was to evaluate treatment outcomes in patients with ORN and MRONJ, as well as to identify risk factors for complications, with particular emphasis on differences in clinical presentation and management.

Material and methods. A retrospective analysis of medical records over a five-year period was conducted, including the charts of patients diagnosed with MRONJ and ORN who were hospitalized at the Department of Maxillofacial Surgery, Poznan University of Medical Sciences, Poland, and subsequently followed up in the outpatient setting. Selected parameters included C-reactive protein (CRP), smoking status, type of necrosis, sex, and hospitalization time.

Results. Differences in patient characteristics, including age, sex, smoking status, and clinical presentation, were observed between the groups. Patients with MRONJ were, on average, 5 years older than those with ORN and exhibited a significantly higher concentration of CRP, indicating a more pronounced inflammatory response. Smoking was identified as a weak but notable predictor of the need for mandibular resection. Additionally, elevated concentrations of CRP were associated with longer hospitalization and a higher incidence of complications, potentially contributing to prolonged hospital stays and an increased risk of adverse outcomes.

Conclusions. The findings highlight distinct clinical courses for MRONJ and ORN, underscoring the need for differentiated treatment strategies. Given the lack of standardized treatment protocols, the observed variations in clinical outcomes suggest the necessity for more targeted therapeutic approaches. Further research is essential to establish effective treatment protocols.

Keywords: ORN, osteonecrosis, maxillofacial surgery, MRONJ, medication-related osteonecrosis of the jaw

Highlights

- The incidence of medication-related osteonecrosis of the jaw (MRONJ) is increasing, whereas the occurrence of osteoradionecrosis (ORN) remains stable.
- Patients with MRONJ are generally older and present with elevated inflammatory markers, such as C-reactive protein (CRP), which may be associated with longer hospital stays.
- Smoking is associated with an increased risk of complications and a higher likelihood of requiring surgical resection.
- Distinct clinical profiles of MRONJ and ORN may support earlier identification of treatment challenges and the need for differentiated therapeutic approaches.

Introduction

Bisphosphonates, antiresorptive drugs, anticancer agents, immunomodulators, and steroids, among others, provide significant benefits to patients with cancer and rheumatic diseases. However, their use is also related to adverse effects, including medication-related osteonecrosis of the jaw (MRONJ).[1–5] Osteonecrosis is defined as exposed bone in the oral cavity that does not heal within 8 weeks in a patient receiving medications associated with MRONJ and with no history of head and neck radiotherapy.[6]

The pathophysiology of MRONJ is multifactorial, and its exact mechanism remains unknown. One of the contributing factors is the inhibition of bone remodeling. Bisphosphonates and denosumab impair osteoclast function, leading to delayed bone tissue healing. Inflammation and infection also play significant roles. Most patients who develop MRONJ present with periapical lesions, poor oral hygiene or a history of dental procedures. Additionally, bisphosphonates and antiangiogenic drugs impair the formation of new blood vessels, resulting in reduced blood supply to affected areas and diminished defense mechanisms. The weakening of natural immunity, particularly in patients with cancer and autoimmune diseases, further increases susceptibility to MRONJ. Lastly, genetic factors are mentioned in the literature. Several single-gene polymorphisms have been identified as potential risk factors. However, studies involving large patient groups are lacking.[2,6]

Overall, MRONJ results from the interplay of multiple factors. Impaired bone regeneration due to osteoclast inhibition, combined with infection in the oral cavity and limitations in blood flow lead to compromised healing ability and, ultimately, jaw necrosis.

The treatment of MRONJ is challenging and not clearly defined. According to the 2022 guidelines of the American Association of Oral and Maxillofacial Surgeons (AAOMS), conservative treatment is recommended as the first-line approach, including pain management, antibacterial treatment and local debridement of the affected area. In cases where conservative therapy fails, surgical intervention involving the resection of affected bone is recommended.[6]

Another type of jaw necrosis is osteoradionecrosis (ORN), a rare complication of head and neck radiotherapy,[7] first described in 1922.[8] Although no universally accepted definition exists, ORN is generally characterized as exposed bone within an irradiated area, unrelated to tumor presence, persisting for 3–6 months.

According to the current state of knowledge, the pathogenesis of ORN is associated with vascular disturbances associated with damage to small blood vessels. Radiation doses exceeding 50 Gy primarily damage small vessels.[9] It has been demonstrated that reduced blood flow in these vessels likely contributes to impaired bone turnover, resulting in necrosis.[10] The higher incidence of ORN in the mandible, as compared to the maxilla, supports this theory.[11,12] Infections and invasive dental procedures are also considered contributing factors.[7]

The treatment protocol for ORN is not well-established. Initial management is typically conservative and involves antiseptic rinses and antibiotic therapy. Additional treatment options include hyperbaric oxygen therapy as well as the use of pentoxifylline and tocopherol.[7,13,14] Surgical treatment involves local debridement, removal of necrotic bone, reconstruction using adjacent tissues, and, in cases of extensive defects, reconstructive surgery. The primary goals of treatment are pain relief, restoration of function and improvement of quality of life.[7,15]

Attention should be drawn to the exceptionally rare occurrence of both MRONJ and ORN in the pediatric patient population, which remains an ongoing subject of research.[16–18] Cases of both MRONJ and ORN differ significantly, and the number of detailed clinical descriptions is limited. The pathophysiology and treatment processes differ.

In MRONJ, bone exhibits blocked osteoclast activity. Upon trauma or infection, an inflammatory response arises, but the inability of osteoclasts to address the inflammation leads to its spread. In this scenario, the bone remains viable but becomes inflamed. This process is associated with an increase in inflammatory mediators, such as interleukins (ILs), and the recruitment of immune cells to combat inflammation. Persistent inflammation and the inability to manage it effectively result in necrosis. Consequently, elevated levels of inflammatory markers may be detectable in blood tests.[19]

Osteoradionecrosis primarily results from vascular damage and reduced blood flow. The theory of radiation-induced fibrosis has been proposed as a three-phase mechanism describing the molecular events leading to clinical manifestations of the disease. The initial pre-fibrotic phase is followed by an organized phase characterized by abnormal fibroblast activity and disorganization of the extracellular matrix. The late fibroatrophic phase involves tissue remodeling, resulting in fragile, healed tissues susceptible to re-injury. Histologically, ORN is characterized by significant osteocyte destruction and the absence of osteoblasts along bone margins.[20,21]

The number of affected patients varies across centers. In most countries, the incidence of ORN remains similar or is decreasing, while the number of MRONJ cases is increasing. The observed rise is presumably due to the growing number of patients receiving bisphosphonates and anticancer therapies.[22]

An increasing number of studies emphasize the importance of preventing both MRONJ and ORN. Preventive strategies should include patient education as well as the training for healthcare providers delivering primary medical and dental care.[23]

The aim of the present study was to present and compare cases of MRONJ and ORN in a 5-year retrospective cohort of patients from the Wielkopolska region of Poland, with the goal of providing data to support future research.

Material and methods

The study involved an analysis of medical records obtained from the Department of Maxillofacial Surgery, Poznan University of Medical Sciences, Poland, and its affiliated outpatient clinic, where follow-up visits were conducted. The research was performed in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. The study protocol was approved by the Bioethics Committee at Poznan University of Medical Sciences (approval No. KB761/24).

Medical records were identified using the International Classification of Diseases, 10th Revision (ICD-10) diagnostic codes within the hospital's electronic system. Subsequently, the etiology for necrosis was verified through chart review. All included cases met the diagnostic criteria for either MRONJ or ORN. To reduce potential bias, statistical analyses were performed independently.

Patients eligible for inclusion met the diagnostic criteria for ORN or MRONJ. Surgical qualification was determined by a maxillofacial surgery specialist. Surgery was indicated in patients who developed pathological fractures, experienced exacerbation of inflammatory processes, or did not respond to conservative treatment.

Patient records from March 2018 to March 2023 were analyzed, as full electronic documentation was available from that period. The following variables were evaluated: type of necrosis; sex[24]; location; age[25]; C-reactive protein (CRP) concentration, due to its higher levels in a previous study involving MRONJ patients[26]; smoking status; body mass index (BMI) (underweight, normal weight, overweight, obesity)[27]; length of hospital stay[28]; histopathological findings[29]; and treatment outcomes. Given the higher incidence of surgical complications in older individuals, patients were divided into 2 groups, namely those aged ≥65 years and those aged <65 years. A similar analysis was performed for CRP levels, using a threshold of >10 mg/dL to indicate greater severity of inflammatory changes.[26] Oral hygiene was not included due to the absence of questionnaires applicable to this type of research.

The CRP concentrations were measured on admission, and the information about smoking status was given and noted in medical charts on admission and in the anesthesiology form before the administration of anesthesia. All patients presented with comorbidities; however, to perform surgery, these had to be clinically stabilized.

Statistical analysis

During the analyzed period, 162 records of patients hospitalized due to bone necrosis were identified. The normality of data distribution was assessed using the Kolmogorov–Smirnov test. Depending on the distribution, parametric (Student's *t*-test) or non-parametric tests (Mann–Whitney *U* test, χ^2 test) were applied. Potential risk of bias was limited to the retrospective character of the study. Selected parameters were chosen as the most objective.

Results

A total of 162 patients were included in the analysis, of whom 108 were diagnosed with MRONJ and 54 with ORN. Over the 5-year period, the number of patients with MRONJ increased during the first 3 years, followed by a decrease, while the number of ORN patients remained stable (Fig. 1). A noticeable decline observed in the 4th year (2022) coincided with the coronavirus disease 2019 (COVID-19) pandemic and potential limitations in access to healthcare services.

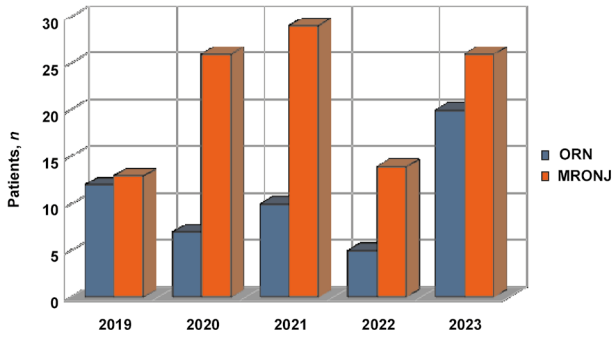

Fig. 1. Prevalence of osteoradionecrosis (ORN) and medication-related osteonecrosis of the jaw (MRONJ) over a five-year period in the analyzed study population

A statistically significant difference in average age was observed between patients with MRONJ and ORN (68 years vs. 62.9 years; $p < 0.05$, Mann–Whitney *U* test), with those affected by MRONJ being, on average, 5 years older. The majority of patients with ORN were smokers. Additionally, smoking was identified as a weak but statistically significant risk factor for bone resection and was associated with a higher rate of complications (Cramér's $V = 0.17$; $p = 0.04$). Male patients predominated in the ORN group.

Table 1. Characteristics of the study population

Variable	MRONJ	ORN	*p*-value (95% *CI*)
Patients, *n*	108	54	<0.05*
Sex (F/M)	58/50	7/47	<0.05*
Age [years] *M* ±*SD*	68.01 ±8.23	62.94 ±10.28	0.0006* (60.13–69.57)
Time of hospitalization [days] *M* ±*SD*	7.76 ±5.08	8.66 ±5.31	>0.05
Time to discharge [days] *M* ±*SD*	3.21 ±2.89	3.92 ±3.31	>0.05
CRP concentration [mg/dL]	21.03	9.35	0.0023* (4.50–29.02)
Smoking status (YES/NO), *n*	17/91	31/23	<0.0001* (0.06–0.29)
Complication on follow-up (YES/NO), *n*	53/40	31/19	>0.05
Extraoral fistula on admission (YES/NO), *n*	26/82	24/30	0.008* (0.19–0.79)
Resection (YES/NO), *n*	38/70	26/28	>0.05
Pain on admission, *n*	53	26	>0.05

* statistically significant ($p < 0.05$, Mann–Whitney *U* test or χ^2 test);
MRONJ – medication-related osteonecrosis of the jaw;
ORN – osteoradionecrosis; CRP – C-reactive protein; *M* – mean; *SD* – standard deviation; 95% *CI* – 95% confidence interval; F – female; M – male.

A total of 143 patients attended at least 1 follow-up appointment. The incidence of complications during follow-up was comparable between the 2 groups. Treatment success was defined as the absence of necrosis and complications at the first follow-up appointment, that is, approx. 6 weeks post-surgery. Subsequent visits were scheduled every 3–6 months, depending on the clinical condition.

Complications occurred in 53 of 93 patients with MRONJ (57%) and in 31 of 50 patients with ORN (62%), with no statistically significant difference between the groups ($p > 0.05$, McNemar–Bowker test). Patients with MRONJ had notably higher CRP levels at admission compared to those with ORN (21.03 mg/dL vs. 9.35 mg/dL, respectively; $p < 0.05$, Mann–Whitney *U* test; 95% confidence interval (95% *CI*): 4.50–29.02). Elevated CRP concentration was correlated with prolonged hospitalization. Similarly, longer hospital stays were observed in patients who underwent bone resection. Approximately half of the patients reported pain on admission (53/108 in the MRONJ group and 26/54 in the ORN group) and presented with intraoral or extraoral fistulas. Detailed results are summarized in Tables 1 and 2.

Discussion

In recent decades, the incidence of ORN has decreased, while MRONJ has become more common, reversing the proportions observed over the past century. Similar findings have been reported in other studies.[30,31] This trend is primarily due to the more precise use of radiotherapy techniques and proper dental preparation of patients before treatment.[32] The increasing use of antiresorptive drugs in cancer treatment, along with extended life expectancy, has contributed to the rising incidence of MRONJ.[28,31] The decline in MRONJ cases observed

Table 2. Comparison of selected parameters between patients with medication-related osteonecrosis of the jaw (MRONJ) and osteoradionecrosis (ORN)

Parameter	MRONJ	MRONJ	ORN	ORN	*p*-value (95% *CI*)
CRP [mg/dl], *n*	>10, 32	<10, 48	>10, 9	<10, 37	0.003* (1.37–8.05)
Time of hospitalization [days]	9.67	6.46	9.60	8.84	MRONJ: 0.004* (5.39–11.40)
Smoking status, *n*	YES, 17	NO, 91	YES, 31	NO, 23	<0.001* (0.06–0.29)
Resection (YES/NO), *n*	8/9	30/61	17/14	9/14	>0.05
Complication on follow-up (YES/NO), *n*	10/5	43/35	20/10	11/9	MRONJ: <0.0001* (0.04–0.29)
Pain on admission [NRS] *Me*	0	1	0	3	ORN: 0.036* (1.75–4.24)
Resection, *n*	YES, 38	NO, 70	YES, 26	NO, 28	>0.05
Time to discharge [days]	5.45	2.00	4.73	3.17	MRONJ: <0.0001* (1.74–6.68) ORN: <0.001* (1.87–5.98)
Age ≥65 years, *n*	YES, 73	NO, 35	YES, 27	NO, 27	<0.02* (1.06–4.06)
Complication on follow-up, *n*	36	17	14	17	>0.05
Localization, *n*	maxilla, 13 mandible, 90 both, 5		maxilla, 1 mandible, 51 both, 2		>0.05

NRS – numerical rating scale; *Me* – median; * statistically significant ($p < 0.05$, Mann–Whitney *U* test or χ^2 test).

in the 4th year coincided with the COVID-19 pandemic, which led to a decrease in the number of patients visiting maxillofacial surgery departments.[28,33–35]

Lockdowns and restrictions in access to healthcare services may have led to delays in treatment initiation. Unfortunately, the use of telemedicine was unable to provide any assistance to these patients. Moreover, treatment delays often resulted in the development of more advanced conditions, including abscesses and pathological fractures, which significantly limited the options for surgical treatment.[28,35]

Medication-related osteonecrosis of the jaw predominantly affects older individuals. In the present study, the average age of patients with MRONJ was 68 years. This age group is the primary target for antiresorptive drug use. Age is also correlated with a longer development period for MRONJ.[25,30,36,37]

Previous studies report a mean age of approx. 60 years for patients with ORN, whereas in the present analysis the mean age was approx. 63 years. This difference may reflect the limited application of radiotherapy or the use of lower doses in older individuals, as the potential risks of complications and limited benefits in this group make aggressive treatment less favourable.[38] The findings of this study are consistent with previously published data.[30,31,39] Patients with MRONJ were, on average, 5 years older than those with ORN. Moreover, patients aged ≥65 years presented complications more frequently during follow-up. This may be attributed to reduced regenerative potential associated with the aging process and a higher prevalence of comorbidities in older individuals, which in turn may increase the need for visits and the associated treatment costs.

Previous reports have indicated a higher prevalence of smokers among ORN patients,[30] and similar results were obtained in this analysis. Smoking is a known risk factor for both MRONJ and ORN.[27,30,31,40,41] This phenomenon may partly reflect the higher proportion of smokers among patients with head and neck cancers, as smoking is a significant carcinogenic factor in the development of these malignancies.[30,42] Additionally, smoking impairs oral tissue healing,[43] leading to a higher rate of complications, especially in MRONJ patients. There is a weak correlation between smoking status and the need for bone resection. This may be due to the abovementioned factors impairing healing process and the relatively small sample size. Nevertheless, patients should be consistently encouraged to cease smoking.

Smoking alone is unlikely to account for the observed outcomes, which could explain the weak correlation observed. The medical records analyzed in the study did not include standardized questionnaires regarding hygiene. Additionally, exacerbations of conditions such as diabetes in the postoperative period were not monitored. These factors could have an additional impact and should be addressed in future prospective studies, particularly with regard to disease exacerbation during healing process.

Previous studies have reported a higher prevalence of ORN in men,[24,29,31,41] which is consistent with the findings of the present study. Similar observations have been described by Grisar et al. and Akashi et al.[30,39] In contrast, earlier research shows that MRONJ occurs more frequently in women, with female sex often cited as a risk factor for disease development.[29] However, in this study, the incidence of MRONJ was comparable between sexes. This could be explained by the increasing use of antiresorptive drugs among men, particularly in the treatment of prostate cancer, renal cancer and multiple myeloma.[44–46] In addition, increased life expectancy among men has led to a higher incidence of neoplastic diseases. Lifestyle factors, including higher rates of tobacco and alcohol use, may also contribute to the development of necrotic lesions[30] in the oral cavity, leading to an increase in MRONJ cases. Equally significant risk factor is the generally lower level of oral hygiene among men.

The role of specialized breast units should also be considered. Physicians specialized in the treatment of female cancers may be more aware of MRONJ risk and more likely to implement preventive measures. Furthermore, studies suggest that women are generally more aware of the risk of MRONJ and more frequently seek information from medication package inserts.[47]

The treatment of bone necrosis is not free of complications. In this study, complications were defined as the occurrence of intraoral or extraoral fistulas, pain, sensory disturbances, disease recurrence, impaired wound healing or dehiscence, and pathological fractures during follow-up. The complication rates were high and comparable between the groups – 57% for MRONJ and 62% for ORN, highlighting the difficulty of treating both conditions. These results differ from some previous reports for both MRONJ[40] and ORN.[41] Improved outcomes reported in ORN studies may be attributed to the use of reconstructive techniques, such as microvascular flaps, which were not employed in the treatment of ORN in the present study. Furthermore, limited access to oral and maxillofacial surgery treatment may have contributed to poorer outcomes. Patients often initially consult primary care physicians or dentists, and limited awareness of the condition may delay the initiation of appropriate treatment.

Inflammatory processes likely play a significant role in the development of MRONJ.[1–4,42] In this study, the concentration of CRP was significantly higher in patients with MRONJ than in those with ORN. Previous research has identified elevated CRP as a marker of postoperative complications.[48] However, no studies have examined the impact of CRP concentration on treatment outcomes in ORN. The current analysis also revealed a correlation between hospitalization time and CRP concentration in MRONJ patients, with higher concentrations of CRP leading to longer hospital stays. Although a similar trend was observed in ORN patients, it did not reach statistical significance.

When analyzing this data, it is important to consider the low specificity of CRP. Elevated CRP concentrations are also observed in rheumatic and neoplastic diseases,[49] which affect a large proportion of patients with MRONJ. However, 1 study demonstrated higher CRP concentrations in patients with MRONJ compared to individuals receiving bisphosphonates without clinical signs of the disease.[50] Additionally, elevated CRP levels have been observed in patients receiving intravenous bisphosphonates,[51] and this route of administration is itself a risk factor for the development of MRONJ.

The limited specificity of CRP reduces its utility as a sole marker of complications. Nonetheless, future analyses should consider the concentration of CRP alongside other variables that potentially affect MRONJ or ORN treatment outcomes. This is especially relevant given that elevated CRP concentrations are commonly observed in patients with necrotic lesions.[50–52]

Due to the inflammatory component and frequent bacterial infections, patients with MRONJ often present with purulent fistulas. Swabs obtained from these areas allow for antibiogram analysis and targeted treatment. In many cases, this necessitates intravenous antibiotic therapy with agents available only in hospital settings, potentially prolonging the hospitalization period for these patients.

Elevated CRP levels in this patient group indicate the severity of inflammation and the necessity of initiating antibiotic therapy. Effective control of the inflammatory process significantly facilitates surgical procedures; however, it requires time and may delay surgery. Individuals with MRONJ frequently undergo chemotherapy, which compromises the body's ability to combat infection. Therefore, early assessment of inflammatory markers upon admission, followed by timely initiation of antibiotic therapy rather than limiting treatment to the perioperative period, appears justified.

The current analysis found a correlation only with CRP concentration; no such relationship was observed for white blood cell (WBC) counts. Future studies on MRONJ could consider measuring markers such as IL-6, as increased expression of IL-6 receptors has been observed in patients affected by MRONJ.[53]

Data on the duration of hospitalization in patients with jaw necrosis is scarce. One study reported a 10-day hospital stay for MRONJ patients, attributed to the need for a 5-day course of intravenous antibiotics prior to surgery.[54] No comparable data was identified for ORN. In the present analysis, patients undergoing bone resection had longer hospital stays in both MRONJ and ORN cases, likely due to the extent of the surgery. Prolonged hospitalization is also associated with increased treatment costs.

Patients with an elevated CRP concentration and MRONJ experienced longer hospital stays, likely due to the need for inflammatory markers to decrease. Another important factor is the presence of systemic diseases, particularly oncological conditions. These patients often develop anemia as a result of ongoing treatment, which may necessitate blood transfusions in the preoperative or postoperative period.

Bone resections are performed in the most advanced stages of necrosis.[6,7] According to previous studies, between 12% and 25% of ORN patients require bone resection.[39,55,56] Similar proportions have been reported for MRONJ,[57] although some studies state that nearly half of MRONJ patients undergo resection.[39] In our analysis, resection rates were similar in both groups but higher than those reported in the literature, reaching 35% for MRONJ and 48% for ORN. This may result from patients being referred late to our facility, with delayed diagnosis resulting from low awareness of the disease.[57] Furthermore, due to the limited involvement of the public sector in dental services, patients often initially consult general practitioners, which may prolong the diagnostic pathway.

The center providing care for patients with necrosis serves the Greater Poland and Lubuskie voivodeships, encompassing a significant portion of western Poland. These regions have a combined population of approx. 4.5 million, of whom 52% are women. A predominance of women becomes evident in the population pyramid from the age of 55 years. During the study period, our clinic provided services to 4,564 individuals.[58,59]

There are relatively few maxillofacial surgery outpatient clinics within the public healthcare system. Moreover, these clinics are located in large cities, while the issues of MRONJ and ORN affect patients across all regions. Transportation barriers and limited access to specialized care prolong the time needed to reach a specialized center. Additionally, it can postpone the implementation of basic treatments, such as rinses or antibiotic therapy, particularly in cases with exacerbated local conditions. Patients without access to specialist care are at risk of pathological bone fractures due to the progression of inflammation, which often necessitates resection.

Pain on admission was present in nearly half of the patients hospitalized with necrosis. The level of pain on admission did not differ significantly between the 2 groups. In available studies, pain was present in the majority of MRONJ (61% and 81%) and ORN (81% and 96%) patients.[30,39] The presence of pain is an important diagnostic signal in at-risk populations. Additionally, pain, as an unpleasant sensation, significantly reduces the quality of life for these patients.[60]

A common clinical manifestation of necrosis is the formation of intraoral fistulas. In this analysis, such lesions were present in the majority of MRONJ and ORN patients, consistent with previous reports.[30,39] No significant differences between the groups were found. However, skin fistulas were more frequently observed in ORN patients. This observation aligns with the outcomes of other studies,[30,39] and may be related to the poorer condition of the skin in irradiated areas. The mandible remains the most commonly affected site, whereas the maxilla is involved

less frequently. This is primarily due to the mandible's poorer blood supply and its structure, including a lower proportion of cancellous bone.[30,39]

Limitations

When considering potential risk factors, it is necessary to account for other clinical situations that may influence treatment outcomes and hospitalization time. As mentioned earlier, several factors may have an impact on the results of this analysis. These include oral hygiene, alcohol consumption and comorbidities. The socioeconomic status of the patient is also of significance, as patients from smaller towns may face difficulties in accessing specialized care.

In the present study, oral hygiene was not assessed using any available standardized indices applicable to scientific studies; thus, this variable was excluded from our analysis. The qualification for performing surgery, except in emergency cases, required stabilization of chronic diseases. Consequently, these conditions were not considered risk factors in the analysis. However, they should be taken into account in future studies, particularly prospective ones, due to the potential for exacerbation and their impact on healing outcomes.

Additionally, relying solely on patient medical history and test results may limit data utility. In this study, we aimed to prioritize objective factors. Nevertheless, a significant portion of the data was based on assessments not conducted by the primary researcher. This limitation is inherent to retrospective studies. However, the potential risk of bias is considered low due to the nature of the data.

Recommendations and future research directions

Many publications emphasize the importance of preventing MRONJ and ORN,[61] as the treatment of these conditions remains highly challenging. Nevertheless, instances of improper management are still reported.[5]

The number of patients at risk for MRONJ and ORN is growing. Education on these conditions should be incorporated into medical and dental curricula. This topic should also form an integral part of postgraduate training, including specialized courses and certification programs. Additionally, not only dentists but also primary care physicians, oncologists and rheumatologists should regularly update their knowledge of MRONJ or ORN, as these specialists most frequently prescribe medications associated with the risk of MRONJ. Improved awareness will facilitate earlier identification of high-risk patients.[23]

In response to projections regarding oncological diseases, the establishment of care centers for high-risk patients should be considered. Such centers would address the growing number of patients requiring specialized care due to an increased risk of complications. They should ensure interdisciplinary approach to the patient by involving specialists from fields such as dentistry, oncology, and oral and maxillofacial surgery.

In addition to educating healthcare professionals, it is also crucial to increase patient awareness about potential complications. For example, similarly to patients receiving anticoagulants, individuals at risk of MRONJ could be provided with a short leaflet for their dentist, outlining their medication history. This is especially relevant, as some medications are administered every 3 months or for limited durations.

Patients should also be instructed on the importance of meticulous oral hygiene. Highlighting the benefits of this approach should be a key responsibility of the physician initiating treatment.

Historically, the issue of "phossy jaw", associated with occupational exposure to phosphorus, was mitigated through mandatory dental examinations.[62,63] Similarly, implementing preventive measures, such as ensuring dental clearance of oral inflammatory foci before initiating antiresorptive therapy for oncological treatment, should be considered.

Patients receiving medications associated with MRONJ risk or those who have undergone radiotherapy should be informed about the importance of regular dental check-ups.

There is a lack of large-scale population-based studies on patients with MRONJ and ORN that encompass aspects such as quality of life, oral hygiene levels and systematically collected clinical data. Such studies would facilitate meta-analyses and provide deeper insights.

The introduction of standardized data collection forms, completed by both patients and clinicians, should be considered. These should include information on duration and route of medication administration, type of radiotherapy, pain intensity, oral hygiene (assessed using available scales), smoking status, comorbidities, and other relevant factors.

Currently, non-surgical methods of prevention primarily include enhanced oral hygiene and the use of antibiotics. Antibiotic therapy before and after invasive procedures related to the oral cavity is widely recommended, typically starting 12 h before the procedure and continuing for up to 14 days.[64] Antibiotics and chlorhexidine oral mouthwashes are also used in conservative treatment, with moderate effectiveness. Other non-surgical methods include hyperbaric oxygen therapy[65,66] and pharmacological interventions.

The ongoing PENTO study is aiming to assess the possibility of using pentoxifylline and tocopherol in patients with MRONJ.[67] The combination of these 2 drugs has already demonstrated positive effects in ORN.[68,69]

Surgical attempts include the use of platelet-rich fibrin (PRF) in the socket after tooth extraction to reduce the risk of MRONJ or as a treatment method alone.[65,68–70]

Future research should focus on identifying specific risk factors and evaluating therapeutic approaches, as well as

exploring novel prognostic markers, such as IL-6. The absence of standardized treatment protocols highlights the need for randomized clinical trials, which should also consider long-term follow-up.

The majority of available papers is based on case reports and non-randomized studies,[71] making it challenging to establish optimal treatment methods. Future research should consider factors such as CRP, IL-6, lesion location, specific interventions (e.g., the use of PRF), and extended follow-up periods.

Conclusions

Medication-related osteonecrosis of the jaw and ORN are distinct conditions, both representing serious complications of modern therapies. While advances in radiotherapy have contributed to a decrease in the incidence of ORN, the number of MRONJ cases has been alarmingly increasing.

Medication-related osteonecrosis of the jaw primarily affects patients with a higher disease burden, particularly those with active cancer. In contrast, ORN typically develops after the completion of treatment. Neither condition has a fully understood pathophysiology, and there is no gold standard treatment. However, both share certain similarities in clinical aspects.

Since diagnosis relies on patient history and clinical examination, clear differentiation between MRONJ and ORN is crucial. Physician awareness of these conditions remains limited but is gradually improving. Therefore, dissemination of clinical data regarding these diseases is important for continued education of healthcare professionals, enhancement of treatment outcomes, and the promotion of wider use of preventive strategies.

Further research should primarily include randomized trials with extended observation periods. During the study design phase, maximum standardization of parameters such as quality of life, oral hygiene status and inflammatory markers should be considered to enable better selection of therapeutic approaches in the future.

Until an effective treatment method is identified, and even thereafter, prevention should be actively promoted. Current preventive measures include antibiotic therapy, minimally invasive procedures and enhanced monitoring of patients in high-risk groups.

To the best of our knowledge, this is the first study to describe patient characteristics and treatment outcomes of MRONJ and ORN in Poland.

Ethics approval and consent to participate

The study protocol was approved by the Bioethics Committee at Poznan University of Medical Sciences, Poland (approval No. KB761/24).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Artificial intelligence (ChatGPT; OpenAI, San Francisco, USA) was used for language editing and to improve readability.

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References

1. Bernardi S, Di Girolamo M, Necozione S, Continenza MA, Cutilli T. Antiresorptive drug-related osteonecrosis of the jaws, literature review and 5 years of experience. *Musculoskelet Surg*. 2019;103(1):47–53. doi:10.1007/s12306-018-0548-6
2. Sharma S, Shankar R, Ravi Kiran BS, Breh R, Sarangi S, Kumar Upadhyay A. A narrative review of osteonecrosis of the jaw: What a clinician should know. *Cureus*. 2023;15(12):e51183. doi:10.7759/cureus.51183
3. Ahdi HS, Wichelmann TA, Pandravada S, Ehrenpreis ED. Medication-induced osteonecrosis of the jaw: A review of cases from the Food and Drug Administration Adverse Event Reporting System (FAERS). *BMC Pharmacol Toxicol*. 2023;24(1):15. doi:10.1186/s40360-023-00657-y
4. Eguia A, Bagán-Debón L, Cardona F. Review and update on drugs related to the development of osteonecrosis of the jaw. *Medicina Oral Patol Oral Cir Bucal*. 2020;25(1):e71–e83. doi:10.4317/medoral.23191
5. Soliman T, Ali Z, Zayed M, Sabry D, AbuBakr N. Assessing the bone-healing potential of bone marrow mesenchymal stem cells in jawbone osteoporosis in albino rats. *Dent Med Probl*. 2022;59(1):75–83. doi:10.17219/dmp/139200
6. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' position paper on medication-related osteonecrosis of the jaws – 2022 update. *J Oral Maxillofac Surg*. 2022;80(5):920–943. doi:10.1016/j.joms.2022.02.008
7. Frankart AJ, Frankart MJ, Cervenka B, Tang AL, Krishnan DG, Takiar V. Osteoradionecrosis: Exposing the evidence not the bone. *Int J Radiat Oncol Biol Phys*. 2021;109(5):1206–1218. doi:10.1016/j.ijrobp.2020.12.043
8. Regaud C. Sur la sensibilité du tissu osseux normal vis-à-vis des radiations X et y et sur le mécanisme de l'ostéoradionécrose [in French]. *Compt Rend Soc de Biol*. 1922;87:629.
9. Dekker H, Bravenboer N, van Dijk D, et al. The irradiated human mandible: A quantitative study on bone vascularity. *Oral Oncol*. 2018;87:126–130. doi:10.1016/j.oraloncology.2018.10.030
10. Xu J, Zheng Z, Fang D, et al. Early-stage pathogenic sequence of jaw osteoradionecrosis in vivo. *J Dent Res*. 2012;91(7):702–708. doi:10.1177/0022034512448661

11. Beech N, Porceddu S, Batstone MD. Preradiotherapy dental extractions and health-related quality of life. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;122(6):672–679. doi:10.1016/j.oooo.2016.07.020
12. Wahl MJ. Osteoradionecrosis prevention myths. *Int J Radiat Oncol Biol Phys*. 2006;64(3):661–669. doi:10.1016/j.ijrobp.2005.10.021
13. Patel V, Gadiwalla Y, Sassoon I, Sproat C, Kwok J, McGurk M. Use of pentoxifylline and tocopherol in the management of osteoradionecrosis. *Br J Oral Maxillofac Surg*. 2016;54(3):342–345. doi:10.1016/j.bjoms.2015.11.027
14. Słowik Ł, Totoń E, Nowak A, Wysocka-Słowik A, Osmola K, Ślebioda Z. Pharmacological treatment of medication-related osteonecrosis of the jaw (MRONJ) with pentoxifylline and tocopherol – report of two cases. *J Int Dent Med Res*. 2024;17(1):370–376. http://www.jidmr.com/journal/wp-content/uploads/2024/03/57-D23_2257_Lukasz_Slowik_Poland-Case.pdf. Accessed September 26, 2024.
15. Baumann DP, Yu P, Hanasono MM, Skoracki RJ. Free flap reconstruction of osteoradionecrosis of the mandible: A 10-year review and defect classification. *Head Neck*. 2010;33(6):800–807. doi:10.1002/hed.21537
16. Kış HC, Coşgunarslan A, Delikan E, Aksu S. Does childhood chemotherapy affect mandibular bone structures in a lifetime? *Dent Med Probl*. 2022;59(4):495–501. doi:10.17219/dmp/146752
17. Rosales HD, Garcia Guevara H, Requejo S, Jensen MD, Acero J, Olate S. Medication-related osteonecrosis of the jaws (MRONJ) in children and young patients – a systematic review. *J Clin Med*. 2023;12(4):1416. doi:10.3390/jcm12041416
18. Liu Y, Cheng H, Zhang Z, et al. Mandible osteoradionecrosis and asymmetry in pediatric patients treated with proton therapy for head and neck malignancies. *Int J Radiat Oncol Biol Phys*. 2022;114(3):e481. doi:10.1016/j.ijrobp.2022.07.1748
19. Tetradis S, Allen MR, Ruggiero SL. Pathophysiology of medication-related osteonecrosis of the jaw – a minireview. *JBMR Plus*. 2023;7(8):e10785. doi:10.1002/jbm4.10785
20. Vahidi N, Lee TS, Daggumati S, Shokri T, Wang W, Ducic Y. Osteoradionecrosis of the midface and mandible: Pathogenesis and management. *Semin Plast Surg*. 2020;34(4):232–244. doi:10.1055/s-0040-1721759
21. Pagare SS, Khosa SK. Osteoradionecrosis. *Int J Head Neck Surg*. 2011;2(1):27–32. doi:10.5005/jp-journals-10001-1043
22. Steel BJ. Management of medication-related osteonecrosis of the jaw (MRONJ) risk in patients due to commence anti-resorptive/anti-angiogenic drugs – how should pre-drug-treatment dental preventive care be organised? *Community Dent Health*. 2019;36(4):245–254. doi:10.1922/CDH_4582Steel11
23. Poxleitner P, Engelhardt M, Schmelzeisen R, Voss P. The prevention of medication-related osteonecrosis of the jaw. *Dtsch Arztebl Int*. 2017;114(5):63–69. doi:10.3238/arztebl.2017.0063
24. Kim R, Kim SW, Kim H, Ku SY. The impact of sex steroids on osteonecrosis of the jaw. *Osteoporos Sarcopenia*. 2022;8(2):58–67. doi:10.1016/j.afos.2022.05.003
25. AlRowis R, Aldawood A, AlOtaibi M, et al. Medication-related osteonecrosis of the jaw (MRONJ): A review of pathophysiology, risk factors, preventive measures and treatment strategies. *Saudi Dent J*. 2022;34(3):202–210. doi:10.1016/j.sdentj.2022.01.003
26. Shin WJ, Kim CH. Prognostic factors for outcome of surgical treatment in medication-related osteonecrosis of the jaw. *J Korean Assoc Oral Maxillofac Surg*. 2018;44(4):174–181. doi:10.5125/jkaoms.2018.44.4.174
27. Ciobanu GA, Camen A, Ionescu M, et al. Risk factors for medication-related osteonecrosis of the jaw – a binomial analysis of data of cancer patients from craiova and constanta treated with zoledronic acid. *J Clin Med*. 2023;12(11):3747. doi:10.3390/jcm12113747
28. Nowak A, Słowik Ł, Szcześniak M, Okła M, Osmola K, Wyganowska M. Osteonecrosis of the jaw: Surgical treatment results before and during the COVID-19 pandemic in one of the reference hospitals in Poland. *Eur Rev Med Pharmacol Sci*. 2024;28(2):772–777. doi:10.26355/eurrev_202401_35077
29. De Antoni CC, Matsumoto MA, da Silva AA, et al. Medication-related osteonecrosis of the jaw, osteoradionecrosis, and osteomyelitis: A comparative histopathological study. *Braz Oral Res*. 2018;32:e23. doi:10.1590/1807-3107bor-2018.vol32.0023
30. Grisar K, Schol M, Schoenaers J, et al. Osteoradionecrosis and medication-related osteonecrosis of the jaw: Similarities and differences. *Int J Oral Maxillofac Surg*. 2016;45(12):1592–1599. doi:10.1016/j.ijom.2016.06.016
31. Kün-Darbois JD, Fauvel F. Medication-related osteonecrosis and osteoradionecrosis of the jaws: Update and current management. *Morphologie*. 2020;105(349):170–187. doi:10.1016/j.morpho.2020.11.008
32. Peterson DE, Doerr W, Hovan A, et al. Osteoradionecrosis in cancer patients: The evidence base for treatment-dependent frequency, current management strategies, and future studies. *Support Care Cancer*. 2010;18(8):1089–1098. doi:10.1007/s00520-010-0898-6
33. Visholm TM, Sandhu N, Dhariwal DK. COVID-19: The oral and maxillofacial surgery experience, Oxford, UK. *Craniomaxillofac Trauma Reconstr*. 2021:14(4):317–324. doi:10.1177/1943387521991267
34. Blackhall KK, Downie IP, Ramchandani P, et al. Provision of emergency maxillofacial service during the COVID-19 pandemic: A collaborative five centre UK study. *Br J Oral Maxillofac Surg*. 2020;58(6):698–703. doi:10.1016/j.bjoms.2020.05.020
35. Lentge F, Jehn P, Zeller AN, Spalthoff S, Rahlf B, Korn P. Changes in emergency patient presentation to a maxillofacial surgery department during the COVID-19 pandemic. *J Oral Maxillofac Surg*. 2021;79(10):2123.ei–2123.e6. doi:10.1016/j.joms.2021.05.026
36. Kwack DW, Park SM. Prediction of medication-related osteonecrosis of the jaw (MRONJ) using automated machine learning in patients with osteoporosis associated with dental extraction and implantation: A retrospective study. *J Korean Assoc Oral Maxillofac Surg*. 2023;49(3):135–141. doi:10.5125/jkaoms.2023.49.3.135
37. Khan AA, Rios LP, Sándor GKB, et al. Bisphosphonate-associated osteonecrosis of the jaw in Ontario: A survey of oral and maxillofacial surgeons. *J Rheumatol*. 2011;38(7):1396–1402. doi:10.3899/jrheum.100221
38. Metges JP, Eschwege F, de Crevoisier R, Lusinchi A, Bourhis J, Wibault P. Radiotherapy in head and neck cancer in the elderly: A challenge. *Crit Rev Oncol Hematol*. 2000;34(3):195–203. doi:10.1016/s1040-8428(00)00061-5
39. Akashi M, Wanifuchi S, Iwata E, et al. Differences between osteoradionecrosis and medication-related osteonecrosis of the jaw. *Oral Maxillofac Surg*. 2018;22(1):59–63. doi:10.1007/s10006-017-0667-5
40. Wessel JH, Dodson TB, Zavras AI. Zoledronate, smoking, and obesity are strong risk factors for osteonecrosis of the jaw: A case–control study. *J Oral Maxillofac Surg*. 2008;66(4):625–631. doi:10.1016/j.joms.2007.11.032
41. Fitzgerald KT, Lyons C, England A, et al. Risk factors associated with the development of osteoradionecrosis (ORN) in Head and Neck cancer patients in Ireland: A 10-year retrospective review. *Radiother Oncol*. 2024;196:110286. doi:10.1016/j.radonc.2024.110286
42. Du E, Mazul AL, Farquhar D, et al. Long-term survival in head and neck cancer: Impact of site, stage, smoking, and human papillomavirus status. *Laryngoscope*. 2019;129(11):2506–2513. doi:10.1002/lary.27807
43. Balaji SM. Tobacco smoking and surgical healing of oral tissues: A review. *Indian J Dent Res*. 2008;19(4):344–348. doi:10.4103/0970-9290.44540
44. Lee OL, Horvath N, Lee C, et al. Bisphosphonate guidelines for treatment and prevention of myeloma bone disease. *Intern Med J*. 2017;47(8):938–951. doi:10.1111/imj.13502
45. Vrdoljak E, Gore M, Leyman S, et al. Bisphosphonates in patients with renal cell carcinoma and bone metastases: A sunitinib global expanded-access trial subanalysis. *Future Oncol*. 2015;11(20):2831–2840. doi:10.2217/fon.15.140
46. Nayyar R, Gupta NP. Use of bisphosphonates in prostate cancer: Current status. *Indian J Urol*. 2007;23(1):51–54. doi:10.4103/0970-1591.30267
47. Abdullateef AA, Alhareky MS. Awareness among patient at risk of developing medication related osteonecrosis of the jaw (MRONJ) – A primary prevention strategy. *Saudi Pharm J*. 2020;28(6):771–778. doi:10.1016/j.jsps.2020.05.004
48. Shim YH, Jung SH, Boboeva O, et al. Analysis of factors associated with the postoperative healing of medication-related osteonecrosis of the jaw in patients with osteoporosis. *J Clin Med*. 2021;10(16):3651. doi:10.3390/jcm10163651

49. Mouliou DS. C-reactive protein: Pathophysiology, diagnosis, false test results and a novel diagnostic algorithm for clinicians. *Diseases*. 2023;11(4):132. doi:10.3390/diseases11040132
50. Choi SY, An CH, Kim SY, Kwon TG. Bone turnover and inflammatory markers of bisphosphonate-related osteonecrosis of the jaw in female osteoporosis patients. *J Oral Maxillofac Surg Med Pathol*. 2012;25(2):123–128. doi:10.1016/j.ajoms.2012.06.007
51. Lee SH, Choi SY, Bae MS, Kwon TG. Characteristics of patients with osteonecrosis of the jaw with oral versus intravenous bisphosphonate treatment. *Maxillofac Plast Reconstr Surg*. 2021;43(1):24. doi:10.1186/s40902-021-00310-w
52. Hata H, Kitao T, Sato J, et al. Monitoring indices of bone inflammatory activity of the jaw using SPECT bone scintigraphy: A study of ARONJ patients. *Sci Rep*. 2020;10(1):111385. doi:10.1038/s41598-020-68428-x
53. Gkouveris I, Soundia A, Gouveris P, Zouki D, Hadaya D, Tetradis S. Macrophage involvement in medication-related osteonecrosis of the jaw (MRONJ): A comprehensive, short review. *Cancers (Basel)*. 2022;14(2):330. doi:10.3390/cancers14020330
54. Laimer J, Höller A, Pichler U, et al. Nutritional status in patients with medication-related osteonecrosis of the jaw (MRONJ). *Nutrients*. 2021;13(5):1585. doi:10.3390/nu13051585
55. Kumar S, Chandran C, Chacko R, Jesija JS, Paul A. Osteoradionecrosis of jaw: An institutional experience. *Contemp Clin Dent*. 2018;9(2):242–248. doi:10.4103/ccd.ccd_843_17
56. Zaghi S, Miller M, Blackwell K, Palla B, Lai C, Nabili V. Analysis of surgical margins in cases of mandibular osteoradionecrosis that progress despite extensive mandible resection and free tissue transfer. *Am J Otolaryngol*. 2012;33(5):576–580. doi:10.1016/j.amjoto.2012.03.001
57. Ekici Ö. Awareness of medication related osteonecrosıs of the jaw among dentist working at public oral and dental health care centers in Ankara, Turkey. *Turkiye Klinikleri J Dental Sci*. 2021;27(1):98–107. doi:10.5336/dentalsci.2020-74342
58. Statistics Poland – Spatial and Environmental Surveys Department. *Regions of Poland 2022*. Warsaw, Poland: Zakład Wydawnictw Statystycznych; 2022. https://stat.gov.pl/download/gfx/portalinformacyjny/en/defaultaktualnosci/3315/5/16/1/regions_of_poland_2022.pdf. Accessed November 18, 2024.
59. Statistics Poland, Demographic Surveys Department. *Population. Size and structure and vital statistics in Poland by territorial division in 2024. As of 30 June*. Warsaw, Poland: Zakład Wydawnictw Statystycznych; 2024. https://stat.gov.pl/download/gfx/portalinformacyjny/pl/defaultaktualnosci/5468/6/37/1/ludnosc._stan_i_struktura_vi2024.pdf. Accessed December 9, 2024.
60. Murphy J, Mannion CJ. Medication-related osteonecrosis of the jaws and quality of life: Review and structured analysis. *Br J Oral Maxillofac Surg*. 2020;58(6):619–624. doi:10.1016/j.bjoms.2020.03.010
61. Nieckula P, Stempniewicz A, Tubaja M. Prophylaxis of osteonecrosis in the case of patients treated with bisphosphonates: A review paper. *Dent Med Probl*. 2018;55(4):425–429. doi:10.17219/dmp/99021
62. McLeod NMH, Brennan PA, Ruggiero SL. Bisphosphonate osteonecrosis of the jaw: A historical and contemporary review. *Surgeon*. 2011;10(1):36–42. doi:10.1016/j.surge.2011.09.002
63. Devlin H. A historical review of 'phossy jaw'. *Br Dent J*. 2023;234(11):825–826. doi:10.1038/s41415-023-5859-9
64. The Polish Dental Society. *Rekomendacje Grupy Roboczej Polskiego Towarzystwa Stomatologicznego i Narodowego Programu Ochrony Antybiotyków w Zakresie Stosowania Antybiotyków w Stomatologii*. Warsaw, Poland: Narodowy Instytut Leków; 2019. https://antybiotyki.edu.pl/wp-content/uploads/2019/01/stomatologia-zalecenia-25_01-net.pdf. Accessed December 9, 2024.
65. On SW, Cho SW, Byun SH, Yang BE. Various therapeutic methods for the treatment of medication-related osteonecrosis of the jaw (MRONJ) and their limitations: A narrative review on new molecular and cellular therapeutic approaches. *Antioxidants (Basel)*. 2021;10(5):680. doi:10.3390/antiox10050680
66. Inchingolo AM, Malcangi G, Ferrara I, et al. MRONJ treatment strategies: A systematic review and two case reports. *Appl Sci*. 2023;13(7):4370. doi:10.3390/app13074370
67. ClinicalTrials.gov. Pentoxifylline and tocopherol (PENTO) in the treatment of medication-related osteonecrosis of the jaw (MRONJ). https://clinicaltrials.gov/ct2/show/NCT03040778. Accessed December 9, 2024.
68. Meleca JB, Zhang E, Fritz MA, Ciolek PJ. Overview and emerging trends in the treatment of osteoradionecrosis. *Curr Treat Options Oncol*. 2021;22(12):115. doi:10.1007/s11864-021-00915-3
69. Peterson DE, Koyfman SA, Yarom N, et al. Prevention and management of osteoradionecrosis in patients with head and neck cancer treated with radiation therapy: ISOO–MASCC–ASCO Guideline. *J Clin Oncol*. 2024;42(16):1975–1996. doi:10.1200/jco.23.02750
70. Besi E, Pitros P. The role of leukocyte and platelet-rich fibrin in the prevention of medication-related osteonecrosis of the jaw, in patients requiring dental extractions: An observational study. *Oral Maxillofac Surg*. 2024;28(2):785–793. doi:10.1007/s10006-023-01204-z
71. Frutuoso F, Freitas F, Vilares M, et al. Medication-related osteonecrosis of the jaw: A systematic review of case reports and case series. *Diseases*. 2024;12(9):205. doi:10.3390/diseases12090205

Original paper

Effect of a platform switch and a laser-microgrooved coronal design on short dental implants: A multicenter retrospective study

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Abstract

Background. The presence of severe atrophy in proximity to the maxillary sinus or the inferior alveolar nerve is a primary indication for the use of short dental implants. The placement of standard-length implants in such cases often requires complex regenerative procedures, leading to prolonged healing time and an increased invasiveness of the surgical procedure.

Objectives. The aim of the study was to evaluate crestal bone remodeling, implant success, complications, and implant- and patient-related risk factors associated with the use of short, platform-switched dental implants with a laser-microgrooved collar for prosthetic rehabilitation of atrophic posterior jaws.

Material and methods. This multicenter retrospective study involved a chart review of medical records from 5 private dental clinics. Variables such as age, sex, smoking, periodontal disease, systemic diseases, medications, anatomical location, implant diameter and length, crown-to-implant ratio, and type of placement were collected. A total of 132 short implants placed in 84 patients were analyzed, with a follow-up of 3–5 years (mean (*M*): 4.2 ±0.8 years).

Results. Eight short implants failed, including 62.5% of those affected by peri-implantitis. The implant-level and patient-level success rates were 93.9% and 95.0%, respectively. The mean crestal bone loss (CBL) was 0.385 ±0.7 mm. Univariate regression analysis revealed that female sex, smoking status and periodontal disease were significantly associated with implant success at both the implant and patient levels.

Conclusions. Platform-switched short implants with a laser-microgrooved coronal design demonstrated high success rates in atrophic posterior jaws after 3–5 years of function, with stable crestal bone levels.

Keywords: short dental implant, platform switch, laser-microgrooved collar

Highlights

- Short implants (<10 mm) may be considered as an alternative to regenerative procedures in the rehabilitation of the atrophic maxilla.
- The use of platform switching, appropriate macrogeometry and surface treatment promotes connective tissue stabilization at the implant collar.
- Short implants can effectively support single crowns in atrophied posterior alveolar ridges with high long-term success rates.

Introduction

Osseointegrated dental implants with a conventional length of 10 mm or greater represent a well-established modality for the prosthetic rehabilitation of partially and completely edentulous patients. However, their use in cases of severe posterior jaw atrophy, particularly in proximity to the inferior alveolar nerve or the maxillary sinus, often requires additional bone augmentation techniques, which increase both the biological and economic burden for patients.[1–4] In recent years, short dental implants (<10 mm) have been proposed as an alternative approach to avoid advanced bone augmentation procedures in atrophic jaws.[5–7] However, the literature reports conflicting data regarding the success rates of short implants in the atrophic posterior alveolar ridge.[5–16] First studies on short dental implants with machined (smooth) surfaces indicated that reduced bone-to-implant contact could represent an important risk factor for failure, especially in low-density bone, such as the atrophic posterior maxilla.[8–12] In recent years, the use of short dental implants with textured surfaces, which augment the integrated surface area, has made it possible to overcome this limitation.[13,14] Preliminary studies have indicated that short implants with textured surfaces present clinical outcomes and success rates comparable to those of standard-length implants in the posterior maxilla.[13,14]

The criteria for defining implant success remain a matter of ongoing debate. However, especially for short implants, the maintenance of crestal bone stability is of utmost importance.[15] It is generally accepted that bone loss of up to 2 mm during the first year of functional loading, followed by an annual loss of approx. 0.2 mm around the implant neck, can be considered physiological.[16] Nevertheless, even this level of crestal bone loss (CBL) may pose a significant concern for the long-term success of short implants compared to standard-length implants. For example, after 5 years of loading, a bone loss of approx. 2.0–2.5 mm around a 6-mm implant would correspond to nearly half of the implant length.

Various implant neck designs and surface modifications have been proposed to minimize CBL. Among these, a platform switch associated with laser-ablated microchannels placed within the implant collar has shown favorable results.[17] Platform switching involves the use of a prosthetic abutment with a smaller diameter than the implant platform, thereby influencing the peri-implant biological width through geometric modification of the supracrestal connective tissue space. Additionally, laser-microgrooved surfaces have been developed to guide the alignment and the subsequent growth of fibroblasts at the implant neck (Fig. 1).[18–20] Histological studies in humans have demonstrated a different organization of peri-implant soft tissues around laser-microgrooved surfaces compared to machined (smooth) surfaces, characterized by an intimate physical contact of connective tissue and perpendicularly oriented collagen fibers along the laser-ablated, microgrooved implant necks.[21]

Previous studies evaluating standard-length, platform-switched implants with a laser-microgrooved collar have indicated that the configuration of the coronal part of the implant may counteract CBL.[18] To the best of our knowledge, no studies have investigated the combined effect of platform switching and a laser-microgrooved collar on the stability of the marginal bone in short dental implants. Therefore, the objective of the current multicenter retrospective study was to evaluate the clinical and radiographic outcomes of short dental implants featuring a platform switch and a laser-microgrooved collar in atrophic posterior maxillae and mandibles after 3–5 years of loading.

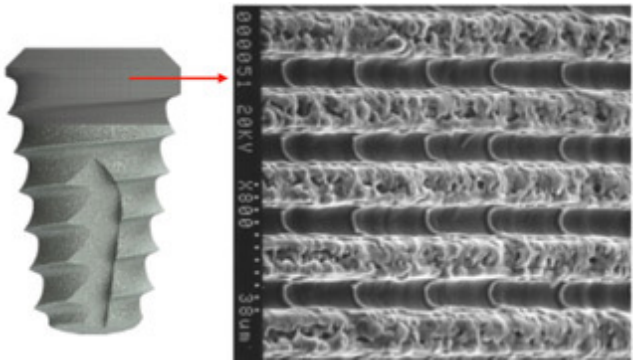

Fig. 1. Short dental implant with a laser-microgrooved surface; on the right, scanning electron microscopy (SEM) image at ×800 magnification

Material and methods

A retrospective, multicenter, clinical and radiographic study was conducted. A clinical database of subjects previously treated within an approved research framework was used; therefore, this study did not require additional approval from an ethics committee. All patients provided written informed consent, and the study followed the guidelines of the Declaration of Helsinki on experimentation involving human subjects. Patients were treated in 5 private dental clinics.

The inclusion criteria were as follows: patients aged ≥18 years; absence of medical conditions considered contraindications to implant surgery; no history of alcohol or drug abuse; presence of resorbed posterior edentulous jaws requiring prosthetic rehabilitation with dental implants; and the presence of residual bone height to allow placement of implants of at least 6 mm or 7.5 mm in length, as well as residual bone width of at least 4.6 mm, as assessed by cone beam computed tomography (CBCT).

The following exclusion criteria were implemented: poor oral hygiene (full-mouth plaque score and full-mouth bleeding score >25%); and discontinuation of supportive periodontal therapy.

Patients were classified as periodontally compromised when ≥3 mm of attachment loss and ≥30% of radiographic bone loss affecting at least 30% of sites were present.

The analysis included 84 patients (44 men (52.4%) and 40 women (47.6%); mean age: 52.49 ±11.23 years) who underwent implant therapy between December 2015 and December 2018. Patients with severely atrophied, partially edentulous ridges (Classes III and IV according to Cawood and Howell[19]), with ≥8 mm of residual bone height and a thickness of ≥6 mm who received at least 1 short implant (≤7.5 mm) supporting a single crown were included in the study (Fig. 2). Smokers, patients with periodontal disease (treated or untreated) and individuals with systemic conditions not considered absolute contraindications to implant surgery were included. Intraoral radiographs, CBCT scans, diagnostic wax patterns, and surgical templates were used for presurgical evaluation. At the end of the follow-up period (mean (*M*): 4.2 ±0.8 years), a total of 132 short implants were evaluated.

Surgical protocol

The same surgical protocol was applied in all patients. Antibiotic therapy consisted of amoxicillin and clavulanic acid (Augmentin; GlaxoSmithKline, Verona, Italy) at a dose of 1 g twice a day for 7 days, and rinses with 0.12% chlorhexidine digluconate solution (Dentosan; Johnson & Johnson, New Brunswick, USA) twice daily for 1 min prior to surgery. Infiltrations of articaine with epinephrine (Citocartin 1.7 mL; Molteni Dental, Milan, Italy) were used for local anesthesia. A full-thickness flap was then elevated at the level of the keratinized mucosa, with care

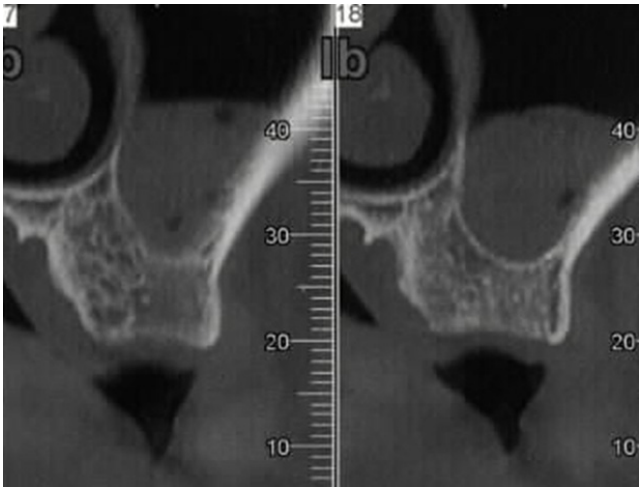

Fig. 2. Radiographic images of severely atrophic edentulous maxillary ridges

taken to preserve the periodontal integrity of adjacent teeth. Implant placement was carried out according to the manufacturer's instructions. Finally, 4-0 or 5-0 monofilament sutures were used and left in place for 10 days. Postoperatively, ibuprofen 600 mg was prescribed, with an additional dose administered after 8 h. Second-stage surgery for placement of healing abutments was performed after 4–6 months, followed by prosthetic restoration delivery at 5–7 months after implant placement.

Implants

Short implants with a platform switch and a laser-microgrooved coronal design (Tapered Short; BioHorizons, Birmingham, USA) were used in all patients (Fig. 3). The implants varied in diameter (4.6 mm and 5.8 mm) and length (6.0 mm and 7.5 mm), depending on the bone dimensions at each site.

Follow-up

Patients were recalled at 5–10, 30, and 90 days following implant placement for clinical evaluation. After crown

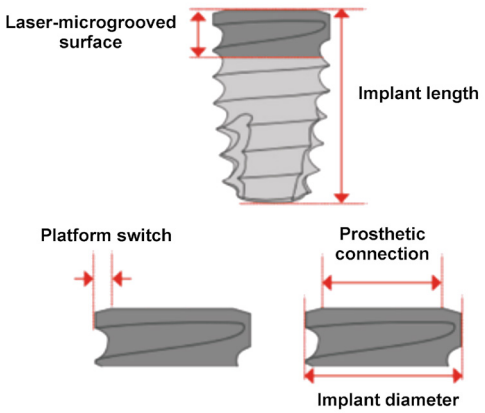

Fig. 3. Schematic representation of the design features of a short implant

delivery, patients were enrolled in a 6-month recall program for professional oral hygiene maintenance. The following parameters were recorded at crown delivery and at each control visit: number of sites with plaque; number of sites with bleeding on probing (BOP); probing depth (PD); and mucosal recession (REC). Additional variables included anatomical location, implant diameter and length, type of placement (immediate or conventional), smoking habits, presence of periodontal disease, systemic diseases, pharmacological therapies, and implant success (time). Based on the criteria proposed by Albrektsson et al.,[16] implant success was defined as the absence of mobility, peri-implant radiolucency and pain, as well as the absence of infection, neuropathy, paresthesia, or any violation of the mandibular canal. In addition, annual bone loss after the first year of function was required to be <0.2 mm.

For radiographic assessment, an individualized acrylic device was fixed to the residual dentition, and a radiograph holder was constructed for each patient. This technique ensured that the same position of the radiograph film could be reproduced at each visit, and the angle of the radiograph would not deviate.

Crestal bone loss was assessed radiographically on the mesial and distal sides of each implant by subtracting the CBL value at baseline from CBL at the 3/5-year follow-up visits. Measurements were calculated using imaging software (Gendex™ VixWin™ Platinum; Gendex, Des Plaines, USA).

To ensure reproducibility, calibration of the examiners was performed (1 examiner per clinic) by repeated assessment of clinical and radiographic parameters in the same 10 patients and using the same radiographs at a 1-week interval, achieving an intraexaminer reliability of 90%.

The following clinical variables were recorded:

- plaque score: assessed at 4 sites per implant (mesial, midfacial, distal, and palatal) using a dichotomous scale (0 = no visible plaque at the soft tissue margin; 1 = visible plaque at the soft tissue margin);
- probing depth: measured to the nearest 0.5 mm at 4 sites per implant (mesial, midfacial, distal, and palatal) using a manual probe (CP 15 UNC; Hu-Friedy Manufacturing, Co., LLC, Chicago, USA);
- bleeding on probing: recorded at 4 sites per implant (mesial, midfacial, distal, and palatal) using a dichotomous scale (0 = no bleeding; 1 = bleeding).

Statistical analysis

The sample size was determined based on previously published data.[13,14] The following parameters were used: a minimal detectable difference between means of 0.5; standard deviations on the difference between means of 0.7; an effect size of 0.71; a beta error of 10%; and a one-tailed alpha error of 5% with an 80% power. These calculations indicated that a minimum of 84 patients was required. Data analysis was conducted using the SAS/STAT® software (v. 9.2; SAS Institute Inc., Cary, USA). The data was analyzed to evaluate the survival rate of this type of rehabilitation at 5 years post-loading. Changes in CBL between baseline and 3/5-year follow-up were assessed using the Wilcoxon signed-rank test. The level of significance was set at 0.05. Homogeneity of variance and normality were assessed using Levene's test and the Shapiro–Wilk test, respectively.

Repeated measures analysis of variance (ANOVA) was used for inter- and intragroup comparisons over time. Variability within and between subjects was used to estimate standard errors for regression coefficients. Bonferroni correction was used to maintain the overall type I error at $\alpha = 0.05$.

Results

Out of the 84 patients included, 25 (29.8%) were smokers, 31 (36.9%) had been diagnosed with periodontal disease, while 8 (9.5%) and 3 (3.6%) presented type I and type II diabetes mellitus, respectively. Twenty individuals (23.8%) had other systemic diseases (Table 1). A total of 72 implants were placed in the maxilla (54.5%) and 60

Table 1. Frequency distribution of patient-related variables

Variable		Patients, *n*	%
Sex	male	44	52.4
	female	40	47.6
Age [years] *M* ±*SD*		52.2 ±10.93	
Systemic diseases	none	53	63.1
	type I diabetes mellitus	3	3.6
	type II diabetes mellitus	8	9.5
	hypertension	11	13.1
	arrhythmia	1	1.2
	asthma	1	1.2
	hypercholesterolemia	2	2.4
	hyperthyroidism	1	1.2
	gastric ulcer	2	2.4
	osteoporosis	1	1.2
	rheumatic diseases	1	1.2
Smoking habits	no	59	70.2
	yes	25	29.8
	light smoker	7	28.0
	moderate smoker	8	32.0
	heavy smoker	10	40.0
History of periodontitis	no	53	63.1
	yes	31	36.9
Total		84	100.0

M – mean; *SD* – standard deviation.

Table 2. Frequency distribution of implant-related variables

Variable		Implants, *n*	%
Anatomical location	posterior mandible	60	45.5
	posterior maxilla	72	54.5
Implant placement	conventional	94	71.2
	immediate	38	28.8
Bone regenerative therapy at the surgical site	no	118	89.4
	yes	14	10.6
Implant diameter [mm]	4.6	88	66.7
	5.8	44	33.3
Implant length [mm]	7.5	28	21.2
	6.0	104	78.8
Total		132	100.0

in the mandible (45.5%). The distribution of patient- and implant-related variables is summarized in Table 1 and Table 2. At the end of the study period, 8 implants were lost in 6 patients. Of these, 5 implants (62.5%) were lost due to peri-implantitis. The implant-level and patient-level success rates were 93.9% and 95.0%, respectively (Table 3). Female sex, periodontal disease and smoking were significantly associated with implant failure at both the implant and patient levels (Table 4, Table 5). No statistically significant correlations were found between the remaining variables and short implant failure.

Regarding CBL, the mean values recorded after 5 years of follow-up were 0.38 ±0.7 mm at the mesial aspect and 0.39 ±0.6 mm at the distal aspect (Fig. 4). Mean MBL values were higher in smokers (0.64 ±0.6 mm), periodontally compromised patients (0.66 ±0.4 mm) and females (0.61 ±0.7 mm). These differences were statistically significant.

Table 3. Distribution variables associated with failed (unsuccessful) short implants

Variable		Implant No.							
		1	2	3	4	5	6	7	8
Patient	age	56	68	61	72	68	63	52	63
	sex	F	M	F	F	F	M	F	M
	medical history	DM-II	healthy	healthy	DM-II	healthy	healthy	DM-II	healthy
	smoking	yes	yes	yes	no	yes	yes	no	yes
	periodontal disease	yes	no	yes	no	yes	yes	yes	yes
Implant	localization	27	16	26	47	17	16	26	16
	diameter [mm]	4.6	5.4	5.4	4.6	5.4	4.6	4.6	4.6
	length [mm]	7.5	6.0	6.0	6.0	6.0	7.5	6.0	7.5
	placement	conventional	immediate	conventional	conventional	conventional	conventional	conventional	conventional
	bone regenerative therapy at the surgical site	no	yes	yes	no	no	no	no	no
	PI	yes	yes	yes	no	yes	yes	no	no
	follow-up [months]	58	55	52	49	52	54	54	60

DM-II – type II diabetes mellitus; F – female; M – male; PI – peri-implantitis.

Table 4. Results of univariate binary logistic regression analysis of short implant success at the implant level

Variable	B	*SE*	Wald	*p*-value	*OR*	95% *CI* for *OR*	
						lower	upper
Anatomical location	–0.442	0.301	1.799	0.235	0.668	0.331	1.156
Bone regenerative therapy at the surgical site	–0.298	0.208	1.881	0.270	0.751	0.501	1.122
Implant diameter	–0.111	0.403	0.014	0.982	0.896	0.047	1.996
Implant length	0.288	0.227	1.924	0.198	1.333	0.787	2.001
Sex (female)	1.118	0.436	6.879	0.009*	3.076	1.325	7.054
Age	–0.025	0.002	1.594	0.202	0.989	0.939	1.018
Periodontal disease	–0.911	0.389	5.202	0.021*	0.398	0.182	0.872
Smoking	–0.058	0.011	25.873	0.001*	0.997	0.911	0.977
Diabetes mellitus	0.196	0.385	0.258	0.611	1.215	0.567	2.448
Other systemic diseases	2.801	0.914	9.345	0.062	11.562	2.775	33.892

SE – standard error; *OR* – odds ratio; *CI* – confidence interval; * statistically significant ($p < 0.05$).

Table 5. Results of univariate binary logistic regression analysis of short implant success at the patient level

Variable	B	*SE*	Wald	*p*-value	*OR*	95% *CI* for *OR*	
						lower	upper
Sex (female)	1.347	0.490	7.997	0.007*	3.985	1.526	9.872
Age	–0.035	0.024	2.294	0.289	0.968	0.929	1.122
Periodontal disease	–0.933	0.456	4.185	0.045*	0.318	0.163	0.972
Smoking	–0.081	0.003	15.662	0.001*	0.820	0.818	0.991
Diabetes mellitus	0.176	0.469	0.141	0.708	1.194	0.467	2.848
Other systemic diseases	2.401	1.014	7.294	0.058	11.915	1.775	43.123

* statistically significant ($p < 0.05$).

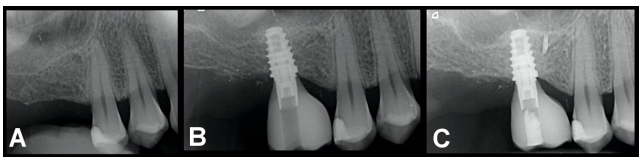

Fig. 4. Radiographic images obtained before treatment (A), at delivery of the definitive crown (B), and after 3 years of follow-up (C)

Discussion

The present retrospective study aimed to evaluate crestal bone remodeling, implant success, complications, and implant- and patient-related risk factors associated with short, platform-switched dental implants with a laser-microgrooved collar used for prosthetic rehabilitation of atrophic posterior jaws over a follow-up period of 3–5 years. The majority of previously published studies have included 8-, 9- and 10-mm-long implants and patient populations without unfavorable conditions that could negatively influence implant success.[9,10,19,21,22] In contrast, the present multicenter study exclusively evaluated 6.0- and 7.5-mm-long implants and included a broader patient population regardless of systemic conditions, periodontal status, smoking habits, or type of restoration. The results demonstrated that periodontal disease, smoking and female sex were significantly associated with short implant failure. The effect of a history of periodontitis on implant success has been widely documented.[22,23] However, only few studies reported data on short implants. Carra et al. evaluated the long-term outcomes of 213 short implants (≤10 mm) and reported comparable outcomes in patients with and without periodontal disease.[22] Similar findings have been reported by Correia et al.[24] in a sample of 202 patients (53% with a history of periodontal disease) and 689 implants, as well as by Zuffetti et al.,[25] who analyzed 254 short implants (≤7.5 mm) in 174 patients, including 51 with a history of periodontitis. On the contrary, Hasanoglu Erbasar et al. noted a significant relationship between a history of periodontal disease and short implant failure.[26] The findings of the current study indicate that patients with a history of periodontal disease are at increased risk of short implant failure. Despite strict adherence to supportive maintenance therapy during follow-up, peri-implantitis was diagnosed in 62.5% of implant failures. The etiology of periodontitis and peri-implantitis is bacterial.[27] It can therefore be hypothesized that a similar complex pathogenic dysbiotic microbiota may trigger a dysregulated inflammatory or immune response, ultimately leading to the loss of both periodontal and peri-implant supporting tissues.[27] However, further analysis is needed to confirm these results.

It is extensively documented that smoking is an important patient-related risk factor for the failure of standard-length dental implants.[28] Its negative impact has also been documented for short implants.[29] A recent systematic review reported a failure rate of 0.008 implants/year in smokers compared to 0.004 implants/year in non-smokers.[29] In the present study, smokers comprised two-thirds of peri-implantitis cases. This data is in agreement with the study by Rinke et al.[30] and Karoussis et al.,[31] who reported a higher prevalence of peri-implantitis among smokers. The significant association between female sex and short implant failure may be related to a higher rate of periodontal disease and smoking within this subgroup in the present sample.

The use of short dental implants for the rehabilitation of atrophic jaws is supported by biomechanical evidence indicating that most of the load stress around dental implants is concentrated at the crestal bone level, while minimal stress is distributed apically.[32] Therefore, preserving crestal bone is critical for the success of short implants. In implants measuring 6.0–7.5 mm in length, even physiologically acceptable levels of CBL may, over time, drastically reduce the percentage of bone anchorage.

Crestal bone loss has been associated with several factors, such as implant design, bone density, surgical trauma during implant placement, occlusal overload, and apical migration of the junctional epithelium to isolate bacterial infection or to establish the biological width.[33]

Platform switching[34] and laser-microgrooved surfaces[35] are implant design modifications aimed at reducing CBL. Platform switching involves the use of prosthetic abutments with a smaller diameter than the implant platform at the implant–abutment interface. This configuration results in a geometric manipulation of the supracrestal connective tissue, which thickens horizontally and may help protect the crestal bone from reabsorption. Another method attempting to reduce crestal bone remodeling is the use of a laser-microgrooved coronal surface at the implant

neck. Laser-microgrooved surfaces were developed to modify cellular interactions with the titanium surface. These surfaces are created using controlled excimer laser ablation, which allows for the creation of a microgeometry characterized by parallel linear grooves with resolution in the micrometer range (Fig. 1). Pre-clinical studies have shown that laser-microgrooved surfaces present a high potential for the growth of fibroblasts and effective inhibition of epithelial cell migration across the grooves.[19] In contrast to implants with machined (smooth) necks, which typically exhibit a parallel distribution of collagen fibers, histological analyses have demonstrated that laser-microgrooved surfaces promote a more intimate connective tissue attachment, with fibers oriented perpendicularly to the implant surface.[20] This configuration may contribute to improved protection of peri-implant crestal bone remodeling.[35–37]

Available literature indicates that CBL around short implants does not exceed the criteria accepted for standard-length implants. A literature review with a meta-analysis of 16 studies[38–40] reported a mean CBL of 0.83 mm around short implants after 4 years of follow-up. In the present study, after 3–5 years of function, short platform-switched implants with laser-microgrooved collars exhibited a mean CBL of 0.385 mm. These results highlight the effectiveness of this configuration in preserving peri-implant marginal bone levels, even in short implants.[41,42]

Limitations

However, the outcomes of the present study must be interpreted with caution due to several limitations, including the study's retrospective design and the exclusive use of single crowns supported by short implants, which limits the possibility of evaluating the influence of different types of restorations on implant success. On the other hand, all eligible patients treated with short implants were included, regardless of their medical condition, smoking status or history of periodontitis, thereby minimizing selection bias and increasing the applicability of the study findings to the general population. Additional limitations include the multicenter nature of the study, involving different private clinics, and the lack of evaluation of potentially relevant variables, such as the presence or absence of keratinized mucosa.

It could be assumed that, due to aforementioned limitations, the present study may report more variable outcomes compared to efficacy studies with small, well-controlled and selective groups. However, effectiveness studies such as this one may better reflect outcomes achievable in routine clinical practice.

Conclusions

Short, platform-switched implants with laser-microgrooved collars supporting single crowns in atrophied posterior alveolar ridges demonstrate high long-term success rates. Furthermore, this implant design maintains stable crestal bone levels over time.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. *Int J Oral Maxillofac Implants*. 2009;24 Suppl:237–259. PMID:19885448.
2. Anitua E, Alkhraisat MH, Orive G. Novel technique for the treatment of the severely atrophied posterior mandible. *Int J Oral Maxillofac Implants*. 2013;28(5):1338–1346. doi:10.11607/jomi.3137
3. Esposito M, Felice P, Worthington HV. Interventions for replacing missing teeth: Augmentation procedures of the maxillary sinus. *Cochrane Database Syst Rev*. 2014;2014(5):CD008397. doi:10.1002/14651858.CD008397.pub2
4. Esposito M, Grusovin MG, Felice P, Karatzopoulos G, Worthington HV, Coulthard P. Interventions for replacing missing teeth: Horizontal and vertical bone augmentation techniques for dental implant treatment. *Cochrane Database Syst Rev*. 2009;2009(4):CD003607. doi:10.1002/14651858.CD003607.pub4
5. Stellingsma K, Raghoebar GM, Visser A, Vissink A, Meijer HJA. The extremely resorbed mandible, 10-year results of a randomized controlled trial on 3 treatment strategies. *Clin Oral Implants Res*. 2014;25(8):926–932. doi:10.1111/clr.12184
6. Friberg B, Gröndahl K, Lekholm U, Brånemark PI. Long-term follow-up of severely atrophic edentulous mandibles reconstructed with short Brånemark implants. *Clin Implant Dent Relat Res*. 2000;2(4):184–189. doi:10.1111/j.1708-8208.2000.tb00116.x
7. Van Assche N, Michels S, Quirynen M, Naert I. Extra short dental implants supporting an overdenture in the edentulous maxilla: A proof of concept. *Clin Oral Implants Res*. 2012;23(5):567–576. doi:10.1111/j.1600-0501.2011.02235.x
8. Anitua E, Piñas L, Begoña L, Orive G. Long-term retrospective evaluation of short implants in the posterior areas: Clinical results after 10–12 years. *J Clin Periodontol*. 2014;41(4):404–411. doi:10.1111/jcpe.12222

9. Lops D, Bressan E, Pisoni G, Cea N, Corazza B, Romeo E. Short implants in partially edentuolous maxillae and mandibles: A 10 to 20 years retrospective evaluation. *Int J Dent*. 2012;2012:351793. doi:10.1155/2012/351793
10. Mertens C, Meyer-Bäumer A, Kappel H, Hoffmann J, Steveling HG. Use of 8-mm and 9-mm implants in atrophic alveolar ridges: 10-year results. *Int J Oral Maxillofac Implants*. 2012;27(6):1501–1508. PMID:23189302.
11. Reda R, Zanza A, Bellanova V, et al. Zinc oxide non-eugenol cement versus resinous cement on single implant restoration: A split-mouth study. *J Compos Sci*. 2023;7(3):128. doi:10.3390/jcs7030128
12. Anitua E, Alkhraist MH, Piñas L, Begoña L, Orive G. Implant survival and crestal bone loss around extra-short implants supporting a fixed denture: The effect of crown height space, crown-to-implant ratio, and offset placement of the prosthesis. *Int J Oral Maxillofac Implants*. 2014;29(3):682–689. doi:10.11607/jomi.3404
13. Kotsovilis S, Fourmousis I, Karoussis IK, Bamia C. A systematic review and meta-analysis on the effect of implant length on the survival of rough-surface dental implants. *J Periodontol*. 2009;80(11):1700–1718. doi:10.1902/jop.2009.090107
14. Bratu E, Chan HL, Mihali S, et al. Implant survival rate and marginal bone loss of 6-mm short implants: A 2-year clinical report. *Int J Oral Maxillofac Implants*. 2014;29(6):1425–1428. doi:10.11607/jomi.3729
15. Monje A, Suarez F, Galindo-Moreno P, García-Nogales A, Fu JH, Wang HL. A systematic review on marginal bone loss around short dental implants (<10 mm) for implant-supported fixed prostheses. *Clin Oral Implants Res*. 2014;25(10):1119–1124. doi:10.1111/clr.12236
16. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: A review and proposed criteria of success. *Int J Oral Maxillofac Implants*. 1986;1(1):11–25. PMID:3527955.
17. Iorio-Siciliano V, Marenzi G, Blasi A, et al. Influence of platform-switched, laser-microtextured implant on marginal bone level: A 24-month case series study. *Int J Oral Maxillofac Implants*. 2016;31(1):162–166. doi:10.11607/jomi.4130
18. Ricci JL, Charvert J, Frenkel SR, et al. Bone response to laser microtextured surfaces. In: Davies JE, ed. *Bone Engineering*. Toronto, Canada: Em^2 Inc.; 2000:282–294.
19. Cawood JI, Howell RA. A classification of the edentulous jaws. *Int J Oral Maxillofac Surg*. 1988;17(4):232–236. doi:10.1016/s0901-5027(88)80047-x
20. Nevins M, Nevins ML, Camelo M, Boyesen JL, Kim DM. Human histologic evidence of a connective tissue attachment to a dental implant. *Int J Periodontics Restorative Dent*. 2008;28(2):111–121. PMID:18546807.
21. Sánchez-Garcés MA, Costa-Berenguer X, Gay-Escoda C. Short implants: A descriptive study of 273 implants. *Clin Implant Dent Relat Res*. 2012;14(4):508–516. doi:10.1111/j.1708-8208.2010.00301.x
22. Carra MC, Rangé H, Swerts PJ, Tuand K, Vandamme K, Bouchard P. Effectiveness of implant-supported fixed partial denture in patients with history of periodontitis: A systematic review and meta-analysis. *J Clin Periodontol*. 2022;49 Suppl 24:208–223. doi:10.1111/jcpe.13481
23. Reda R, Zanza A, Di Nardo D, Bellanova V, Xhajanka E, Testarelli L. Implant survival rate and prosthetic complications of OT equator retained maxillary overdenture: A cohort study. *Prosthesis*. 2022;4(4):730–738. doi:10.3390/prosthesis4040057
24. Correia F, Gouveia S, Felino AC, Costa AL, Almeida RF. Survival rate of dental implants in patients with history of periodontal disease: A retrospective cohort study. *Int J Oral Maxillofac Implants*. 2017;32(4):927–934. doi:10.11607/jomi.3732
25. Zuffetti F, Testarelli L, Bertani P, Vassilopoulos S, Testori T, Guarnieri R. A retrospective multicenter study on short implants with a laser-microgrooved collar (≤7.5 mm) in posterior edentulous areas: Radiographic and clinical results up to 3 to 5 years. *J Oral Maxillofac Surg*. 2020;78(2):217–227. doi:10.1016/j.joms.2019.08.007
26. Hasanoglu Erbasar GN, Hocaoğlu TP, Erbasar RC. Risk factors associated with short dental implant success: A long-term retrospective evaluation of patients followed up for up to 9 years. *Braz Oral Res*. 2019;33:e030. doi:10.1590/1807-3107bor-2019.vol33.0030
27. Lasserre JF, Brecx MC, Toma S. Oral microbes, biofilms and their role in periodontal and peri-implant diseases. *Materials (Basel)*. 2018;11(10):1802. doi:10.3390/ma11101802
28. Mustapha AD, Salame Z, Chrcanovic BR. Smoking and dental implants: A systematic review and meta-analysis. *Medicina (Kaunas)*. 2021;58(1):39. doi:10.3390/medicina58010039
29. Cruz RS, Lemos CAA, Batista VES, et al. Short implants versus longer implants with maxillary sinus lift. A systematic review and meta-analysis. *Braz Oral Res*. 2018;32:e86. doi:10.1590/1807-3107bor-2018.vol32.0086
30. Rinke S, Ohl S, Ziebolz D, Lange K, Eickholz P. Prevalence of periimplant disease in partially edentulous patients: A practice-based cross-sectional study. *Clin Oral Implants Res*. 2011;22(8):826–833. doi:10.1111/j.1600-0501.2010.02061.x
31. Karoussis IK, Salvi GE, Heitz-Mayfield LJA, Brägger U, Hämmerle CHF, Lang NP. Long-term implant prognosis in patients with and without a history of chronic periodontitis: A 10-year prospective cohort study of the ITI Dental Implant System. *Clin Oral Implants Res*. 2003;14(3):329–339. doi:10.1034/j.1600-0501.000.00934.x
32. Kang N, Wu YY, Gong P, Yue L, Ou GM. A study of force distribution of loading stresses on implant-bone interface on short implant length using 3-dimensional finite element analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2014;118(5):519–523. doi:10.1016/j.oooo.2014.05.021
33. Guarnieri R, Reda R, Zanza A, Miccoli G, Nardo DD, Testarelli L. Can peri-implant marginal bone loss progression and a-MMP-8 be considered indicators of the subsequent onset of peri-implantitis? A 5-year study. *Diagnostics (Basel)*. 2022;12(11):2599. doi:10.3390/diagnostics12112599
34. Messias A, Rocha S, Wagner W, et al. Peri-implant marginal bone loss reduction with platform-switching components: 5-year post-loading results of an equivalence randomized clinical trial. *J Clin Periodontol*. 2019;46(6):678–687. doi:10.1111/jcpe.13119
35. Chen Z, Zhang Y, Li J, Wang HL, Yu H. Influence of laser-microtextured surface collar on marginal bone loss and peri-implant soft tissue response: A systematic review and meta-analysis. *J Periodontol*. 2017;88(7):651–662. doi:10.1902/jop.2017.160805
36. Weiner S, Simon J, Ehrenberg DS, Zweig B, Ricci JL. The effects of laser microtextured collars upon crestal bone levels of dental implants. *Implant Dent*. 2008;17(2):217–228. doi:10.1097/ID.0b013e3181779016
37. Guarnieri R, Reda R, Di Nardo D, Miccoli G, Zanza A, Testarelli L. Clinical, radiographic, and biochemical evaluation of two-piece versus one-piece single implants with a laser-microgrooved collar surface after 5 years of functional loading. *Clin Implant Dent Relat Res*. 2022;24(5):676–682. doi:10.1111/cid.13118
38. Guarnieri R, Reda R, Di Nardo D, Miccoli G, Zanza A, Testarelli L. In vitro direct and indirect cytotoxicity comparative analysis of one pre-hydrated versus one dried acellular porcine dermal matrix. *Materials (Basel)*. 2022;15(5):1937. doi:10.3390/ma15051937
39. Mezzomo LA, Miller R, Triches D, Alonso F, Shinkai RSA. Meta-analysis of single crowns supported by short (<10 mm) implants in the posterior region. *J Clin Periodontol*. 2014;41(2):191–213. doi:10.1111/jcpe.12180
40. Guarnieri R, Reda R, Di Nardo D, Pagnoni F, Zanza A, Testarelli L. Effects of maintenance implant therapy with and without periodic removal and decontamination of prosthetic components on inflammatory peri-implant parameters. *Int J Periodontics Restorative Dent*. 2023;(7):s118–s128. doi:10.11607/prd.6395
41. Tonin BSH, Peixoto RF, Fu J, De Mattos MGC, Macedo AP. Influence of the frameworks of implant-supported prostheses and implant connections on stress distribution. *Dent Med Probl*. 2023;60(4):551–557. doi:10.17219/dmp/153060
42. Dovigo S, Massariol M, Gandini A, Zuffellato N. Instantaneous dental implant loading technique by fixed dentures: A retrospective cohort study. *Dent Med Probl*. 2023;60(3):375–383. doi:10.17219/dmp/154981

Original paper

Periodontitis associated with the diagnosis of systemic lupus erythematosus

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Abstract

Background. Systemic lupus erythematosus (SLE) is an autoimmune disease that has been linked to the progression of periodontitis, negatively affecting patients' quality of life. Further studies are required to determine the correlation between these conditions and to support the incorporation of periodontal control in patients with SLE.

Objectives. The aim of the study was to determine the association between periodontitis and the diagnosis of SLE in adult female patients in Peru attending outpatient rheumatology consultations.

Material and methods. This cross-sectional study was conducted between July and October 2023 and included 342 patients from the Department of Rheumatology of the Regional Teaching Hospital of Trujillo, Peru, and Víctor Lazarte Echegaray Hospital, Trujillo, Peru. Intra- and inter-rater calibration for the clinical measurement of periodontitis yielded a κ value of 0.846 in both cases. The rheumatology specialist established the diagnosis of SLE. The χ^2 test and binary logistic regression were used to analyze the results, with a significance level set at $p < 0.05$.

Results. No association was found between periodontitis and SLE ($p = 0.453$). However, correlations were noted between periodontitis and patients aged 27–59 years ($p = 0.006$), smoking ($p = 0.035$), systemic medication use ($p < 0.001$), arterial hypertension ($p = 0.018$), and type 2 diabetes mellitus (T2DM) ($p = 0.003$).

Conclusions. No association was established between periodontitis and SLE. However, correlations were observed between periodontitis and SLE in specific subgroups, including patients aged 27–59 years, non-smokers, and those without hypertension or diabetes. Furthermore, a higher prevalence of periodontitis was noted in older patients, smokers, those receiving systemic medication for SLE, and patients with hypertension or T2DM.

Keywords: risk factors, systemic lupus erythematosus, autoimmune disease, periodontal disease, periodontitis

Highlights

- No overall association was identified between periodontitis and systemic lupus erythematosus (SLE).
- Bivariate analysis revealed a correlation between periodontitis and SLE in specific subgroups, including individuals aged 27–59 years, non-smokers, and those without hypertension or diabetes.
- Multivariate analysis showed a higher prevalence of periodontitis among older individuals, smokers, patients receiving systemic therapy for SLE, and those with type 2 diabetes mellitus (T2DM) or hypertension.

Introduction

Periodontal disease is a multifactorial chronic inflammatory condition that represents a significant global public health problem, as it negatively affects patients' quality of life[1] and leads to progressive destruction of the tooth-supporting apparatus, making it a leading cause of tooth loss.[2]

The global prevalence of periodontal disease ranges from 20% to 50%.[3] It occurs with greater frequency and severity in certain age groups, systemic conditions and educational levels.[4] In this context, the disease produces a systemic inflammatory burden that may influence existing conditions such as arterial hypertension,[5] diabetes mellitus, coronary heart disease,[6] and cerebrovascular accidents.[7]

Systemic lupus erythematosus (SLE) is considered a prototypical autoimmune disease associated with increased disability and mortality.[8] It is a complex condition that presents challenges for diagnosis and treatment,[9] and is characterized by an exacerbated response of B and T cells, as well as a loss of tolerance to autoantigens. Defective production and clearance of antibodies, the circulation and deposition of immune complexes in tissues, as well as the activation of cytokines and complements contribute to clinical manifestations ranging from mild fatigue and joint pain to severe health damage.[8]

Typical manifestations of SLE include skin rash, malar (butterfly) rash, arthritis, pleurisy, serositis, alopecia, and lupus nephritis. The response to treatment is often variable and difficult to predict, likely due to the complex immune dysregulation involved in SLE pathogenesis.[10,11] Early diagnosis is crucial to prevent outbreaks and resulting tissue damage; however, delays in diagnosis remain common.[10,12]

Although SLE has no cure, it can be effectively managed with pharmacological therapy, with the primary goals being the prevention of organ damage and the reduction of secondary comorbidities.[13] Recommendations for SLE management were published by the European League Against Rheumatism (EULAR) in 2008 and updated in 2019 in collaboration with the American College of Rheumatology (ACR) based on emerging new data. These guidelines indicate that lupus patients can receive hydroxychloroquine, prednisone, methotrexate, azathioprine, mycophenolate, belimumab, rituximab, and cyclophosphamide.[12]

Oral manifestations are included among the diagnostic criteria for SLE. The prevalence of xerostomia, dental caries, mucositis, cheilitis, and oral mucosal ulceration ranges from 81.3% to 87.5% in patients with SLE. Additionally, up to 93.8% of patients with SLE present with periodontitis.[14,15]

Periodontitis and SLE have been reported to share genetic and environmental risk factors. Systemic lupus erythematosus induces an imbalance between pro-inflammatory and anti-inflammatory cytokines, which contributes to tissue damage. It also activates autoreactive B cells and promotes dysregulation of other immune cells, including macrophages, neutrophils, $CD4^+$ T cells, and dendritic cells, which may influence the subgingival microbiota.[16,17]

It is estimated that at least 5 million people worldwide are affected by lupus in some of its manifestations.[18] Several studies suggest a possible association between the pathology of SLE and periodontal disease[19,20]; however, the available evidence remains limited. Therefore, the main objective of this study was to determine the association between periodontitis and the diagnosis of SLE in female Peruvian patients, and to provide information about the associated factors such as age group, smoking habits, use of systemic medication for SLE, hypertension, and diabetes. These findings may contribute to improving clinical care guidelines, emphasizing multidisciplinary collaboration between dentists and rheumatologists, and ultimately enhancing the quality of life of affected patients.

Material and methods

The present cross-sectional observational study was conducted at the Department of Rheumatology of the Regional Teaching Hospital of Trujillo, Peru, and Víctor Lazarte Echegaray Hospital, Trujillo, Peru, between July and October 2023.

The sample consisted of 342 patients, including 114 diagnosed with SLE and 228 with other rheumatological diseases. The sample size was calculated using a formula for group comparison, based on the data obtained from

a pilot study conducted on 40 patients, using the following parameters: n1 – number of patients without SLE; n2 – number of patients with SLE; k = 2 (number of patients without SLE per patient with SLE); α = 0.05 (type I error); β = 0.20 (type II error, corresponding to 80% power); $z_{\alpha/2}$ = 1.96 (normal value with a type I error of 5%); z_{β} = 0.842 (normal value with a test power of 80%); p1 = 0.526 (prevalence of periodontitis among patients without SLE); p2 = 0.680 (prevalence of periodontitis among patients with SLE); and ε = 0.154 (difference in prevalence between groups). A non-probabilistic convenience sampling method was used, selecting the same number of cases from each hospital.

The study included female adults aged over 18 years who attended outpatient consultations in the rheumatology services of the aforementioned hospitals. Pregnant women, hospitalized women, and those who did not agree to participate were excluded.

The study protocol was approved by the Faculty of Human Medicine of Antenor Orrego Private University (Resolution No. 2598-2023-FMH-UPAO) and the Bioethics Committee of Antenor Orrego Private University (Resolution No. 0658-2023-UPAO), Trujillo, Peru, as well as by the Research Committee of the La Libertad–ESSALUD Healthcare Network (PI No. 121 CIYE-O.C.I.Y. D-RALL-ESSALUD-2023) and the Research Ethics Committee of Regional Teaching Hospital of Trujillo, Peru (September 19, 2023). All procedures complied with the principles established in the Declaration of Helsinki and the General Health Law of Peru (No. 26842).

All patients were informed about the objectives of the study and provided written informed consent prior to participation. The diagnosis of SLE and its severity, as well as the diagnosis of other rheumatological diseases, were established by a rheumatology specialist based on clinical examination, signs and symptoms, and corresponding blood and urine tests, such as antinuclear antibody (ANA), direct Coombs, anti-Smith (anti-Sm), and DNA antibody (anti-dsDNA) tests.

Subsequently, the principal researcher evaluated the presence of periodontitis according to the classification of Page and Eke,[21] taking into account the following criteria: no/mild periodontitis (≥2 interproximal sites with clinical attachment loss ≤3 mm or probing depth ≤4 mm); moderate periodontitis (≥2 interproximal sites with clinical attachment loss ≥4 mm or ≥2 proximal sites with probing depth ≥5 mm); and severe periodontitis (≥2 interproximal sites with clinical attachment loss ≥6 mm and ≥1 interdental sites with probing depth ≥5 mm). To avoid information bias, intra- and inter-rater reliability were assessed through calibration of the principal investigator with an expert in periodontology affiliated with the Stomatology Program at Antenor Orrego Private University. The evaluation was conducted on 12 patients, yielding a κ value of 0.846 for both calibrations.

Statistical analysis

The obtained data was processed using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). The correlation between periodontitis and SLE was determined using the χ^2 test of independence. In addition, associations between study variables, adjusted for covariates, were evaluated using binary logistic regression (Wald test). The level of statistical significance was set at $p < 0.05$.

Results

In the present study, 342 female patients aged 18–92 years (mean (*M*) = 48.89; standard deviation (*SD*) = 16.27) who attended the rheumatology services of the abovementioned hospitals between July and October 2023 were evaluated. The prevalence of periodontitis in the study sample was 56.7% (Table 1).

Table 2 shows the association between periodontitis and SLE ($p = 0.002$). The percentage of patients with SLE who presented with periodontitis (68.4%) was twice as high as those without periodontitis (31.6%). When stratified by age group, a significant association between periodontitis and SLE was observed only in patients aged 27–59 years ($p < 0.001$). Regarding smoking habits, a significant correlation between periodontitis and SLE was found only among non-smokers ($p = 0.017$).

Table 1. Prevalence of periodontitis among patients attending rheumatology consultations between July and October 2023, according to the Page and Eke classification[21]

Variable		Regional Teaching Hospital of Trujillo		Víctor Lazarte Echegaray Hospital		Total	
		n	%	*n*	%	*n*	%
Periodontitis	yes	94	55.0	100	58.5	194	56.7
	no	77	45.0	71	41.5	148	43.3
Periodontitis category	no/mild	142	83.0	132	77.2	275	80.4
	moderate	25	14.6	34	19.9	58	17.0
	severe	4	2.3	5	2.9	9	2.6
Total		171	100.0	171	100.0	342	100.0

Table 2. Association between periodontitis and systemic lupus erythematosus (SLE) in rheumatology patients, stratified by age group and smoking status

Variable		Systemic lupus erythematosus	Periodontitis				Total	χ^2	*p*-value
			yes		no				
			n	%	*n*	%			
Systemic lupus erythematosus		yes	78	68.4	36	31.6	114	9.529	0.002*
		no	116	50.9	112	49.1	228		
		total	194	56.7	148	43.3	342		
Age group	younger adults: 18–26 years	yes	9	42.9	12	57.1	21	1.155	0.282
		no	2	22.2	7	77.8	9		
		total	11	36.7	19	63.3	30		
	adults: 27–59 years	yes	55	70.5	23	29.5	78	16.92	<0.001*
		no	59	41.5	83	58.5	142		
		total	114	51.8	106	48.2	220		
	older adults: ≥60 years	yes	14	93.3	1	6.7	15	3.213	0.073
		no	55	71.4	22	28.6	77		
		total	69	75.0	23	25.0	92		
Smoking habit	yes	yes	22	88.0	3	12.0	25	0.267	0.605
		no	18	78.3	5	21.7	23		
		total	40	83.3	8	16.7	48		
	no	yes	56	62.9	33	37.1	89	5.685	0.017*
		no	98	47.8	107	52.2	205		
		total	154	52.4	140	47.6	294		

* statistically significant ($p < 0.05$, χ^2 test).

Table 3. Association between periodontitis and systemic lupus erythematosus (SLE) in rheumatology patients, according to systemic medication use, hypertension and type 2 diabetes mellitus (T2DM)

Variable		Systemic lupus erythematosus	Periodontitis				Total	χ^2	*p*-value
			yes		no				
			n	%	*n*	%			
Systemic medication	yes	yes	77	70.0	33	30.0	110	2.557	0.110
		no	41	82.0	9	18.0	50		
		total	118	73.8	42	26.3	160		
	no	yes	1	25.0	3	75.0	4	0.030	0.861
		no	75	42.1	103	57.9	178		
		total	76	41.8	106	58.2	182		
Hypertension	yes	yes	29	78.4	8	21.6	37	0.074	0.786
		no	42	73.7	15	26.3	57		
		total	71	75.5	23	24.5	94		
	no	yes	49	63.6	28	36.4	77	8.805	0.003*
		no	74	43.3	97	56.7	171		
		total	123	49.6	125	50.4	248		
T2DM	yes	yes	13	100.0	0	0.0	13	2.680	0.102
		no	22	73.3	8	26.7	30		
		total	35	81.4	8	18.6	43		
	no	yes	65	64.4	36	35.6	101	7.655	0.006*
		no	94	47.5	104	52.5	198		
		total	159	53.2	140	46.8	299		

* statistically significant ($p < 0.05$, χ^2 test).

As shown in Table 3, no association was found between periodontitis and SLE in patients who used medications (p = 0.110) or in those who did not use medications (p = 0.861). However, a significant correlation was identified between periodontitis and SLE in patients without hypertension (p = 0.003) and in those without type 2 diabetes mellitus (T2DM) (p = 0.006).

Nevertheless, the associations identified through the bivariate analysis may be influenced by confounding factors such as age, smoking habits, medication use, and comorbidities. Therefore, a multivariate analysis was performed.

The multivariate binary logistic regression analysis identified no association between periodontitis and SLE (p = 0.453) (Table 4). However, significant differences in the prevalence of periodontitis were observed between older and younger adults (p = 0.006), smokers and non-smokers (p = 0.035), patients receiving medication and those not receiving medication (p = 0.000), hypertensive and non-hypertensive patients (p = 0.018), and diabetic and non-diabetic patients (p = 0.003). Furthermore, compared with their respective reference groups, the odds of periodontitis were 4.475-, 2.601-, 19.941-, 2.072-, and 3.760-fold higher in older adults, smokers, patients receiving medications for SLE, and those with hypertension and diabetes, respectively.

The multivariate analysis is more robust than the bivariate analyses presented in Table 2 and Table 3. As shown in Table 4, no association between periodontitis and SLE was observed after adjusting for age group, smoking status, medication use, and comorbidities. Furthermore, the analysis allowed for the evaluation of the association between these factors and periodontitis, regardless of SLE status.

Discussion

Systemic lupus erythematosus is a complex multisystem autoimmune condition that poses different challenges for diagnosis and treatment planning.[8] Some studies have reported an association between SLE and periodontitis, particularly in terms of pathogenesis, physiological mechanisms and risk factors.[16,17] Dentists play an important role in the evaluation and management of these patients, taking into account the specific conditions associated with the disease.[22]

In the present study, the bivariate analysis revealed an association between periodontitis and the diagnosis of SLE. Similar results have been reported by Bolstad et al.,[23] Rutter-Locher et al.,[24] Wu et al.,[25] and Calderaro et al.[26] This may be explained by the fact that both diseases share genetic and environmental factors, as well as similar pathophysiological characteristics and inflammatory profiles. In this study, the number of patients diagnosed with SLE who presented with periodontitis was more than twice that of those without periodontitis, which is consistent with the findings of Bolstad et al. and Rutter-Locher et al., who reported that the risk of developing periodontitis in patients with SLE is approximately twice as high.[23,24]

Regarding age groups, the association between periodontitis and SLE was observed only in patients aged 27–59 years. In this regard, Bolstad et al. found that SLE was associated with a higher risk of periodontitis in young patients aged 20–30 years.[23] On the other hand, Wu et al. found no association between periodontitis and SLE across age subgroups.[25] The findings of the present study may be explained by the larger proportion of adult patients with SLE attending the rheumatology service compared to younger and older age groups. Thus, future studies with balanced age group distributions are necessary to validate these findings, as the higher number of patients aged 27–59 years may have increased the statistical power. Furthermore, adult patients may have been under the effect of long-term medication, which could increase their susceptibility to periodontitis.

Regarding smoking habits, an association between periodontitis and SLE was observed only in non-smokers. This atypical result could be due to the small number of smoking patients diagnosed with SLE. In this regard, various authors, including Calderaro et al.,[26] have reported greater probing depth in smoking patients, possibly due to alterations in gingival microbiota, immune response and periodontal tissue repair.[27,28] Chen et al. also reported an association between smoking and active skin rash in patients with SLE.[27]

Table 4. Multivariate binary logistic regression analysis of factors associated with periodontitis in rheumatology patients

Variable	Coefficient	*SE*	Wald	*p*-value	*OR*
Systemic lupus erythematosus	–0.327	0.436	0.563	0.453	0.721
Age: adult	0.632	0.453	1.944	0.163	1.882
Age: older adult	1.499	0.550	7.435	0.006*	4.475
Smoking habit	0.956	0.453	4.452	0.035*	2.601
Systemic medication	1.766	0.395	–	<0.001*	19.941
Hypertension	0.728	0.307	5.628	0.018*	2.072
T2DM	1.325	0.448	8.726	0.003*	3.760
Constant	–1.615	0.463	12.150	<0.001*	0.199

* statistically significant ($p < 0.05$); *SE* – standard error; *OR* – odds ratio.

No association was found between periodontitis and SLE in patients receiving pharmacological treatment for SLE or in those not receiving treatment, which is consistent with the findings of Calderaro et al.[26] The pharmaceuticals most frequently administered to these patients include corticosteroids, immunosuppressants and antimalarial agents. Evidence suggests that these drugs may affect the periodontium due to their anti-inflammatory and immunosuppressive properties, potentially increasing susceptibility to microbial infections.[29,30]

Some studies have indicated that periodontitis is a risk factor for the onset and progression of systemic diseases, including cardiovascular diseases (34%), T2DM (19–33%) and respiratory tract infections (50%).[31,32] When evaluating lupus patients with T2DM and hypertension, an association was found between periodontitis and SLE in individuals who did not present these comorbidities. This finding may be explained by the small number of hypertensive and diabetic patients included in the sample. In contrast, Bolstad et al. reported an association between periodontitis and SLE in patients with T2DM.[23] Periodontitis and T2DM have a documented bidirectional association; furthermore, T2DM can promote the progression of periodontitis, which is associated with the invasion of host tissues by bacteria or degradation products into the systemic circulation.[33,34] Further studies with larger samples sizes are needed to clarify the association between periodontitis and SLE in patients with these comorbidities.

Furthermore, to strengthen the statistical analysis, binary logistic regression was performed. The analysis simultaneously evaluated multiple variables and covariates, providing a more accurate estimate of the probability of periodontitis and the associated factors, in addition to determining the risk of developing periodontitis from cofactors using odds ratios (*OR*s). Based on the results, no association between periodontitis and SLE was identified, in contrast to the results of the χ^2 test. This discrepancy may be explained by the influence of covariates in the bivariate analysis, and lack of such influence in the multivariate model. Due to this contradiction, further studies with larger sample sizes and longitudinal designs are necessary to obtain more robust conclusions. On the other hand, the binary logistic regression analysis allowed us to observe an association between periodontitis and age (older adults), smoking, systemic medication use, hypertension, and T2DM. Notably, patients receiving medication for SLE were nearly 20 times more likely to develop periodontitis. This finding may reflect the effects of corticosteroids and immunosuppressants, such as prednisone and mycophenolate mofetil, on immune defense mechanisms in the oral cavity.[35] In addition, glucocorticoids suppress the number and function of osteoblasts, leading to damage to bone turnover and compromising homeostasis in the periodontium.[29]

Although this study included a large sample of patients, its significant limitation is the unequal distribution of participants across covariate categories (e.g., 48 smokers vs. 294 non-smokers), which may have affected statistical power. In this sense, the associations observed with these variables should be interpreted with caution and only serve as preliminary information for the development of future studies.

Based on the results of this study, early diagnosis, treatment and periodontal control are recommended for patients with SLE. Future research should include larger samples and longitudinal designs, as the cross-sectional nature of this study does not allow for causal inference. In addition, collaboration between rheumatologists and periodontists is essential for the multidisciplinary management of patients with SLE. As suggested by Wu et al.,[25] maintaining good periodontal health has a positive effect on controlling SLE, especially in patients undergoing immunosuppressive therapy, thus improving quality of life and increasing survival rates.

Conclusions

No overall association was identified between periodontitis and SLE in the sample population. However, a correlation was observed in specific subgroups, including patients aged 27–59 years, non-smokers, and those without hypertension or diabetes. In addition, a higher prevalence of periodontitis was noted in older patients, smokers, those receiving systemic medication for SLE, and patients with T2DM and hypertension.

Ethics approval and consent to participate

The study protocol was approved by the Faculty of Human Medicine of Antenor Orrego Private University (Resolution No. 2598-2023-FMH-UPAO) and the Bioethics Committee of Antenor Orrego Private University (Resolution No. 0658-2023-UPAO), Trujillo, Peru, as well as by the Research Committee of the La Libertad–ESSALUD Healthcare Network (PI No. 121 CIYE-O.C.I.Y. D-RALL-ESSALUD-2023) and the Research Ethics Committee of Regional Teaching Hospital of Trujillo, Peru (September 19, 2023). All procedures complied with the principles established in the Declaration of Helsinki and the General Health Law of Peru (No. 26842).

Data availability

This work corresponds to the thesis required to obtain the professional degree of Dental Surgeon from Antenor Orrego Private University (UPAO), Trujillo, Peru.

The data supporting the findings of this study are available in the institutional repository of UPAO at https://hdl.handle.net/20.500.12759/36331.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Kwon TH, Lamster IB, Levin L. Current concepts in the management of periodontitis. *Int Dent J*. 2021;71(6):462–476. doi:10.1111/idj.12630
2. Zhang X, Hu Z, Zhu X, Li W, Chen J. Treating periodontitis – a systematic review and meta-analysis comparing ultrasonic and manual subgingival scaling at different probing pocket depths. *BMC Oral Health*. 2020;20(1):176. doi:10.1186/s12903-020-01117-3
3. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. *ScientificWorldJournal*. 2020;2020:2146160. doi:10.1155/2020/2146160
4. Darby I. Risk factors for periodontitis & peri-implantitis. *Periodontol 2000*. 2022;90(1):9–12. doi:10.1111/prd.12447
5. Narendran N, Shenoy S, Kodangala S, Raghavendra Vamsi A, Kamath V. Assessment of myocardial strain in hypertensive patients with periodontitis. *Dent Med Probl*. 2023;60(1):61–69. doi:10.17219/dmp/152707
6. Ghorbani P, Rezaei Esfahrood ZR, Foroughi M. Paraoxonase-1, a novel link between periodontitis and ischemic heart disease: A case–control study. *Dent Med Probl*. 2023;60(1):55–59. doi:10.17219/dmp/152181
7. Dewan M, Pandit AK, Goyal L. Association of periodontitis and gingivitis with stroke: A systematic review and meta-analysis. *Dent Med Probl*. 2024;61(3):407–415. doi:10.17219/dmp/158793
8. Narváez J. Systemic lupus erythematosus 2020. *Med Clin (Barc)*. 2020;155(11):494–501. doi:10.1016/j.medcli.2020.05.009
9. Dörner T, Furie R. Novel paradigms in systemic lupus erythematosus. *Lancet*. 2019;393(10188):2344–2358. doi:10.1016/S0140-6736(19)30546-X
10. Lazar S, Kahlenberg JM. Systemic lupus erythematosus: New diagnostic and therapeutic approaches. *Annu Rev Med*. 2023;74:339–352. doi:10.1146/annurev-med-043021-032611
11. Aringer M, Johnson SR. Classifying and diagnosing systemic lupus erythematosus in the 21st century. *Rheumatology (Oxford)*. 2020;59(Suppl5):v4–v11. doi:10.1093/rheumatology/keaa379
12. Fanouriakis A, Tziolos N, Bertsias G, Boumpas DT. Update on the diagnosis and management of systemic lupus erythematosus. *Ann Rheum Dis*. 2021;80(1):14–25. doi:10.1136/annrheumdis-2020-218272
13. Fava A, Petri M. Systemic lupus erythematosus: Diagnosis and clinical management. *J Autoimmun*. 2019;96:1–13. doi:10.1016/j.jaut.2018.11.001
14. Putra Gofur NR, Handono K, Nurdiana N, Kalim H. Periodontal comparison on systemic lupus erythematosus patients and healthy subjects: A cross-sectional study. *Pesqui Bras Odontopediatria Clín Integr*. 2021;21:e0006. doi:10.1590/pboci.2021.108
15. Hussain SB, Leira Y, Zehra SA, et al. Periodontitis and systemic lupus erythematosus: A systematic review and meta-analysis. *J Periodontal Res*. 2022;57(1):1–10. doi:10.1111/jre.12936
16. Pires JR, Nogueira MRS, Nunes AJF, et al. Deposition of immune complexes in gingival tissues in the presence of periodontitis and systemic lupus erythematosus. *Front Immunol*. 2021;12:591236. doi:10.3389/fimmu.2021.591236
17. Sojod B, Pidorodeski Nagano C, Garcia Lopez GM, Zalcberg A, Dridi SM, Anagnostou F. Systemic lupus erythematosus and periodontal disease: A complex clinical and biological interplay. *J Clin Med*. 2021;10(9):1957. doi:10.3390/jcm10091957
18. Ugarte-Gil MF, González LA, Alarcón GS. Lupus: The new epidemic. *Lupus*. 2019;28(9):1031–1050. doi:10.1177/0961203319860907
19. Koppolu P, Sheethi KV, Swapna LA, et al. Treatment of aggressive periodontitis in a patient with systemic lupus erythematosus: A case report with 8 years follow-up. *Ann Afr Med*. 2023;22(4):549–553. doi:10.4103/aam.aam_128_22
20. Sahebari M, Allaf FL, Nasrabadi N, et al. Periodontal disease in patients with newly diagnosed systemic lupus erythematosus. *Mediterr J Rheumatol*. 2023;34(3):315–321. doi:10.31138/mjr.20230724.pd
21. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *J Periodontol*. 2007;78(7 Suppl):1387–1399. doi:10.1902/jop.2007.060264
22. Benli M, Batool F, Stutz C, Petit C, Jung S, Huck O. Orofacial manifestations and dental management of systemic lupus erythematosus: A review. *Oral Dis*. 2021;27(2):151–167. doi:10.1111/odi.13271
23. Bolstad AI, Sehjpal P, Lie SA, Fevang BTS. Periodontitis in patients with systemic lupus erythematosus: A nationwide study of 1,990 patients. *J Periodontol*. 2022;93(3):364–372. doi:10.1002/JPER.21-0181
24. Rutter-Locher Z, Smith TO, Giles I, Sofat N. Association between systemic lupus erythematosus and periodontitis: A systematic review and meta-analysis. *Front Immunol*. 2017;8:1295. doi:10.3389/fimmu.2017.01295
25. Wu YD, Lin CH, Chao WC, Liao TL, Chen DY, Chen HH. Association between a history of periodontitis and the risk of systemic lupus erythematosus in Taiwan: A nationwide, population-based, case–control study. *PLoS One*. 2017;12(10):e0187075. doi:10.1371/journal.pone.0187075
26. Calderaro DC, Ferreira GA, Corrêa JD, et al. Is chronic periodontitis premature in systemic lupus erythematosus patients? *Clin Rheumatol*. 2017;36(3):713–718. doi:10.1007/s10067-016-3385-8
27. Chen J, Liao S, Pang W, et al. Life factors acting on systemic lupus erythematosus. *Front Immunol*. 2022;13:986239. doi:10.3389/fimmu.2022.986239
28. Leite FRM, Nascimento GG, Scheutz F, López R. Effect of smoking on periodontitis: A systematic review and meta-regression. *Am J Prev Med*. 2018;54(6):831–841. doi:10.1016/j.amepre.2018.02.014
29. de Almeida JM, Matheus HR, Fiorin LG, Furquim EMA, Gusman DJR. Influence of immunosuppression on the progression of experimental periodontitis and on healthy periodontal tissue: A rat in vivo study. *J Dent Res Dent Clin Dent Prospects*. 2021;15(2):94–99. doi:10.34172/joddd.2021.016
30. Epsley S, Tadros S, Farid A, Kargilis D, Mehta S, Rajapakse CS. The effect of inflammation on bone. *Front Physiol*. 2021;11:511799. doi:10.3389/fphys.2020.511799
31. Bui FQ, Almeida-da-Silva CLC, Huynh B, et al. Association between periodontal pathogens and systemic disease. *Biomed J*. 2019;42(1):27–35. doi:10.1016/j.bj.2018.12.001
32. Kalhan AC, Wong ML, Allen F, Gao X. Periodontal disease and systemic health: An update for medical practitioners. *Ann Acad Med Singap*. 2022;51(9):567–574. doi:10.47102/annals-acadmedsg.2021503
33. Wu CZ, Yuan YH, Liu HH, et al. Epidemiologic relationship between periodontitis and type 2 diabetes mellitus. *BMC Oral Health*. 2020;20(1):204. doi:10.1186/s12903-020-01180-w
34. Baeza M, Morales A, Cisterna C, et al. Effect of periodontal treatment in patients with periodontitis and diabetes: Systematic review and meta-analysis. *J Appl Oral Sci*. 2020;28:e20190248. doi:10.1590/1678-7757-2019-0248
35. Baumeister SE, Reckelkamm SL, Grabe HJ, et al. Cortisol and periodontitis: Prospective observational and Mendelian randomization studies. *Front Endocrinol (Lausanne)*. 2023;14:1100985. doi:10.3389/fendo.2023.1100985

Original paper

Comparison of the effectiveness of psycho-behavioral-relaxation and occlusal splint therapy in the treatment of myalgia

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Abstract

Background. The current treatment for patients with myalgia comprises splint therapy (ST), cognitive-behavioral therapy (CBT), psychological treatment, and education.

Objectives. The aim of the present study was to compare the effectiveness of relax-psycho-breathing therapy (RPB) and ST in women with myalgia, and to evaluate the efficacy of occlusal splint and/or behavioral therapy in the management of muscle pain.

Material and methods. The study, designed as a randomized controlled trial (RCT), involved 105 women aged 25–55 years with pain in the masticatory muscles during the 30 days prior to examination. Myalgia was diagnosed according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I. The clinical examination assessed muscle and temporomandibular joint (TMJ) pain intensity, TMJ sounds, maximum mouth opening (MMO), and deviation in mouth opening and mandibular movement. After an initial assessment, at the 2nd visit (V2), the 25 patients from the 1st group were introduced to RPB: relaxation techniques; breathing patterns; psychological-behavioral instruction; muscle exercise therapy; puffing therapy; and self-massage. The 2nd group of patients received a custom-made, thermoplastic, flat-plane relaxation splint placed on the mandible. The efficacy of treatment in all subjects was evaluated at 2 follow-up visits (V3 and V4).

Results. In cases of muscle pain, pain during function, TMJ and muscle tenderness, and headache attributed to TMD, a statistically significant difference between the RPB and ST groups was observed only at the final follow-up visit. No significant differences between the 2 groups were found for MMO.

Conclusions. The reduction in myalgia following treatment suggests that both RPB exercises and ST are equally effective. However, considering the cost and duration of myalgia therapy, comprehensive care incorporating relaxation techniques may be a preferable first-line approach rather than ST alone.

Keywords: temporomandibular disorders, myalgia, behavioral therapy, splint

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Highlights

- The present study compared two methods of myalgia treatment with regard to the masticatory muscles.
- Both methods – the relax-psycho-breathing therapy (RPB – relaxation techniques, breathing patterns, psychological-behavioral instruction, muscle exercise therapy, puffing therapy, and self-massage) and splint therapy (ST – a custom-made, thermoplastic, flat-plane relaxation splint placed on the mandible) were equally effective.
- Considering the cost and duration of myalgia treatment, comprehensive care incorporating relaxation techniques may be a preferable first-line approach rather than ST alone.

Introduction

Masticatory myalgia is the primary source of chronic non-odontogenic orofacial pain, frequently observed in dental practice. The prevalence of temporomandibular disorders (TMD) has been estimated to be 31% for adults and 11% for children and adolescencents.[1] About 60–70% of the human population has experienced at least one TMD symptom in their lifetime, but only 5% of cases have been treated.[2] In the Polish population, myalgia has been reported to occur in 47.4% of patients, while myofascial pain occurs in 14.1% of patients (in accordance with the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) protocol).[3,4]

These problems commonly occur between the age of 27.5 and 50 years, with a predominance in females, and particularly in sedentary individuals.[3,4] Masticatory myalgia originates in the masticatory muscles, and in some cases results in limited mandibular movement.[3,5] The diagnoses of muscle pain are classified into 4 subclasses: myalgia; tendonitis; myositis; and spasm. Within the DC/TMD classification (Axis I), myalgia is subdivided into 3 categories (sub-diagnoses): local myalgia; myofascial pain (with "spreading" pain being its hallmark clinical manifestation); and myofascial pain with referral.[6–8]

The etiology of myalgia is multifactorial; the condition may result from trauma, repetitive micro-traumas due to parafunctional habits (PHs), as well as psychological factors (stress, anxiety, tension).[6,8–10] Patients with myalgia may present with one or more clinical complaints, including pain during the movement of the temporomandibular joint (TMJ), pain in the pre-auricular area and/or the masticatory muscles, and limited and/or deviated mandibular movement. Somatic symptoms that impair function are common and often lead to disability, complicating treatment outcomes and increasing the need for more costly therapeutic interventions.[11,12] It has been noted that patients with myofascial pain are more likely to experience changes in the breathing parameters, as well as fatigue, depression and sleep disruptions.[13,14]

The primary goals in the management of masticatory myalgia are to reduce or eliminate pain, increase mouth opening, and improve the overall quality of life (QoL). Therapy should include patient education delivered with understanding and empathy.[15] The most widely accepted approaches to managing myalgia and PHs include behavioral interventions, pharmacological treatment, physical therapy, occlusal appliances, and botulinum toxin injections.[16–18]

Self-management is widely regarded as a core component in the treatment of TMD and myalgia.[19–21] This typically involves elements of cognitive-behavioral therapy (CBT) combined with patient education, relaxation techniques, the reinforcement of adaptive behaviors, and the reduction of maladaptive habits, alongside home-based physical therapy. The CBT approach has been extensively studied and shown to be effective in the treatment of depression, anxiety, low self-esteem, eating disorders, and other psychological conditions.[21–23]

Occlusal splints are among the most commonly used therapeutic approaches for patients diagnosed with myalgia. They promote occlusal disengagement, help restore the vertical dimension of occlusion, facilitate muscle relaxation, reduce joint loading, and may aid in TMJ repositioning.[24]

There is no clear consensus regarding clinical indications for occlusal splints and their function. Türp et al. reported no significant differences in symptom reduction between the splints placed on the maxilla and those placed on the mandible.[25] In patients with myalgia, splints should be used intermittently, primarily during nocturnal wear, provided that PHs are adequately controlled during the day. Their use should generally be limited to a few months, as prolonged wear may lead to patient habituation and the development of undesirable dependence on the appliance.[26]

The present study compares the effectiveness of relax-psycho-breathing therapy (RPB) and splint therapy (ST) in women with myalgia, aiming to assess the clinical value of occlusal splints and/or behavioral therapy in the management of muscle pain. It also evaluates the relative efficacy of both approaches in reducing myalgia and seeks to identify the most appropriate treatment sequence for optimal outcomes.

The null hypothesis of this study is that relax-psycho-breathing therapy (RPB) and splint therapy (ST) are equally effective in women with muscle pain.

Material and methods

The study was conducted at Poznan University of Medical Sciences, Poland, between 2016 and 2022. Approval from the Institutional Ethics Committee (No. 551/15), as well as written informed consent from all patients were obtained. This study was designed as a randomized controlled trial (RCT).

Study design, inclusion and exclusion criteria

The study involved 105 patients seeking treatment due to orofacial pain.

The inclusion criteria were as follows:

– pain in the masticatory muscles scored ≥3 on a numerical rating scale (NRS) of 0–10 points, present during the 30 days prior to selection; myalgia was diagnosed according to the DC/TMD Axis I;
– other pain conditions, such as pain involving the arm, shoulder and upper back;
– jaw movement disorders with regard to TMJ, e.g., limited and/or deviated mandibular movement;
– eye symptoms of TMD – lacrimation, pain behind the eyes; and
– ear symptoms of TMD – otalgia, tinnitus, vertigo, aural fullness, itching in the ear, subjective hearing impairment.

The exclusion criteria were as follows:

– denture (fixed or removable) wearers;
– patients with arthralgia and/or disc displacement;
– patients with an open bite, a cross-bite, a prognathic relationship, and with pain of dental origin;
– patients with systemic diseases (e.g., rheumatic diseases or degenerative joint disease), serious neurological disorders, cervical spine injuries or postural deformities, the presence of drug abuse, a comorbid malignant disease, psychiatric diseases;
– patients taking medications for neurological problems or medications that could affect equilibrium or cause visual impairment; and
– patients with earlier ST or a masticatory organ injury.

On the 1st day of the study (visit 1 – V1), all patients completed a questionnaire and were subjected to a clinical examination. The questionnaire consisted of several parts: (1) personal details, including age and sex; (2) general health; (3) history of trauma; (4) pain evaluation – the patients were asked to rate their regional pain intensity from 0 to 10 (NRS), with regard to both current pain and maximal pain during the past 6 months; moreover, the presence of pain in other parts of the body (the arm, shoulder and upper back) was reported (yes/no); (5) history of ear-related symptoms – otalgia, tinnitus, vertigo, aural fullness, itching in the ear, subjective hearing impairment; (6) history of eye-related symptoms – lacrimation, pain behind the eyes; (7) the occurrence of physical symptoms of TMD (DC/TMD Axis II – Patient Health Questionnaire-15 (PHQ-15)); (8) the occurrence of depression (DC/TMD Axis II – PHQ-4, PHQ-9, Generalized Anxiety Disorder-7 (GAD-7)); (9) history of headaches during the past 30 days, including the temple areas of the head (DC/TMD Axis II); and (10) PHs (DC/TMD Axis II – Oral Behavior Checklist).

Data collection

In total, 105 patients with orofacial pain were recruited, from which 55 were excluded for the following reasons: not meeting the criteria (after completing the questionnaire and/or after the clinical examination) (n = 45); refusing to participate (n = 10). The included patients (50 women, aged 25–55 years) were randomly assigned either to the RPB group (n = 25) or the ST group (n = 25). From each group, 5 patients dropped out due to illness or failure to attend a visit. Finally, 20 patients in each group completed the therapy (Fig. 1). The patient demographics were recorded.

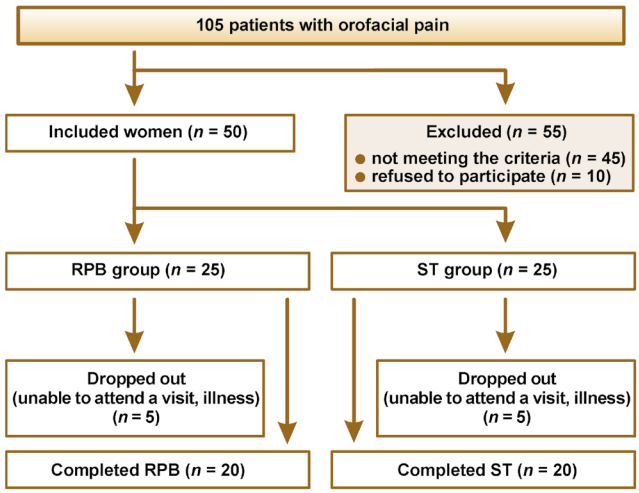

Fig. 1. Study protocol

RPB – relax-psycho-breathing therapy; ST – splint therapy.

Clinical examination

The clinical examination focused on the following data: (1) muscle and TMJ pain intensity; (2) TMJ sounds; (3) maximum mouth opening (MMO); (4) mouth opening limitation/deviation; and (5) deviation in mandibular movement (lateral (right and left) and protrusive).

Maximum mouth opening and opening deviation

Maximum mouth opening was measured from the incisal edge of the maxillary central incisors to the incisal edge of the mandibular central incisors at the midline, and did not include the degree of overlap between the teeth.[27] The patient was instructed to slowly open their

mouth 3 times to allow the visualization of mandibular end-range movement in the frontal plane. Moreover, the presence of deviation during mouth opening, defined as the displacement of the mandible at least 2 mm to the right or left of an imaginary vertical line, was evaluated.

Pain intensity

Jaw muscle pain and tenderness were assessed using an 11-point numerical rating scale (NRS-11). The NRS-11 is a widely used measure of pain intensity in both research and clinical practice.[28] The participants were asked to select a single number that best represented their pain intensity on a scale from 0 ("no pain") to 10 ("worst possible pain").

The Graded Chronic Pain Scale (GCPS), included in the Axis II screening instruments of the DC/TMD protocol,[6] comprises 6 items assessing facial pain experienced over the past 3–6 months. Each item is rated on a scale from 0 to 10.

Moreover, pain intensity during the clinical examination was assessed using the DC/TMD Axis I protocol.[6,7,29] The examination included standardized palpation of the masticatory muscles (masseter, temporalis, lateral pterygoid, and medial pterygoid) and TMJ, using the index and middle fingers.

Physical symptoms (DC/TMD Axis II – PHQ-15)

Physical symptoms were assessed using the DC/TMD Axis II – PHQ-15. Axis II, which addresses psychosocial and behavioral dimensions, comprises instruments for the evaluation of pain intensity, pain-related disability, the functional limitations of the masticatory system, oral parafunctional behaviors, as well as symptoms of depression and anxiety, and the extent of multiple somatic symptoms throughout the body.

The physical symptoms reflecting somatic complaints ("somatization") were assessed using a series of psychoanalytic questions (e.g., the presence of chest pain, palpitations, dizziness, or sleep disturbances), with responses recorded as yes/no.

Occurrence of depressive symptoms (DC/TMD Axis II – PHQ-4, PHQ-9, GAD-7)

The occurrence of depressive symptoms was assessed using the following question: "During the past month, have you experienced decreased energy or slowing down, feelings of loneliness or sadness, or indifference?"

Occurrence of parafunctional habits (DC/TMD Axis II – Oral Behavior Checklist)

The occurrence of PHs was assessed using the following questions: "Do you clench or grind your teeth during the day?"; "Do you clench or grind your teeth during sleep, based on any information available to you?"; "Do you bite your fingernails, fingers, pens, pencils, hair, or other objects?"; and "Do you use chewing gum?"

Treatment protocol

The diagnostic impressions of the maxilla and mandible were performed on day 1 (enrollment, V1). The patients were then randomly assigned, using a computer algorithm, to one of the study groups – the RPB group (relax-psycho-breathing therapy) or the ST group (splint therapy). Treatment commenced at the 2nd visit (V2; day 7). The RPB intervention included relaxation (R), self-care techniques (M), psychological and behavioral therapy (P), and breathing exercises (B).[30–32] In the ST group, in addition to the splint, the patients received basic educational and self-care instructions. Treatment outcomes were assessed at the 3rd and 4th follow-up visits (V3 and V4; day 21 and day 49, respectively). In patients with a limitation in mouth opening (<40 mm), the post-treatment deviation in mandibular movement was evaluated, along with the level of muscle pain.

RPB group – relax-psycho-breathing therapy

During V2, 25 patients were instructed to participate in RPB, with care taken to avoid pain or discomfort. The intervention included relaxation techniques, breathing exercises, psychological-behavioral guidance, muscle exercise therapy, puffing therapy, and self-massage (Fig. 2).

- Relaxation techniques: Performed at least twice daily for 10 min. The patients were instructed to lie in a supine position, and sequentially tense and relax the designated muscle groups, starting from the head and progressing toward the feet.
- Breathing patterns: Performed 3 times daily for 3–5 min. The patients were instructed to sit comfortably, focus on slow, deep inhalation through the nose and exhalation through the mouth, and maintain the awareness of their breathing without attempting to control it.



Fig. 2. Relax-psycho-breathing therapy (RPB): muscle exercise therapy; puffing therapy; and self-massage

While performing the exercises with the eyes closed, the patients were encouraged to visualize the outside world. If tension was experienced, inhalation was guided by counting to 5 or 10, followed by slow exhalation.
- Muscle exercise therapy: Performed in a series of 5 repetitions, with 5 series per day. The patients were instructed to look upward with the jaws slightly apart and open their mouths slowly, maintaining the open position for a few seconds before closing gently. Each repetition was followed by inhalation through the nose and exhalation through the mouth.
- Puffing therapy: Performed 5 times daily for 5 min. The patients produced a gentle puffing sound with the lips to relax the cheeks, jaws and lips, exhaling through the mouth, with the lips lightly in contact. They were then instructed to maintain a small gap of a few millimeters between the maxillary and mandibular teeth while the mandible remained in a resting position, keeping this relaxed posture except during eating or speaking. These exercises were also performed following the episodes of tooth clenching.
- Self-massage: Performed in a series of 5 repetitions, with at least 3 series daily, each lasting 3 min. The patients were instructed to habitually relax the facial and jaw muscles throughout the day. During the massage, TMJs and the surrounding muscles were gently treated by placing the fingertips inward, medially from the angle of the mandible, and slowly pushing the skin upward. Proper breathing was incorporated during the exercise, with inhalation through the nose and exhalation through the mouth.

Psychological-behavioral therapy consisted in keeping the jaws apart unless swallowing or eating. Moreover, wide mouth opening was to be avoided.

ST group – splint therapy

The patients received a custom-made, thermoplastic, flat-plane relaxation splint placed on the mandible.[19,33,34] Occlusal splints were fabricated from a 2.0-mm hard thermoplastic resin sheet (Erkodur; Erkodent Erich Kopp, Pfalzgrafenweiler, Germany), using the thermoforming technique. Occlusal contacts between the teeth and the splint were carefully checked and adjusted as necessary. A smooth occlusal splint with evenly distributed contacts in centric occlusion was designed. The patients were instructed to wear the splint nightly (up to 8 h) and were permitted to use it for an additional hour during the day if pain occurred.

Treatment evaluation

The treatment evaluation was carried out during V3 (day 21) and V4 (day 49). The following parameters were evaluated: pain intensity; TMJ sounds; MMO; mouth opening limitation/deviation; deviation in mandibular movement (lateral – right and left, and protrusive). Maximum mouth opening was measured from the incisal edge of the maxillary central incisors to the incisal edge of the mandibular central incisors at the midline. The presence of deviation during mouth opening was evaluated. Both treatment groups (RPD and ST) were evaluated for the reduction of pain in the masticatory muscles sensitive to touch (NRS). Upon muscular palpation, the patients reported the severity of tenderness. The objective and subjective assessment of symptoms and treatment results was performed by a dentist with 25 years of experience.

Statistical analysis

The results were subjected to statistical analysis using the Statistica software (StatSoft, Krakow, Poland). The distribution of variables was assessed using the Lilliefors and Shapiro–Wilk tests. For normally distributed variables, data is presented as mean ± standard deviation ($M \pm SD$), and parametric tests were applied for group comparisons. Variables that deviated from normality are reported as median (interquartile range) (Me (IQR)). For comparisons between the results before and after the treatment with regard to the whole sample ($N = 40$), the χ^2 test was used, with p-values provided. For the comparisons of nominal variables, the non-parametric χ^2 test was used, while the Mann–Whitney U test was applied for ordinal variables. For longitudinal related variables, the Wilcoxon signed-rank test was employed. Statistical significance was set at $p < 0.05$.

Results

Forty women (mean age: 37 ±9 years) completed either RPB or ST, and were evaluated at all follow-up visits: the RPB group ($n = 20$; mean age: 37 ±9 years); and the ST group ($n = 20$; mean age: 35 ±9 years).

The prevalence of TMD symptoms in all patients before and after treatment is presented in Table 1. When comparing the data gathered before treatment (day 1) with that obtained after treatment (day 49), statistically significant improvement was noticed in terms of pain reduction in the masticatory muscles, TMJ and muscle tenderness, arm and shoulder pain, and headache in both groups (RPB and ST). Both the number of patients reporting pain and the mean pain score decreased. The parameter that decreased the most was headache attributed to TMD. Also the number of patients reporting occlusal parafunctions, non-occlusal parafunctions, eye symptoms of TMD, ear symptoms of TMD, physical symptoms of TMD (DC/TMD), depressive symptoms (DC/TMD), and mandibular deviation during mouth opening decreased following the treatment. Regarding the extent of painless MMO, statistically significant improvement was found when comparing the values before and after

Table 1. Prevalence of symptoms of temporomandibular disorders (TMD) in all the examined patients ($N = 40$) before and after the treatment (χ^2 test)

TMD signs and symptoms	Before treatment (day 1) groups RPB and ST	After treatment (day 49) groups RPB and ST	*p*-value
Muscle pain *n*/mean NRS score	40/6	36/2	0.000*
Pain during function *n*/mean NRS score	40/6	35/2	0.000*
TMJ and muscle tenderness *n*/mean NRS score	40/6	35/2	0.000*
Arm and shoulder pain *n*/mean NRS score	30/2	25/1	0.000*
Headache attributed to TMD *n*/mean NRS score	29/2	17/0	0.000*
Occlusal parafunctions *n*	33	14	0.000*
Non-occlusal parafunctions *n*	24	12	0.002*
Eye symptoms of TMD *n*	19	5	0.001*
Ear symptoms of TMD *n*	17	4	0.001*
Physical symptoms of TMD (DC/TMD) *n*	21	3	0.003*
Depressive symptoms (DC/TMD) *n*	12	0	0.034*
Mandibular deviation during mouth opening *n*	24	19	0.015*
MMO [mm] *Me*	40	44	0.000*

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy. NRS – numerical rating scale; TMJ – temporomandibular joint; DC/TMD – Diagnostic Criteria for Temporomandibular Disorders; MMO – maximum mouth opening; *Me* – median; * statistically significant ($p < 0.05$).

the treatment in both groups. Significant eye (vision) and ear (hearing) symptom reduction ($p = 0.001$) was observed within the whole group (RPB and ST).

The comparisons of selected parameters (nominal variables) between and within the study groups with regard to 3 visits (V1, V3 and V4) are presented in Table 2.

Statistically significant differences in pain reduction during therapy are presented in Fig. 3 (pain during function), Fig. 4 (TMJ and muscle tenderness) and Fig. 5 (arm and shoulder pain). Pain intensity decreased in both groups; however, the reduction was more rapid and more pronounced in the RPB group across all 3 myalgia-related symptoms.

With regard to ordinal variables, differences between the visits within the RPB and ST groups, as well as between-group differences at each time point, are presented in Table 3. Statistical significance was assessed using the Wilcoxon signed-rank test and the Mann–Whitney *U* test, respectively ($p < 0.05$).

The statistical significance of differences between and within the 2 groups was as follows:

- a significant difference in pain during function between the RPB and ST groups was observed only at the final visit (V4) ($z = -2.64$; $p = 0.008$), while within-group comparisons showed significant differences for both the RPB and ST groups between V1 and V3, as well as between V1 and V4 ($z = 3.82$; $p = 0.000$) (Table 3, Fig. 3);
- a significant difference in TMJ and muscle tenderness between the RPB and ST groups was observed only at the final visit (V4) ($z = -3.10$; $p = 0.002$), while within-group comparisons showed significant differences for both the RPB and ST groups between V1 and V3, as well as between V1 and V4 ($z = 3.82$; $p = 0.000$) (Table 3, Fig. 4);
- a significant difference in arm and shoulder pain between the RPB and ST groups was observed only at V3 ($z = -2.38$; $p = 0.002$), while within-group comparisons showed significant differences for the RPB group between V1 and V3, and for both the RPB and ST groups between V1 and V4 ($z = 2.98$; $p = 0.003$, and $z = 3.01$; $p = 0.002$; respectively) (Table 3, Fig. 5).

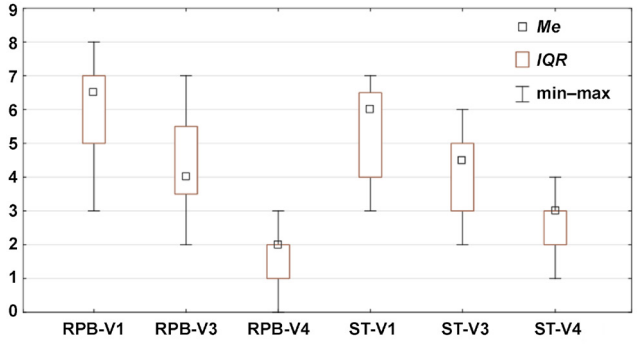

Fig. 3. Differences between the RPB and ST groups in pain during function (VAS (visual analog scale) = 0–10) with regard to 3 visits

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy. V1 –visit 1 (day 1); V3 – visit 3 (day 21); V4 – visit 4 (day 49). *IQR* – interquartile range.

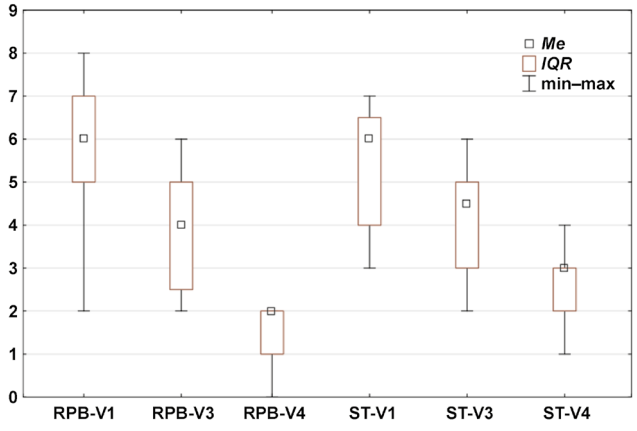

Fig. 4. Differences between the RPB and ST groups in temporomandibular joint (TMJ) and muscle tenderness (VAS = 0–10) with regard to 3 visits

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy. V1 –visit 1 (day 1); V3 – visit 3 (day 21); V4 – visit 4 (day 49).

A decrease in the number of patients presenting myalgia symptoms was observed in both therapy groups; however, the reduction was more rapid in the RPB group, particularly with respect to occlusal and non-occlusal parafunctions and mandibular deviation during mouth opening. In the ST group, a statistically significant reduction was observed only at the final visit (V4). A significant reduction in eye- and ear-related symptoms was observed in both the RPB and ST groups (Fig. 6).

Table 2. Comparisons of nominal variables between and within the study groups with regard to 3 visits

Variable	RPB group vs. ST group (V1, V3 and V4) (χ^2 test)	RPB group (V1 vs. V3 and V1 vs. V4) (Wilcoxon test)	ST group (V1 vs. V3 and V1 vs. V4) (Wilcoxon test)
Occlusal parafunctions	4.33; $p = 0.032$* 8.12; $p = 0.004$* ns	2.52; $p = 0.012$* 2.93; $p = 0.003$*	ns 3.62; $p = 0.003$*
Non-occlusal parafunctions	ns 6.67; $p = 0.010$* 4.28; $p = 0.038$*	2.52; $p = 0.012$* 2.66; $p = 0.008$*	ns ns
Eye symptoms of TMD	ns ns ns	ns 2.20; $p = 0.028$*	ns 2.52; $p = 0.012$*
Ear symptoms of TMD	ns ns ns	ns 2.52; $p = 0.012$*	ns 2.02; $p = 0.043$*
Physical symptoms of TMD	4.91; $p = 0.027$* ns ns	2.20; $p = 0.028$* 2.42; $p = 0.016$	ns ns
Depressive symptoms	ns ns ns	ns ns	ns 2.20; $p = 0.028$*
Mandibular deviation during mouth opening	3.75; $p = 0.053$ 6.40; $p = 0.011$* 8.41; $p = 0.015$*	ns ns	ns ns

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy.
V1 –visit 1 (day 1); V3 – visit 3 (day 21); V4 – visit 4 (day 49).
* statistically significant ($p < 0.05$); ns – non-significant.

Table 3. Comparisons of ordinal variables between and within the study groups with regard to 3 visits

Variable	RPB group ($n = 20$) (V1, V3 and V4)	ST group ($n = 20$) (V1, V3 and V4)	RPB group vs. ST group (V1, V3 and V4) (Mann–Whitney *U* test)	RPB group (V1 vs. V3 and V1 vs. V4) (Wilcoxon test)	ST group (V1 vs. V3 and V1 vs. V4) (Wilcoxon test)
Muscle pain *n* (NRS score range)	6 (5–7) 5 (4–6) 2 (1–2)	6 (4–6) 4 (3–5) 3 (2–4)	ns ns −2.88; $p = 0.004$*	3.92; $p = 0.000$* 3.92; $p = 0.000$*	3.92; $p = 0.000$* 3.92; $p = 0.000$*
Pain during function *n* (NRS score range)	6 (5–7) 4 (4–5) 2 (1–2)	6 (4–6) 4 (3–5) 3 (2–3)	ns ns −2.64; $p = 0.008$*	3.82; $p = 0.000$* 3.82; $p = 0.000$*	3.92; $p = 0.000$* 3.82; $p = 0.000$*
TMJ and muscle tenderness *n* (NRS score range)	6 (5–7) 4 (2–5) 2 (1–2)	6 (4–6) 4 (3–5) 3 (2–3)	ns ns −3.10; $p = 0.002$*	3.82; $p = 0.000$* 3.82; $p = 0.000$*	3.92; $p = 0.000$* 3.82; $p = 0.000$*
Arm and shoulder pain *n* (NRS score range)	2 (1–4) 2 (1–3) 1 (0–1)	2 (0–3) 1 (1–2) 1 (0–1)	ns −2.38; $p = 0.002$* ns	2.02; $p = 0.043$* 2.98; $p = 0.003$*	ns 3.01; $p = 0.002$*
Headache attributed to TMD *n* (NRS score range)	2 (1–2) 1 (1–2) 0 (0–0)	2 (0–3) 2 (0–2) 1 (0–1)	ns ns −2.45; $p = 0.014$*	3.18; $p = 0.001$* 3.41; $p = 0.001$*	2.27; $p = 0.023$* 2.79; $p = 0.005$*
MMO [mm] *Me* (*IQR*)	40 (38–42) 43 (40–44) 45 (44–46)	40 (38–42) 42 (40–43) 44 (42–45)	ns ns ns	3.72; $p = 0.000$* 3.92; $p = 0.000$*	3.92; $p = 0.000$* 3.92; $p = 0.000$*

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy.
V1 –visit 1 (day 1); V3 – visit 3 (day 21); V4 – visit 4 (day 49).
* statistically significant ($p < 0.05$); ns – non-significant.

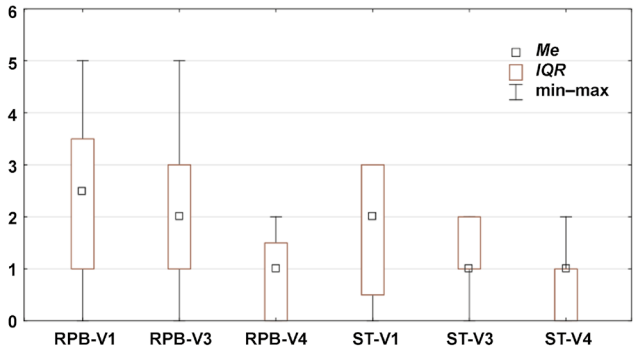

Fig. 5. Differences between the RPB and ST groups in arm and shoulder pain (VAS = 0–10) with regard to 3 visits

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy. V1 –visit 1 (day 1); V3 – visit 3 (day 21); V4 – visit 4 (day 49).

The effectiveness of therapy on pain intensity was evaluated at V3 and V4. Statistically significant differences between the RPB and ST groups were observed only at the final visit for muscle pain (p = 0.004), pain during function (p = 0.008), TMJ and muscle tenderness (p = 0.002), and headache attributed to TMD (p = 0.014). No significant differences were found between the groups in MMO (Table 3).

Discussion

The study focused on a range of symptoms associated with TMD in women, as well as the effectiveness of various therapeutic approaches in reducing pain severity in the masticatory muscles and/or TMJ, alleviating acoustic symptoms, and improving abnormal mandibular movement. Clinically, masticatory muscle dysfunction is characterized, among other features, by increased muscle tension and tenderness. Tenderness in the TMJ region and joint noises are more common in women than in men.[35,36]

More rapid relief and overall reduction of symptoms were observed in the individuals undergoing RPB as compared to those receiving ST. A comparable, statistically significant decrease in pain intensity was noted in the RPB and ST groups, along with a similar increase in MMO. A significant difference between the groups was observed only at the final visit. Symptoms of TMD and headaches were reduced following relaxation exercises.[37] The frequency of headaches decreased significantly in the RPB group as compared to the ST group at the last assessment. It appears that most of the accompanying symptoms were stress-related and subsided in the group in which participants learned to consciously control stress or alleviate complaints through muscle relaxation, whereas the use of a splint alone did not result in comparable improvement.

Wieckiewicz et al. concluded that conservative management – including counseling, exercises, occlusal splint therapy, massage, and manual therapy – should be considered the first-line treatment for TMD-related pain due to its low risk of side effects.[38] According to Michelotti et al., short-term education was slightly more effective than an occlusal splint provided without education in reducing spontaneous muscle pain in patients with TMD, while no significant differences were observed between the two approaches in terms of pain-free mouth opening, headache or chewing pain.[39] A stabilization splint may play a significant role in the short-term management of TMD; however, its effects appear comparable to those of other therapeutic modalities.[40] In contrast, findings from an RCT by Wahlund et al. suggest that in adolescents with TMD pain, standardized clinical treatment involving occlusal appliance therapy is more effective than therapist-

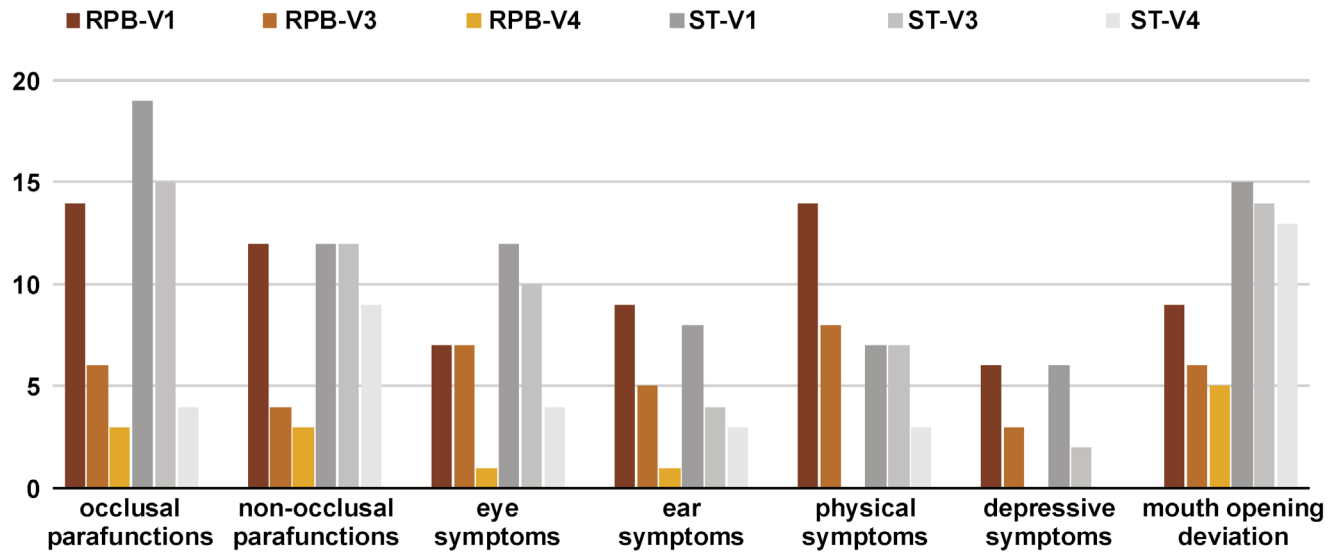

Fig. 6. Numbers of patients showing particular symptoms in both study groups

Groups: RPB – relax-psycho-breathing therapy; ST – splint therapy. V1 –visit 1 (day 1); V3 – visit 3 (day 21); V4 – visit 4 (day 49).

guided relaxation training in terms of self-reported improvement after a 6-month follow-up.[41] Furthermore, a systematic review and meta-analysis of RCTs demonstrated that well-adjusted hard stabilization appliances are more effective in reducing joint and muscle pain in comparison with no appliance, soft stabilization appliances, anterior bite appliances, and non-occluding appliances.[42]

Population-based studies have previously examined the associations between TMD and psychological variables, pain conditions and oral PHs.[43] The findings of the cited study suggest the presence of a shared central mechanism underlying both TMD and headache.[43] Therefore, healthcare providers should not consider these disorders in isolation, but rather adopt a broader perspective to better understand the complexity of the diagnostic and therapeutic process.[44]

In the present research, the RPB group patients, who were aware of their PHs and performed exercises, achieved improvement (in eye- and ear-related as well as physical symptoms) comparable to that observed in the ST group. A reduction in the number of patients presenting with myalgia was noted in both groups; however, this decrease occurred more rapidly in the RPB group, particularly with respect to the reduction of both occlusal and non-occlusal parafunctions.

Makino et al. compared a combination of jaw exercises and psychological interventions aimed at reducing parafunctional activities, demonstrating that this approach is more effective than jaw exercises alone in reducing craniocervical pain in the absence of organic abnormalities.[45] Patients with TMD and myalgia often present with a combination of psychological and somatic components.[11,12] Kalamir et al. evaluated the efficacy of self-care in comparison with manual therapy and splint therapy, reporting similar effectiveness across these approaches.[46] In contrast, Michelotti et al. investigated a combination of self-care with other modalities, such as manual and exercise therapy, and found that combined treatment strategies were more effective than self-care alone.[47]

According to various authors, the effectiveness of relaxation occlusal splints with a minimal standard vertical dimension in reducing TMD symptoms of muscular origin ranges from 70% to 90%.[48,49] These effects include, among others, a reduction in face and head pain, improvement in the range of mandibular movement, and decreased tenderness on palpation of the masticatory muscles and TMJ. Kurita et al. reported a reduction in dysfunction symptoms in 84% of patients and complete resolution in 41% of cases following the use of standard-height splints.[50] According to the guidelines of the American Academy of Orofacial Pain (AAOP), the purpose of a stabilization appliance is to "provide joint stabilization, protect the teeth, redistribute the occlusal forces, relax the elevator muscles, and decrease bruxism." Additionally, "wearing the appliance increases the patient's awareness of jaw habits and helps alter the rest position of the mandible to a more relaxed, open position."[51] The occluding surface of the appliance should contact the opposing dentition uniformly, evenly and simultaneously.

Alqutaibi and Aboalrejal proposed a broad range of management strategies for bruxism and TMD, including conservative and reversible approaches, such as occlusal devices (splints), behavioral techniques, as well as pharmacological and physical therapies.[52]

Self-care therapy, in contrast to pharmacological treatment, is free from side effects, drug interactions and the risk of overdose. Its efficacy is favorable, and the results remain stable provided that the patient consistently adheres to self-management practices. Our study suggests that maintaining a free space between the jaws (puffing therapy) combined with muscle relaxation may be an effective method for treating TMD, particularly when performed regularly. These exercises may also serve as an initial preparatory phase prior to the implementation of the main therapy.[37] It is essential to educate patients about the underlying mechanisms of these disorders – especially the role of emotional stress – and to warn them of habitual parafunctional activities (e.g., non-functional tooth contact or oral mucosa biting). Patients should develop awareness of their oral behaviors and, if such habits recur, make conscious efforts to eliminate them.[53,54]

Bae and Park demonstrated that relaxation exercises for the masticatory muscles were effective treatment for the improvement of limited mouth opening and pain reduction in TMD.[55] Turk et al. compared the efficacy of relaxation techniques (e.g., biofeedback and stress management) with occlusal splint therapy in patients randomly divided into 3 groups.[56] After 6 weeks, pain intensity was significantly reduced in all 3 treatment groups, but occlusal therapy seemed to be more effective. However, at the end of the 6th month, patients treated with occlusal appliances reported the recurrence of symptoms, whereas those under relaxation therapy experienced a further reduction of pain levels. The combined treatment was more effective than either of the single treatment approaches alone, particularly in pain reduction, as observed at the 6-month follow-up.[56]

A systematic review by Aggarwal et al. provides clear evidence supporting the effectiveness of combined biomedical and psychological interventions, including self-management approaches, in improving long-term outcomes in the treatment of chronic orofacial pain, particularly TMD.[31] It is claimed that muscular pain disorders should primarily be managed using conservative approaches. In cases of restricted mouth opening, the additional use of occlusal appliances may rapidly eliminate discomfort.[38] Furthermore, self-management interventions have been shown to be effective in patients with chronic orofacial pain, and therapies incorporating physical and psychosocial self-regulation, as well as patient education, appear beneficial.[31]

We consider the symptoms of TMD – such as pain and tenderness in TMJ and the surrounding muscles and ligaments – to be triggered, among other factors, by the excessive tension in the masticatory muscles associated with parafunctional activities and stress. Therefore, interventions aimed at masticatory muscle relaxation, including targeted exercises and breathing techniques, may represent effective therapeutic strategies.[57]

As the number of patients reporting depressive symptoms during therapy was similar in both groups, and no such symptoms were reported at the end of the observation period, it may be concluded that the alleviation of TMD-related symptoms contributed to improved patient comfort and the resolution of depressive symptoms.

The authors observed that a combination of relaxation exercise therapy, psychological-behavioral interventions and proper breathing patterns (RPB) was as effective as occlusal splints in improving MMO and reducing pain levels in the treatment of myalgia. These findings suggest that a combined therapeutic approach may yield beneficial outcomes at both early and later stages of treatment, owing to the long-term effects of biobehavioral therapy and the immediate benefits provided by occlusal appliances.[58]

Given these premises, we propose that combining conventional conservative treatment with psychologically tailored interventions may represent the most effective strategy for managing myalgia.

Within the limitations of the study, including a small sample size, a short follow-up period, the lack of assessment of the longevity of therapeutic effects, the exclusion of patients with dentures (fixed or removable) and those with an open bite, a cross-bite or a prognathic relationship, and the absence of a placebo control group, it is concluded that occlusal splint therapy should not necessarily be regarded the initial treatment for all cases of myalgia. In certain instances, psycho-behavioral-relaxation exercises may be more effective than splint therapy alone.

Conclusions

Relax-psycho-breathing therapy (RPB) and splint therapy (ST) appear to be comparable in managing myalgia-related muscle pain in women. However, in the initial stage of treatment, a comprehensive approach incorporating relaxation therapy may be preferable to occlusal splint therapy alone.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Poznan University of Medical Sciences, Poland (approval No. 551/15). Written informed consent was obtained from all patients.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request

Consent for publication

Consent for publication was obtained from the patient presented in Fig. 2.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Seweryn P, Orzeszek SM, Waliszewska-Prosół M, et al. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023;60(4):609–617. doi:10.17219/dmp/171894
2. Macfarlane TV, Gray RJ, Kincey J, Worthington HV. Factors associated with the temporomandibular disorder, pain dysfunction syndrome (PDS): Manchester case–control study. *Oral Dis*. 2001;7:321–330. doi:10.1034/j.1601-0825.2001.00758.x
3. Golanska P, Saczuk K, Domarecka M, Kuć J, Lukomska-Szymanska M. Temporomandibular myofascial pain syndrome – aetiology and biopsychosocial modulation. A narrative review. *Int J Environ Res Public Health*. 2021;18:7807. doi:10.3390/ijerph18157807
4. Wieckiewicz M, Grychowska N, Nahajowski M, et al. Prevalence and overlaps of headaches and pain-related temporomandibular disorders among the Polish urban population. *J Oral Facial Pain Headache*. 2020;34(1):31–39. doi:10.11607/ofph.2386
5. Peck CC, Goulet JP, Lobbezoo F, et al. Expanding the taxonomy of the diagnostic criteria for temporomandibular disorders. *J Oral Rehabil*. 2014;41(1):2–23. doi:10.1111/joor.12132
6. Schiffman E, Ohrbach R, Truelove E, et al.; International RDC/TMD Consortium Network, International Association for Dental Research; Orofacial Pain Special Interest Group, International Association for the Study of Pain. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for clinical and research applications: Recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
7. Osiewicz M, Ciapała B, Bolt K, Kołodziej P, Więckiewicz M, Ohrbach R. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD): Polish assessment instruments. *Dent Med Probl*. 2024;61(1):5–8. doi:10.17219/dmp/181149
8. Cigdem Karacay B, Sahbaz T. Investigation of the relationship between probable sleep bruxism, awake bruxism and temporomandibular disorders using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). *Dent Med Probl*. 2023;60(4):601–608. doi:10.17219/dmp/158926
9. Manfredini D, Bandettini di Poggio A, Cantini E, Dell'Osso L, Bosco M. Mood and anxiety psychopathology and temporomandibular disorder: A spectrum approach. *J Oral Rehabil*. 2004;31(10):933–940. doi:10.1111/j.1365-2842.2004.01335.x
10. Molina OF, Peixoto MG, Manzutti Eid NL, Aquilino RN, Costa Rank RC. Headache and bruxing behavior types in craniomandibular disorders (CMDs) patients. *Rev Neurosci*. 2011;19(3):449–457. doi:10.34024/rnc.2011.v19.8345

11. Svensson P, List T, Hector G. Analysis of stimulus-evoked pain in patients with myofascial temporomandibular pain disorders. *Pain*. 2001;92(3):399–409. doi:10.1016/S0304-3959(01)00284-6
12. Zijlema WL, Stolk RP, Löwe B, et al. How to assess common somatic symptoms in large-scale studies: A systematic review of questionnaires. *J Psychosom Res*. 2013;74(6):459–468. doi:10.1016/j.jpsychores.2013.03.093
13. Ramos-Herrada RM, Arriola-Guillén LE, Atoche-Socola KJ, Bellini-Pereira SA, Aliaga-Del Castillo A. Effects of botulinum toxin in patients with myofascial pain related to temporomandibular joint disorders: A systematic review. *Dent Med Probl*. 2022;59(2):271–280. doi:10.17219/dmp/145759
14. Orzeszek S**,** Martynowicz H, Smardz J, et al. Assessment of sleep quality in patients with orofacial pain and headache complaints: A polysomnographic study. *Dent Med Probl*. 2024;61(4):549–562. doi:10.17219/dmp/177008
15. Pal US, Kumar L, Mehta G, et al. Trends in management of myofacial pain. *Natl J Maxillofac Surg*. 2014;5(2):109–116. doi:10.4103/0975-5950.154810
16. Manfredini D, Häggman-Henrikson B, Al Jaghsi A, et al.; International Network for Orofacial Pain and Related Disorders Methodology. Temporomandibular disorders: INfORM/IADR key points for good clinical practice based on standard of care. *Cranio*. 2025;43(1):1–5. doi:10.1080/08869634.2024.2405298
17. Saracutu OI, Manfredini D, Bracci A, Val M, Ferrari M, Colonna A. Comparison between ecological momentary assessment and self-report of awake bruxism behaviours in a group of healthy young adults. *J Oral Rehabil*. 2025;52(3):289–295. doi:10.1111/joor.13895
18. Delcanho R, Val M, Nardini LG, Manfredini D. Botulinum toxin for treating temporomandibular disorders: What is the evidence? *J Oral Facial Pain Headache*. 2022;36(1):6–20. doi:10.11607/ofph.3023
19. Mishra KD, Gatchel RJ, Gardea MA. The relative efficacy of three cognitive-behavioral treatment approaches to temporomandibular disorders. *J Behav Med*. 2000;23(3):293–309. doi:10.1023/a:1005562126071
20. Glaros AG, Owais Z, Lausten L. Reduction in parafunctional activity: A potential mechanism for the effectiveness of splint therapy. *J Oral Rehabil*. 2007;34(2):97–104. doi:10.1111/j.1365-2842.2006.01660.x
21. Ekers D, Richards D, Gilbody S. A meta-analysis of randomized trials of behavioural treatment of depression. *Psychol Med*. 2008;38(5):611–623. doi:10.1017/S0033291707001614
22. Ishikawa SI, Okajima I, Matsuoka H, Sakano Y. Cognitive behavioural therapy for anxiety disorders in children and adolescents: A meta-analysis. *Child Adolesc Ment Health*. 2007;12(4):164–172. doi:10.1111/j.1475-3588.2006.00433.x
23. Morton L, Roach L, Reid H, Stewart SH. An evaluation of a CBT group for women with low self-esteem. *Behav Cogn Psychother*. 2012;40(2):221–225. doi:10.1017/S1352465811000294
24. Wężgowiec J, Małysa A, Więckiewicz M. How does artificial aging affect the mechanical properties of occlusal splint materials processed via various technologies? *Dent Med Probl*. 2025;62(3):527–535. doi:10.17219/dmp/174708
25. Türp JC, Komine F, Hugger A. Efficacy of stabilization splints for the management of patients with masticatory muscle pain: A qualitative systematic review. *Clin Oral Investig*. 2004;8(4):179–195. doi:10.1007/s00784-004-0265-4
26. Widmalm SE. Use and abuse of bite splints. *Compend Contin Educ Dent*. 1999;20(3):249–254. PMID:11692335.
27. Kumar S, Singh M, Nagaraju K, Shukla AK. To determine the effect of a muscle energy technique and therapeutic jaw exercises on the range of mouth opening. *Physiother Occup Ther J*. 2014;7(4):181–188.
28. Chien CW, Bagraith KS, Khan A, Deen M, Strong J. Comparative responsiveness of verbal and numerical rating scales to measure pain intensity in patients with chronic pain. *J Pain*. 2013;14(12):1653–1662. doi:10.1016/j.jpain.2013.08.006
29. Conti PC, De Azevedo LR, De Souza NV, Ferreira FV. Pain measurement in TMD patients: Evaluation of precision and sensitivity of different scales. *J Oral Rehabil*. 2001;28(6):534–539. doi:10.1046/j.1365-2842.2001.00727.x
30. Toussaint L, Nguyen QA, Roettger C, et al. Effectiveness of progressive muscle relaxation, deep breathing, and guided imagery in promoting psychological and physiological states of relaxation. *Evid Based Complement Alternat Med*. 2021;2021:5924040. doi:10.1155/2021/5924040
31. Aggarwal VR, Fu Y, Main CJ, Wu J. The effectiveness of self-management interventions in adults with chronic orofacial pain: A systematic review, meta-analysis and meta-regression. *Eur J Pain*. 2019;23(5):849–865. doi:10.1002/ejp.1358
32. Durham J, Al-Baghdadi M, Baad-Hansen L, et al. Self-management programmes in temporomandibular disorders: Results from an international Delphi process. *J Oral Rehabil*. 2016;43(12):929–936. doi:10.1111/joor.12448
33. Saito K, Ikeda T, Gionhaku N, Iinuma T, Sato J, Lee J. Short-term effects of oral appliances with equal bite-raising distance but with varying protrusions on occlusal force, contact area and load center. *J Oral Sci*. 2008;50(3):253–258. doi:10.2334/josnusd.50.253
34. Orzeszek S, Waliszewska-Prosol M, Ettlin D, et al. Efficiency of occlusal splint therapy on orofacial muscle pain reduction: A systematic review. *BMC Oral Health*. 2023;23(1):180. doi:10.1186/s12903-023-02897-0
35. Gil-Martínez A, Paris-Alemany A, López-de-Uralde-Villanueva I, La Touche R. Management of pain in patients with temporomandibular disorder (TMD): Challenges and solutions. *J Pain Res*. 2018;11:571–587. doi:10.2147/JPR.S127950
36. Loster JE, Wieczorek A. Assessment of the effectiveness of treatment for temporomandibular joint dysfunctions. *Dent Med Probl*. 2014;51(1):72–78. https://dmp.umw.edu.pl/en/article/2014/51/1/72. Accessed January 5, 2025.
37. Agha-Hosseini F, Sheykhbahaei N, Mirzaii-Dizgah I, Fatehi F. The efficacy of oral habit modification on headache. *J Korean Assoc Oral Maxillofac Surg*. 2017;43(6):401–406. doi:10.5125/jkaoms.2017.43.6.401
38. Wieckiewicz M, Boening K, Wiland P, Shiau YY, Paradowska-Stolarz A. Reported concepts for the treatment modalities and pain management of temporomandibular disorders. *J Headache Pain*. 2015;16:106. doi:10.1186/s10194-015-0586-5
39. Michelotti A, Iodice G, Vollaro S, Steenks MH, Farella M. Evaluation of the short-term effectiveness of education versus an occlusal splint for the treatment of myofascial pain of the jaw muscles. *J Am Dent Assoc*. 2012;143(1):47–53. doi:10.14219/jada.archive.2012.0018
40. Kuzmanovic Pficer J, Dodic S, Lazic V, Trajkovic G, Milic N, Milicic B. Occlusal stabilization splint for patients with temporomandibular disorders: Meta-analysis of short and long term effects. *PLoS One*. 2017;12(2):e0171296. doi:10.1371/journal.pone.0171296
41. Wahlund K, Nilsson IM, Larsson B Treating temporomandibular disorders in adolescents: A randomized, controlled, sequential comparison of relaxation training and occlusal appliance therapy. *J Oral Facial Pain Headache*. 2015;29(1):41–50. doi:10.11607/ofph.1285
42. Klasser GD, Greene CS. Oral appliances in the management of temporomandibular disorders. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107(2):212–223. doi:10.1016/j.tripleo.2008.10.007
43. Ciancaglini R, Radaelli G. The relationship between headache and symptoms of temporomandibular disorder in the general population. *J Dent*. 2001;29(2):93–98. doi:10.1016/s0300-5712(00)00042-7
44. Van der Meer HA, Speksnijder CM, Engelbert RH, Lobbezoo F, Nijhuis-van der Sanden MW, Visscher CM. The association between headaches and temporomandibular disorders is confounded by bruxism and somatic symptoms. *Clin J Pain*. 2017;33(9):835–843. doi:10.1097/AJP.0000000000000470
45. Makino I, Arai YCP, Aono S, et al. The effects of exercise therapy for the improvement of jaw movement and psychological intervention to reduce parafunctional activities on chronic pain in the craniocervical region. *Pain Pract*. 2014;14(5):413–418. doi:10.1111/papr.12075
46. Kalamir A, Bonello R, Graham P, Vitiello AL, Pollard H. Intraoral myofascial therapy for chronic myogenous temporomandibular disorder: A randomized controlled trial. *J Manipulative Physiol Ther*. 2012;35(1):26–37. doi:10.1016/j.jmpt.2011.09.004
47. Michelotti A, Steenks MH, Farella M, Parisini F, Cimino R, Martina R. The additional value of a home physical therapy regimen versus patient education only for the treatment of myofascial pain of the jaw muscles: Short-term results of a randomized clinical trial. *J Orofac Pain*. 2004;18(2):114–125. PMID:15250431.
48. Al-Ani Z, Gray RJ, Davies SJ, Sloan P, Glenny AM. Stabilization splint therapy for the treatment of temporomandibular myofascial pain: A systematic review. *J Dent Educ*. 2005;69(11):1242–1250. PMID:16275687.

49. Doepel M, Nilner M, Ekberg E, Le Bell Y. Long-term effectiveness of a prefabricated oral appliance for myofascial pain. *J Oral Rehabil*. 2012;39(4):252–260. doi:10.1111/j.1365-2842.2011.02261.x
50. Kurita H, Kurashina K, Kotani A. Clinical effect of full coverage occlusal splint therapy for specific temporomandibular disorder conditions and symptoms. *J Prosthet Dent*. 1997;78(5):506–510. doi:10.1016/s0022-3913(97)70067-2
51. De Leeuw RD, Klasser GD, eds. Orofacial Pain: Guidelines for Assessment, Diagnosis, and Management. 5th ed. Chicago, IL: Quintessence Publishing Co., Inc.; 2013.
52. Alqutaibi AY, Aboalrejal AN. Types of occlusal splint in management of temporomandibular disorders (TMD). *J Arthritis*. 2015;4:4. doi:10.4172/2167-7921.1000176
53. Freesmeyer WB, Fussnegger MR, Ahlers MO. Diagnostic and therapeutic-restorative procedures for masticatory dysfunctions. *GMS Curr Top Otorhinolaryngol Head Neck Surg*. 2005;4:Doc19. PMID:22073067. PMCID:PMC3201006.
54. Colonna A, Lobbezoo F, Bracci A, Ferrari M, Val M, Manfredini D. Long-term study on the fluctuation of self-reported awake bruxism in a cohort of healthy young adults. *J Oral Rehabil*. 2025;52(1):37–42. doi:10.1111/joor.13872
55. Bae Y, Park Y. The effect of relaxation exercises for the masticator muscles on temporomandibular joint dysfunction (TMD). *J Phys Ther Sci*. 2013;25(5):583–586. doi:10.1589/jpts.25.583
56. Turk DC, Zaki HS, Rudy TE. Effects of intraoral appliance and biofeedback/stress management alone and in combination in treating pain and depression in patients with temporomandibular disorders. *J Prosthet Dent*. 1993;70(2):158-164. doi: 10.1016/0022-3913(93)90012-d.
57. Rosati R, Val M, Manfredini D, et al. Baseline masticatory muscles' performance may predict pain relief in temporomandibular disorders. *Oral Dis*. 2024;30(8):5349–5359. doi:10.1111/odi.15011
58. Nagata K, Maruyama H, Mizuhashi R, et al. Efficacy of stabilisation splint therapy combined with non-splint multimodal therapy for treating RDC/TMD Axis I patients: A randomised controlled trial. *J Oral Rehabil*. 2015;42(12):890–899. doi:10.1111/joor.12332

Original paper

Comparative evaluation of the cytokine profile in platelet-rich fibrin and hyperacute serum from periodontitis patients with or without type 2 diabetes mellitus: An ex vivo cross-sectional study

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Abstract

Background. Periodontitis is regarded as the 6th microvascular complication of type 2 diabetes mellitus (T2DM). In individuals with both periodontitis and T2DM, the condition may adversely influence the regenerative potential of the platelet-rich fibrin (PRF) and hyperacute serum (HAS) derived from these patients.

Objectives. The present cross-sectional study was conducted to evaluate the weight and cytokine levels of the PRF and HAS obtained from periodontitis patients with or without T2DM in a laboratory setting.

Material and methods. Platelet-rich fibrin and HAS were obtained from 17 periodontitis patients with T2DM (group 1: T2DM+/P+), 17 periodontitis patients without T2DM (group 2: T2DM–/P+), and 16 periodontally healthy participants (group 3: T2DM–/P–). Demographic, periodontal and hematological parameters were recorded for all participants. Serum, PRF and HAS samples were quantified and analyzed for inflammatory markers – interleukin-6 (IL-6), resistin (RETN), tumor necrosis factor-alpha (TNF-α), IL-1β, and interleukin-1 receptor antagonist (IL-1ra) – as well as growth factors, including insulin-like growth factor-1 (IGF-1), transforming growth factor-beta 1 (TGF-β1), platelet-derived growth factor-BB (PDGF-BB), and fibroblast growth factor-21 (FGF-21).

Results. The quantities of PRF and HAS obtained were significantly higher in healthy individuals as compared to diabetic patients. The concentrations of inflammatory markers and growth factors in serum, PRF and HAS from periodontitis patients were not significantly different between those with and without T2DM.

Conclusions. Periodontitis in conjunction with T2DM may influence the weight of PRF and HAS; however, no significant association was observed with respect to the levels of inflammatory markers and growth factors.

Keywords: periodontitis, type 2 diabetes mellitus, inflammatory markers, growth factors, platelet-rich fibrin

Highlights

- Patients with periodontitis and type 2 diabetes mellitus (T2DM) exhibited significantly fewer teeth present, a higher body mass index (BMI), elevated systolic blood pressure (SBP), and greater insulin resistance (IR) as compared to non-diabetic healthy controls.
- The coexistence of periodontitis and T2DM may adversely affect the regenerative potential of platelet-rich fibrin (PRF) and hyperacute serum (HAS).
- The mean PRF weight was significantly highest in healthy controls (T2DM−/P−), intermediate in periodontitis participants without T2DM (T2DM−/P+), and lowest in periodontitis participants with T2DM (T2DM+/P+).

Introduction

Insulin resistance (IR) or insufficiency leading to hyperglycemia is the hallmark of type 2 diabetes mellitus (T2DM).[1] Type 2 diabetes mellitus is reported to have affected approx. 462 million individuals by 2017, accounting for 6.28% of the global population,[2] and it constitutes nearly 90% of diabetes cases worldwide.[3,4] It is predicted that by 2025, the countries with the highest number of individuals with diabetes will be India, China and the United States.[5] According to the National Family Health Survey of India (NFHS), 2019–2021, India currently has 74.9 million individuals with diabetes mellitus (DM), and this number is projected to rise to 124.9 million over the next 24 years.[6]

Periodontitis is a microbially induced, localized, chronic inflammatory disease of the supporting tissues of the teeth, characterized by a dysregulated host inflammatory response that leads to periodontal tissue breakdown and eventual tooth loss.[7] The interrelationship between T2DM and periodontitis has been studied for over 50 years.[8] In a joint consensus report by the European Federation of Periodontology (EFP) and the International Diabetes Federation (IDF), Sanz et al. highlighted the association of elevated levels of inflammatory cytokines in the gingival tissues of individuals with periodontitis and T2DM.[9]

Among various platelet concentrates (PCs), autologous platelet-rich fibrin (PRF) is widely used in surgical procedures, not only in the disciplines of oral surgery, implant dentistry and periodontology, but also as a surgical adjunct in sports medicine, in orthopedic surgery and as a healing gel for diabetic foot ulcers.[10–12] In recent years, hyperacute serum (HAS) has been introduced; it is obtained by squeezing the PRF clot through an additive-free, single-step process. Hyperacute serum is a potential regenerative agent that promotes wound healing, mimicking the natural coagulation cascade.[13]

Platelets release a range of pro-inflammatory, anti-inflammatory and pro-regenerative cytokines upon contact with the exposed endothelium, thereby facilitating wound healing. Various cytokines studied in PRF include interleukin-1 beta (IL-1β), IL-4, IL-6, tumor necrosis factor-alpha (TNF-α), interleukin-1 receptor antagonist (IL-1ra), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta 1 (TGF-β1), platelet-derived growth factor-BB (PDGF-BB), fibroblast growth factor-21 (FGF-21), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF), all of which contribute to the natural wound healing process.[14–19]

Resistin (RETN), an inflammatory cytokine first described in 2001 as an adipose-secreted polypeptide, has been linked to IR and periodontitis.[20,21] Increased concentrations of RETN, an important inflammatory marker, in gingival crevicular fluid (GCF) and serum have been associated with poor glycemic control in T2DM patients with periodontitis.[22,23] However, its correlation in serum, PRF and HAS in periodontitis patients with T2DM has not yet been investigated.

Periodontitis is widely regarded as the 6th microvascular complication of DM, and diabetes may exert an inductive effect on the cytokine and growth factor levels in the PRF obtained from the affected patients. Clinical studies have suggested that cytokine release from PRF in diabetic patients with periodontitis may be reduced.[15] To the best of our knowledge, no published report to date has evaluated inflammatory markers and growth factors in both PRF and HAS in periodontitis patients with or without T2DM.

It is hypothesized that periodontitis patients with T2DM may exhibit variations in the regenerative potential of PRF and HAS in terms of inflammatory markers and growth factors. Therefore, the present observational study was designed to evaluate inflammatory markers and growth factors in PRF and HAS among patients with and without T2DM to test this hypothesis. Additionally, the study assessed biochemical (hematological and lipid) parameters, and examined the quantitative association of PRF and HAS with glycated hemoglobin (HbA1c) in patients with periodontitis, with and without T2DM.

Material and methods

Investigation design

This cross-sectional study was carried out from March 2021 to March 2023 in Lucknow, India, according to the STROBE (Strengthening the Reporting of Observational

Studies in Epidemiology) criteria. The study was conducted in accordance with the principles and ethical guidelines outlined in the Declaration of Helsinki 1964, amended in 2013. The study protocol (#FV1PE21122020R) was approved by the Institutional Research and Development Committee (IRDC) and the Institutional Human Ethics Committee (IHEC) at Saraswati Dental College and Hospital, Lucknow, India.

Case definition

Periodontitis was defined as the presence of interdental clinical attachment loss (CAL) >1 mm at 2 or more non-adjacent teeth, or buccal/oral CAL ≥ 3 mm with the probing pocket depth (PPD) >3 mm at 2 or more teeth.[24,25] Patients presenting with at least Stage II periodontitis or higher were enrolled in the present investigation as representative of periodontitis.[25] Volunteers exhibiting bleeding on probing (BoP) at <10% of sites, PPD ≤ 3 mm, and no evidence of horizontal or vertical bone loss on radiographs were considered periodontally healthy.[26] Participants with the fasting plasma glucose (FPG) level ≥126 mg/dL, the random blood sugar (RBS) level >200 mg/dL and HbA1c > 6.5% were classified as having T2DM according to the World Health Organization (WHO) and related criteria, whereas those with values below these thresholds were considered non-diabetic.[27]

Study population

For the present cross-sectional study, periodontitis patients of Indian origin meeting the below-mentioned inclusion and exclusion criteria were recruited from the individuals visiting the Department of Periodontology of Saraswati Dental College and Hospital, Lucknow, India, for the management of periodontal diseases. Those who voluntarily agreed to participate and provided written informed consent, after a thorough explanation of the study aims and objectives, were enrolled. Healthy volunteers from within the institute were included as representatives of the control group.

Inclusion criteria

Participants with moderate to severe periodontitis (Stage II and above, Grade B or C), with T2DM, aged 40–50 years, of either sex, who were non-smokers and non-alcohol consumers, were included. Eligible participants had a minimum of 20 teeth present and at least 8 sites with CAL ≥ 3 mm and PPD ≥ 4 mm. Additionally, they had received no or only stable doses of oral hypoglycemic agents, and had not undergone periodontal therapy or received any systemic or local medications in the preceding 6 months. Volunteers of both sexes with a healthy periodontium (CAL < 1 mm), aged 40–50 years, were also included as controls (T2DM–/P–), as described earlier.[26]

Exclusion criteria

Exclusion criteria comprised the presence of systemic illnesses other than T2DM; pregnant or lactating women; patients with major complications of T2DM (e.g., cardiovascular or peripheral vascular diseases, renal disorders, and hypertension); and individuals with acute infections, heart failure or malignancy at the time of blood collection. Participants who declined to provide written informed consent and those with an inadequate platelet count (<100,000 per milliliter) were also excluded.

Sample size

The sample size was calculated based on the minimum variation observed among growth factors (IGF, PDGF-BB, FGF, EGF, and VEGF) in PRF and HAS at the final follow-up, as reported by Su et al.[17] The maximum standard deviation (*SD*) for growth factor parameters in the PRF group was $\sigma = 0.47$. The difference deemed clinically significant in the HAS group was $d = \max(\sigma 1, \sigma 2)$, which is the maximum *SD* of growth factor values. A type II error (β) of 20% was assumed to achieve 80% study power, while a type I error (α) of 5% corresponded to a 95% confidence level. Based on these assumptions, the minimum required sample size was calculated to be $n = 15$ participants per group.

Recruitment of volunteers

A total of 60 patients visiting the Department of Periodontology were screened for the diabetic status, oral hygiene, weight, height (in centimeters), the body mass index (BMI), and RBS. Comprehensive dental and medical histories were recorded. To exclude systemic diseases other than T2DM, the participants were evaluated for vital parameters, including blood pressure (BP), the pulse rate (beats per minute), body temperature, and the respiratory rate (breaths per minute). All participants underwent blood sampling prior to enrollment in the study.

After careful screening, 51 volunteers were finally recruited for the study. Using a scalp vein infusion set and a 20-mL syringe, 20 mL of blood was drawn from the antecubital region (the median cephalic vein) of each participant. At this stage, 1 participant was excluded due to the inability to collect a sufficient volume of blood. Of the total volume collected, 3 mL of whole blood was transferred into an ethylenediaminetertaacetic acid (EDTA)-coated test tube for the analysis of HbA1c and hemoglobin (Hb). Eight milliliters of blood were equally distributed into 2 clot-activated vacutainers (4 mL each) for serum extraction. Serum obtained from one 4-mL sample was used to assess the complete blood count (CBC), creatinine, the lipid profile, high-sensitivity C-reactive protein (hs-CRP), RBS, FPG, fasting glucose, and IR. Serum from the other 4-mL sample was stored at –80°C for the subsequent

analysis of cytokines and growth factors. The remaining 9 mL of blood was immediately transferred into a plain vacutainer for the preparation of PRF and HAS (Fig. 1). All procedures were performed by a single trained investigator under standardized conditions at room temperature (27°C) to minimize variability.

Grouping

The enrolled participants were allocated into 3 groups according to the study design: group 1 (T2DM+/P+), comprising periodontitis patients with T2DM (n = 17); group 2 (T2DM–/P+), comprising systemically healthy patients with periodontitis (n = 17); and group 3 (T2DM–/P–), comprising systemically healthy individuals with a healthy periodontium (n = 16). To further distinguish the glycemic control status, group 1 (T2DM+/P+) was subdivided into 2 subgroups based on the HbA1c level: <8% (poorly controlled); and ≥8% (uncontrolled).

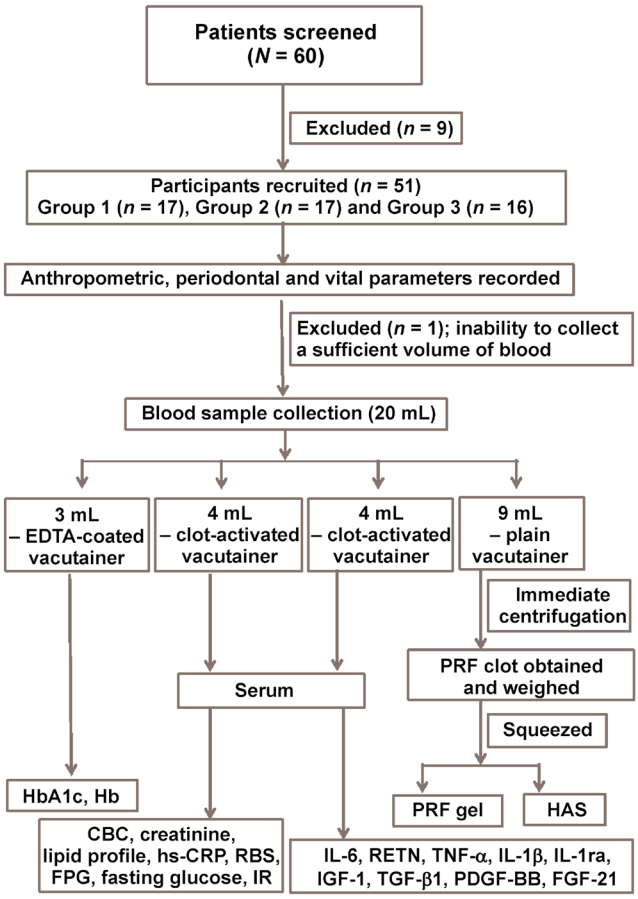

Fig. 1. Flow chart showing the methodology of the study

Groups: group 1 (T2DM+/P+); group 2 (T2DM–/P+); group 3 – control (T2DM–/P–). T2DM – type 2 diabetes mellitus; P – periodontitis. EDTA – ethylenediaminetertaacetic acid; PRF – platelet-rich fibrin; HAS – hyperacute serum; HbA1c – glycated hemoglobin; Hb – hemoglobin; CBC – complete blood count; hs-CRP – high-sensitivity C-reactive protein; RBS – random blood sugar; FPG – fasting plasma glucose; IR – insulin resistance; IL-6 – interleukin-6; RETN – resistin; TNF-α – tumor necrosis factor-alpha; IL-1ra – interleukin-1 receptor antagonist; IGF-1 – insulin-like growth factor-1; TGF-β1 – transforming growth factor-beta 1; PDGF-BB – platelet-derived growth factor-BB; FGF-21 – fibroblast growth factor-21.

Preparation of PRF and HAS

As recommended by Choukroun et al.,[28] autologous PRF was prepared from the patient's blood using a tabletop centrifuge with a fixed-angle rotor head (REMI R-303; IndiaMART, Noida, India). Blood was centrifuged in 9-mL pre-sterilized vacutainers (Labtech Healthcare India, Jaipur, India) at 3,000 rpm (relative centrifugal force (RCF) = 755 g; rotor radius = 7.5 cm) for 12–14 min at room temperature. For HAS preparation, the resulting fibrin clot was gently compressed using flat instruments, and the serum part was collected in microcentrifuge tubes and stored at –80°C for further analysis. To ensure standardization, all samples were collected by a single trained investigator and processed by another trained investigator under identical conditions, using the same centrifuge at room temperature.

Parameters recorded

Demographic analysis

The following demographic data was collected and analyzed: age; sex; weight; height; and BMI.

Periodontal parameters

Each participant underwent a comprehensive full-mouth oral examination using standard diagnostic instruments. All clinical parameters were recorded by a single trained investigator to eliminate inter-observer variability.

The gingival index (GI) was assessed at the facial (labial/buccal), lingual/palatal, mesial, and distal gingival margins.[29] Similarly, the plaque index (PI) was evaluated using a blunt probe/explorer on the facial (labial/buccal), lingual/palatal, mesial, and distal surfaces of all teeth.[30] The probing pocket depth (PPD) was measured at 6 sites per tooth, using a calibrated instrument (University of North Carolina-15 probe, UNC-15), as previously described.[15] Clinical attachment loss (CAL) was determined by measuring the distance from the cementoenamel junction (CEJ) to the base of the pocket or sulcus.

Systemic parameters

Glycated hemoglobin, Hb, CBC, hs-CRP, creatinine, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, RBS, FPG, fasting insulin, and IR were recorded as explained in previous studies.[31,32]

Quantification of PRF/HAS

An analytical balance (Wensar; Chennai, India) with a readability of 0.0001 g was used to measure the weight of the collected PRF clot. The prepared HAS obtained

Table 1. Comparison of the analyzed variables among the various study groups (ANOVA)

Variable	T2DM+/P+	T2DM–/P+	T2DM–/P–	*F*-value	*p*-value
Age [years]	46.53 ±4.62	45.76 ±2.88	45.50 ±3.74	0.33	0.723
BMI [kg/m^2]	25.81 ±2.44	24.14 ±2.44	23.36 ±1.59	5.33	0.008*
SBP [mmHg]	134.00 ±13.50	125.18 ±11.13	125.50 ±4.65	3.80	0.030*
DBP [mmHg]	84.82 ±8.56	81.47 ±5.89	81.94 ±4.33	1.30	0.281
Pulse rate [beats/min]	81.29 ±10.46	79.59 ±5.03	77.94 ±7.63	0.72	0.492
Body temperature [°C]	36.83 ±0.36	36.92 ±0.28	36.95 ±0.15	0.86	0.430
Hb [g/dL]	13.48 ±1.78	12.89 ±1.95	13.68 ±1.56	0.89	0.418
HbA1c [%]	8.15 ±1.53	5.79 ±0.40	5.62 ±0.30	38.06	<0.001*
TLC [cells/mm^3]	7,270.59 ±2,351,80	6,696.47 ±1,187.31	6,812.50 ±1,428.69	0.52	0.599
Neutrophils [×10^3/μL]	4,488.35 ±1,862.09	3,865.88 ±1,167.80	4,220.25 ±1,052.89	0.83	0.443
Lymphocytes [×10^3/μL]	2,248.35 ±699.25	1,977.99 ±648.75	2,108.44 ±554.98	0.76	0.473
Eosinophils [×10^3/μL]	363.88 ±333.71	296.24 ±178.18	320.94 ±222.58	0.31	0.736
Monocytes [×10^3/μL]	170.06 ±128.14	192.29 ±95.47	148.75 ±83.26	0.72	0.493
Basophils [×10^3/μL]	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	NA	NA
RBC count [million cells/mm^3]	4.72 ±0.57	4.48 ±0.50	4.74 ±0.51	1.30	0.283
ESR [mm/h]	25.00 ±28.90	25.76 ±23.81	19.29 ±22.17	0.32	0.726
PLT [×10^5/mm^3]	1.94 ±0.87	2.19 ±0.85	1.87 ±0.69	0.72	0.490
PCV [%]	41.69 ±4.70	36.22 ±10.37	38.16 ±10.63	1.63	0.207
hs-CRP [mg/L]	11.18 ±23.67	3.05 ±2.97	2.33 ±2.73	2.07	0.138
Creatinine [mg/dl]	0.85 ±0.24	4.97 ±17.03	0.90 ±0.20	0.96	0.392
Total cholesterol [mg/dL]	214.24 ±34.48	206.65 ±47.30	215.50 ±41.17	0.22	0.799
HDL [mg/dL]	48.28 ±9.72	63.61 ±51.10	48.23 ±10.84	1.38	0.261
LDL [mg/dL]	115.29 ±26.61	115.43 ±35.86	130.13 ±28.60	1.26	0.293
VLDL [mg/dL]	62.00 ±63.23	43.74 ±20.13	37.43 ±13.90	1.74	0.187
Triglycerides [mg/dL]	310.00 ±316.14	208.82 ±108.48	182.56 ±65.70	1.92	0.158
RBS [mg/dL]	189.60 ±50.10	129.74 ±23.56	123.26 ±18.31	19.51	<0.001*
FPG [mg/dL]	154.48 ±28.69	109.65 ±10.37	108.28 ±10.49	33.02	<0.001*
Fasting insulin [μIU/mL]	17.86 ±16.34	20.18 ±18.42	9.20 ±5.06	2.55	0.089

Table 1 – continuation

Variable	T2DM+/P+	T2DM–/P+	T2DM–/P–	*F*-value	*p*-value
IR	6.75 ±5.73	5.60 ±5.28	2.48 ±1.39	3.77	0.030*
Number of teeth present	26.65 ±1.06	28.47 ±1.74	29.81 ±0.83	25.64	<0.001*
GI	2.51 ±0.21	2.13 ±0.30	1.04 ±0.30	125.80	<0.001*
PI	2.64 ±0.38	2.24 ±0.24	1.15 ±0.37	86.82	<0.001*
PPD [mm]	4.82 ±0.83	4.32 ±0.43	2.02 ±0.26	114.92	<0.001*
CAL [mm]	3.72 ±0.41	3.02 ±0.38	0.45 ±0.10	440.89	<0.001*
PRF clot weight [g]	2.16 ±0.73	2.84 ±0.83	3.20 ±0.78	7.68	0.001*
PRF preparation time [min]	15.65 ±2.15	14.00 ±0.00	14.00 ±0.00	9.68	<0.001*
HAS [mL]	1.57 ±0.56	1.82 ±0.45	1.79 ±0.37	1.42	0.252

Data presented as mean ± standard deviation (*M* ±*SD*).
Groups: group 1 (T2DM+/P+); group 2 (T2DM–/P+); group 3 – control (T2DM–/P–). T2DM – type 2 diabetes mellitus; P – periodontitis.
BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; TLC – total leukocyte count; RBC – red blood cell; ESR – erythrocyte sedimentation rate; PLT – platelet count; PCV – packed cell volume; HDL – high-density lipoprotein; LDL – low-density lipoprotein; VLDL – very low-density lipoprotein; GI – gingival index; PI – plaque index; PPD – probing pocket depth; CAL – clinical attachment loss; NA – data not available; * statistically significant.

from the fibrin clot was measured using a micropipette and subsequently stored at –80°C in microcentrifuge tubes for further analysis.

The quantification of PRF/HAS is highly sensitive; therefore, meticulous care was taken to standardize the sampling conditions, including the participants' demographics, blood collection and centrifugation procedures.

Sample preparation for the evaluation of markers

The samples stored at –80°C were thawed, homogenized and centrifuged at 3,000 rpm for 10 min at room temperature. The resulting supernatants were aliquoted and stored again at –80°C until analysis. Specific biomarkers were quantified using appropriate enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions (Elabscience Biotechnology Inc., Houston, USA). All standards and samples were assayed in duplicate for each marker, and mean values were calculated. The results were adjusted by applying the appropriate dilution factor. All biomarker analyses and data interpretation were performed by trained investigators who were blinded to group allocation, using the Origin® software (OriginLab Corporation, Northampton, USA).

Inflammatory markers and growth factors (ELISA kit)

Ninety-six-well ELISA kits were used to quantify the following human biomarkers: IL-6; RETN; TNF-α; IL-1β; IL-1ra; IGF-1; TGF-β1; PDGF-BB; and FGF-21.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 26.0 (IBM Corp., Armonk, USA). The data was analyzed using descriptive statistics, with comparisons made among study groups. Categorical variables were summarized as frequencies and percentages (*n* (%)), while quantitative data was expressed as mean ± standard deviation (*M* ±*SD*). A *p*-value <0.05 was considered statistically significant.

Intergroup differences were assessed using the one-way analysis of variance (ANOVA). When a significant difference was detected, Tukey's post hoc test was applied for pairwise comparisons between the group means. Comparisons between two groups were performed using the unpaired *t* test. Correlation analysis was conducted to evaluate the strength and direction of associations, whether causal or not, between two random variables or bivariate data.

Results

The mean age of individuals in group 1 (T2DM+/P+), group 2 (T2DM–/P+) and group 3 (T2DM–/P–) was 46.53 ±4.62 years, 45.76 ±2.88 years and 45.50 ±3.74 years, respectively. No statistically significant differences were observed in age or gender distribution among the 3 groups. The BMI of individuals in group 1 was statistically significantly higher, with a mean value of 25.81 ±2.44 kg/m^2, as compared to 24.14 ±2.44 kg/m^2 and 23.36 ±1.59 kg/m^2 in groups 2 and 3, respectively. No statistically significant differences were observed among the groups with respect to the pulse rate, body temperature or diastolic blood pressure (DBP). However, statistically significant variations in BMI and systolic blood pressure (SBP) may be associated with T2DM and periodontitis (i.e., group 1). No statistically significant difference in the mean BMI values was observed between group 2 and group 3.

The intergroup comparisons of demographic, vital, biochemical/hematological, lipid profile, glycemic, and periodontal parameters, as well as the quantity of PRF and HAS, and the time required for PRF preparation among the study groups, are summarized in Table 1. The analysis indicated significant differences among the groups (p = 0.001), with group 3 demonstrating the highest PRF clot weight, followed by group 2 and group 1. A similar trend was observed for HAS; however, the difference was not statistically significant. The time required for the procedures differed significantly among the groups (p < 0.001). Notably, the average time required for PRF preparation was 14 min in groups 2 and 3, as compared to a mean of 15.65 ±2.15 min in group 1.

Although diabetic patients could not be stratified into controlled, moderately controlled and poorly controlled categories due to the small sample size, convenience sampling allowed further subdivision of the T2DM+/P+ group into 2 subgroups based on the HbA1c level: <8% (9 participants); and ≥8% (8 participants). The average time required for PRF preparation in periodontitis patients with uncontrolled diabetes (HbA1c ≥ 8%) was 16.00 ±2.14 min. The comparisons of the mean values of demographic, vital, biochemical/hematological, lipid profile, glycemic, and periodontal parameters, as well as the quantity of PRF and HAS, and the time required for PRF preparation between the subgroups (HbA1c < 8% and HbA1c ≥ 8%) within group 1 (T2DM+/P+), are summarized in Table 2.

The post hoc Tukey's test results, used to identify specific pairwise differences between the group means for the parameters showing significant differences in ANOVA, are summarized in Table 3.

Table 2. Comparison of the analyzed variables between the subgroups within group 1 (T2DM+/P+) (HbA1c < 8% or HbA1c ≥ 8%) (unpaired *t* test)

Variable	HbA1c < 8%	HbA1c ≥ 8%	*t*-value	*p*-value
Age [years]	47.78 ±5.61	45.13 ±2.95	1.20	0.250
BMI [kg/m^2]	25.77 ±2.93	25.85 ±1.96	–0.07	0.947
SBP [mmHg]	129.89 ±7.94	138.63 ±17.26	–1.37	0.191
DBP [mmHg]	81.67 ±5.79	88.38 ±10.10	–1.71	0.109
Pulse rate [beats/min]	82.67 ±11.22	79.75 ±10.05	0.56	0.583
Body temperature [°C]	36.84 ±0.37	36.81 ±0.38	0.18	0.863
Hb [g/dL]	12.43 ±1.26	14.66 ±1.56	–3.26	0.005*
TLC [cells/mm^3]	6,933.33 ±2,424.87	7,650.00 ±2,368.85	–0.61	0.548
Neutrophils [×10^3/μL]	4,205.33 ±2,260.72	4,806.75 ±1,365.88	–0.65	0.524
Lymphocytes [×10^3/μL]	2,193.00 ±508.17	2,310.63 ±902.28	–0.34	0.741
Eosinophils [×10^3/μL]	407.22 ±418.14	315.13 ±222.68	0.56	0.587
Monocytes [×10^3/μL]	127.89 ±79.16	217.50 ±159.71	–1.49	0.156
Basophils [×10^3/μL]	0.00 ±0.00	0.00 ±0.00	NA	NA
RBC count [million cells/mm^3]	4.39 ±0.41	5.08 ±0.52	–3.03	0.009*
ESR [mm/h]	24.33 ±25.44	25.75 ±34.18	–0.10	0.923
PLT [×10^5/mm^3]	2.22 ±0.94	1.63 ±0.71	1.43	0.172
PCV [%]	38.97 ±3.50	44.76 ±4.03	–3.17	0.006*
hs-CRP [mg/L]	16.68 ±31.75	4.99 ±6.74	1.02	0.325
Creatinine [mg/dl]	0.77 ±0.11	0.94 ±0.31	–1.51	0.151
Total cholesterol [mg/dL]	215.78 ±23.60	212.50 ±45.53	0.19	0.852
HDL [mg/dL]	49.94 ±7.77	46.40 ±11.81	0.74	0.471
LDL [mg/dL]	113.89 ±27.86	116.88 ±26.95	–0.22	0.826
VLDL [mg/dL]	51.69 ±17.48	73.60 ±92.19	–0.70	0.494
Triglycerides [mg/dL]	258.44 ±87.38	368.00 ±460.94	–0.70	0.494
RBS [mg/dL]	152.73 ±13.43	231.07 ±42.65	–5.24	<0.001*
FPG [mg/dL]	145.01 ±16.75	165.14 ±36.27	–1.50	0.155
Fasting insulin [μIU/mL]	22.12 ±19.68	13.06 ±10.86	1.15	0.267
IR	7.92 ±6.72	5.44 ±4.43	0.89	0.389
Number of teeth present	26.70 ±1.30	26.57 ±0.76	0.53	0.605
GI	2.53 ±0.14	2.50 ±0.28	0.28	0.786
PI	2.73 ±0.34	2.55 ±0.43	0.96	0.351
PPD [mm]	4.62 ±0.85	5.05 ±0.79	–1.07	0.301
CAL [mm]	3.59 ±0.20	3.86 ±0.54	–1.42	0.177
PRF clot weight [g]	2.23 ±0.68	2.07 ±0.82	0.43	0.676
PRF preparation time [min]	15.33 ±2.24	16.00 ±2.14	–0.63	0.541
HAS [mL]	1.78 ±0.66	1.34 ±0.32	1.71	0.109

Data presented as *M* ±*SD*.
Groups: group 1 (T2DM+/P+); group 2 (T2DM–/P+); group 3 – control (T2DM–/P–). T2DM – type 2 diabetes mellitus; P – periodontitis.
NA – data not available; * statistically significant.

Table 3. Pairwise comparisons between the group means for the parameters with significant differences in ANOVA (Tukey's post hoc test)

Variable	Comparison	*MD*	*SE*	*p*-value
BMI [kg/m^2]	group 1 vs. group 2	1.67	0.76	0.081
	group 1 vs. group 3	2.45	0.77	0.007*
	group 2 vs. group 3	0.78	0.77	0.572
SBP [mmHg]	group 1 vs. group 2	8.82	3.61	0.048*
	group 1 vs. group 3	8.50	3.67	0.063
	group 2 vs. group 3	–0.32	3.67	0.996
HbA1c [%]	group 1 vs. group 2	2.36	0.32	<0.001*
	group 1 vs. group 3	2.53	0.33	<0.001*
	group 2 vs. group 3	0.17	0.33	0.854
RBS [mg/dL]	group 1 vs. group 2	59.86	11.63	<0.001*
	group 1 vs. group 3	66.34	11.81	<0.001*
	group 2 vs. group 3	6.48	11.81	0.848
FPG [mg/dL]	group 1 vs. group 2	44.83	6.43	<0.001*
	group 1 vs. group 3	46.20	6.53	<0.001*
	group 2 vs. group 3	1.37	6.53	0.976
IR	group 1 vs. group 2	1.15	1.58	0.748
	group 1 vs. group 3	4.27	1.61	0.028*
	group 2 vs. group 3	3.12	1.61	0.138
GI	group 1 vs. group 2	0.38	0.09	0.001*
	group 1 vs. group 3	1.47	0.10	<0.001*
	group 2 vs. group 3	1.09	0,10	<0.001*
PI	group 1 vs. group 2	0.40	0.11	0.003*
	group 1 vs. group 3	1.49	0.12	<0.001*
	group 2 vs. group 3	1.09	0.12	<0.001*
PPD [mm]	group 1 vs. group 2	0.50	0.19	0.032*
	group 1 vs. group 3	2.80	0.20	<0.001*
	group 2 vs. group 3	2.30	0.20	<0.001*
CAL [mm]	group 1 vs. group 2	0.70	0.11	<0.001*
	group 1 vs. group 3	3.27	0.12	<0.001*
	group 2 vs. group 3	2.57	0.12	<0.001*
PRF clot weight [g]	group 1 vs. group 2	–0.68	0.27	0.038*
	group 1 vs. group 3	–1.04	0.27	0.001*
	group 2 vs. group 3	–0.36	0.27	0.371
PRF preparation time [min]	group 1 vs. group 2	1.65	0.43	0.001*
	group 1 vs. group 3	1.65	0.44	0.001*
	group 2 vs. group 3	0.00	0.44	1.000

Groups: group 1 (T2DM+/P+); group 2 (T2DM–/P+); group 3 – control (T2DM–/P–). T2DM – type 2 diabetes mellitus; P – periodontitis. *MD* – mean difference; *SE* – standard error; * statistically significant.

The intergroup comparisons of the concentrations of inflammatory markers (human IL-6, RETN, TNF-α, IL-1β, and IL-1ra) and growth factors (human IGF-1, TGF-β1, PDGF-BB, and FGF-21) in serum, PRF and HAS in periodontitis patients with or without T2DM, as well as in healthy participants, are summarized in Table 4.

The correlations of the weight of PRF and HAS with periodontal and glycemic parameters, as well as the correlations of periodontal parameters with glycemic parameters, are summarized in Tables 5 and 6, respectively. The results suggest that PRF exhibited a strong negative correlation with GI, PI, PPD, CAL, HbA1c, RBS, and FPG, indicating that as these parameters increased, the weights of PRF and HAS decreased.

Discussion

The present observational study evaluated variations in the levels of cytokines in the PRF and HAS obtained from periodontitis patients with or without T2DM, in comparison with healthy individuals (no periodontitis and no T2DM). Primarily, it was observed that periodontitis, with or without T2DM, may influence the concentrations of the inflammatory markers and growth factors released from platelet concentrates. Secondarily, it was also observed that the T2DM+/P+ status may influence the weight of platelet concentrates.

Although the influence of centrifuge characteristics has already been studied in detail,[33,34] to the best of the authors' knowledge, this is the first study to evaluate both the quantity and quality of PRF and HAS in terms of 5 inflammatory markers and 4 growth factors, while also correlating the weight of platelet concentrates with periodontal and glycemic parameters in quadragenarian (40–50-year-old) individuals.

The present study showed no significant differences in the TNF-α, IL-1Ra, IGF-1, and FGF-21 levels in the serum, PRF and HAS obtained from the participants across the 3 groups. Consistent with previous reports,[15,35,36] the TGF-β level was significantly higher in the healthy T2DM–/P– group, whereas the inflammatory marker IL-1β was elevated in the diseased groups 1 and 2, as compared to healthy individuals, across serum, PRF and HAS. Gupta et al. reported a significant impact of periodontitis and diabetes on 2 growth factors – TGF-β1 and PDGF-BB – and on the three-dimensional (3D) structure of PRF.[15] Unlike Gupta et al., where the study population was stratified by age groups,[15] the present study was conducted in a narrowly defined cohort to minimize the potential influence of diabetes-associated complications and other comorbidities on the outcomes.

Singh et al. reported that the mean age of patients with T2DM presenting to a tertiary care center in North India was 49.03 ±0.49 years, with most patients falling within the 40–50-year age group.[37] In line with previous reports,[38–40] the present study also observed a mean age of 44–46 years for early-onset T2DM.

Both autologous PRF and HAS are considered to possess biological healing properties owing to their cytokine content. Similar to Narayan and Malaiappan,[41] the present study also quantitatively analyzed PRF and HAS in T2DM+/P+ (group 1), T2DM–/P+ (group 2) and T2DM–/P– (group 3), and observed a significantly

Table 4. Intergroup comparison of the concentrations inflammatory markers and growth factors (in picograms [pg]) in serum, platelet-rich fibrin (PRF) and hyperacute serum (HAS) from chronic periodontitis patients with or without type 2 diabetes mellitus (T2DM) (ANOVA)

Inflammatory markers and growth factors		T2DM+/P+	T2DM–/P+	T2DM–/P–	*F*-value	*p*-value
IL-6	serum	34.62 ±34.54	46.94 ±78.57	52.25 ±87.55	0.15	0.864
	PRF	25.76 ±29.00	15.30 ±2.28	14.29 ±2.52	1.28	0.297
	HAS	26.14 ±4.02	22.83 ±4.26	20.54 ±4.25	4.08	0.030*
RETN	serum	380.21 ±121.80	347.81 ±125.07	282.32 ±106.97	1.60	0.222
	PRF	369.50 ±172.66	2,259.40 ±2,249.21	1,375.47 ±858.04	4.14	0.028*
	HAS	1,027.47 ±909.93	538.19 ±383.81	2,545.60 ±2,327.43	4.63	0.020*
TNF-α	serum	51.07 ±5.09	46.98 ±5.83	51.14 ±8.23	1.20	0.319
	PRF	44.89 ±6.67	42.39 ±7.13	38.48 ±4.75	2.38	0.114
	HAS	48.92 ±7.24	46.34 ±2.52	49.77 ±7.78	0.72	0.496
IL-1β	serum	24.14 ±4.56	19.77 ±3.11	24.07 ±11.64	1.02	0.377
	PRF	21.89 ±3.66	24.01 ±4.07	18.97 ±3.21	4.28	0.026*
	HAS	26.11 ±5.83	26.06 ±7.15	20.48 ±4.79	2.62	0.094
IL-1ra	serum	276.74 ±82.09	233.31 ±64.82	247.05 ±110.32	0.58	0.570
	PRF	140.15 ±33.93	138.51 ±32.02	120.63 ±26.90	1.09	0.352
	HAS	298.75 ±100.21	256.20 ±54.79	280.83 ±85.57	0.61	0.554
IGF-1	serum	13.07 ±5.06	10.42 ±3.33	12.25 ±3.41	1.03	0.373
	PRF	7.38 ±0.69	7.37 ±1.44	6.76 ±1.04	0.95	0.401
	HAS	12.26 ±3.31	11.37 ±3.19	14.17 ±7.81	0.67	0.520
TGF-β1	serum	24.77 ±20.13	27.05 ±22.17	27.38 ±5.00	0.06	0.943
	PRF	60.86 ±40.86	56.63 ±5.87	149.87 ±68.30	11.75	<0.001*
	HAS	13.20 ±5.26	13.85 ±6.46	16.44 ±4.63	0.88	0.429
PDGF-BB	serum	254.45 ±57.65	4,257.06 ±1,054.11	3,781.45 ±1,160.10	52.47	<0.001*
	PRF	2,438.93 ±1,309.21	3,771.52 ±1,094.54	3,580.20 ±999.92	3.58	0.043*
	HAS	2,437.03 ±973.85	4,705.28 ±1,291.78	4,143.75 ±1,292.39	8.79	0.001*
FGF-21	serum	760.18 ±87.72	867.73 ±275.68	802.94 ±277. 62	0.49	0.617
	PRF	1,004.26 ±632.31	1,635.06 ±514.82	1,248.07 ±606.24	2.65	0.091
	HAS	588.36 ±189.25	641.21 ±140.40	754.73 ±499.35	0.64	0.536

Data presented as *M* ±*SD*.
Groups: group 1 (T2DM+/P+); group 2 (T2DM–/P+); group 3 – control (T2DM–/P–). T2DM – type 2 diabetes mellitus; P – periodontitis.
IL – interleukin; RETN – resistin; TNF-α – tumor necrosis factor-alpha; IL-1ra – interleukin-1 receptor antagonist; IGF-1 – insulin-like growth factor-1; TGF-β1 – transforming growth factor-beta 1; PDGF-BB – platelet-derived growth factor-BB; FGF-21 – fibroblast growth factor-21; * statistically significant.

Table 5. Correlation between the weight of platelet-rich fibrin (PRF) and hyperacute serum (HAS) and periodontal and glycemic parameters

Correlation		*r*-value	*p*-value
PRF	GI	–0.454	0.001*
	PI	–0.440	0.001*
	PPD	–0.427	0.002*
	CAL	–0.453	0.001*
	HbA1c	–0.407	0.003*
	RBS	–0.398	0.004*
	FPG	–0.464	0.001*
	fasting insulin	–0.066	0.646
HAS	GI	–0.166	0.249
	PI	–0.118	0.415
	PPD	–0.185	0.199
	CAL	–0.176	0.221
	HbA1c	–0.270	0.058
	RBS	–0.367	0.009*
	FPG	–0.230	0.109
	fasting insulin	0.188	0.190

* statistically significant.

Table 6. Correlation between periodontal parameters and glycemic parameters

Glycemic parameters	Correlation indicators	Periodontal parameters			
		GI	PI	PPD	CAL
HbA1c	*r*-value	0.543	0.515	0.580	0.584
	p-value	0.000*	0.000*	0.000*	0.000*
RBS	*r*-value	0.461	0.445	0.513	0.521
	p-value	0.001*	0.001*	0.000*	0.000*
FPG	*r*-value	0.475	0.476	0.479	0.507
	p-value	0.000*	0.000*	0.000*	0.000*
Fasting insulin	*r*-value	0.323	0.357	0.259	0.278
	p-value	0.022*	0.011*	0.070	0.051
IR	*r*-value	0.387	0.429	0.341	0.354
	p-value	0.006*	0.002*	0.015*	0.012*

* statistically significant.

higher PRF weight in healthy individuals as compared to T2DM+/P+ and T2DM−/P+ patients. Based on these findings, it can be hypothesized that chronic inflammatory conditions may influence the quantity of PRF and HAS. These results are consistent with previous studies suggesting alterations in PRF constituents in T2DM+/P+ patients.[15,32,42]

In contrast to Das and Janardhana Amaranath, who reported higher leukocyte and platelet counts and a larger size of advanced PRF (A-PRF) in uncontrolled T2DM female subjects as compared to healthy individuals and tobacco smokers,[42] the findings of the present study are in agreement with those of Gupta et al.[15]

Another important finding of the study was the longer duration (14 min) required for the procurement of PRF, as compared to the standard established protocol of 10–12 min. This may be attributed to the use of presterilized 9-mm plastic vacutainers for PRF preparation in the present study. Thus, the findings are in agreement with previous studies suggesting that the time required to prepare PRF in plastic tubes is considerably longer than that in glass tubes or glass-coated plastic tubes.[43–45] More interestingly, another notable observation was the longer time required for PRF procurement in the T2DM+/P+ group patients as compared to non-diabetic patients – with or without periodontitis. This suggests a potential impact of systemic inflammatory conditions in T2DM+/P+ (group 1) patients.[15,46]

The present study comprehensively explains the relationship between T2DM, periodontitis, and wound healing potential. It also highlights several important clinical implications. Significantly lower mean PRF weight and reduced mean levels of growth factors (TGF-β1 and PDGF-BB) in serum, PRF, and HAS in diabetic patients support the impaired or delayed wound healing commonly observed in T2DM, which may be further exacerbated by the presence of periodontitis. Thus, the determination of growth factors in PRF and HAS may be particularly beneficial in the management of diabetic patients with periodontal disease using PRF, and may provide insights into future therapeutic approaches. Baran et al., in an animal study, reported an enhanced regenerative capacity with photomodulation,[47] which could be further explored in the context of the PRF obtained from T2DM+/P+ patients.

One limitation of the study was the small number of participants. The extensive experimental requirements, including the analysis of 9 markers, limited the inclusion of a larger sample size. Another limitation was the lack of inclusion of T2DM patients without periodontitis, which would have provided a more robust comparison to better understand the interplay between T2DM and periodontitis. However, all participants were within the age group of 40 to 50 years and were recruited from the Department of Periodontology, where patients primarily report for the management of periodontal diseases. Therefore, a group of T2DM patients without periodontitis could not be included. Previous studies have reported that the prevalence of periodontitis in established T2DM patients exceeds 90% in the North Indian population.[37] Furthermore, the referral of T2DM patients without periodontitis for oral health evaluation remains uncommon in the Indian subcontinent, possibly due to a lack of collaborative practice between physicians and other healthcare professionals.[48]

Conclusions

Within the limitation of the narrow age selection of participants, which may have underestimated the inflammatory and growth factor levels, it can be surmised from the present study that periodontitis along with T2DM may have detrimental effects on the regenerative potential of PRF and HAS. The present study demonstrated significantly lower mean levels of PDGF and TGF-β1 in the PRF obtained from the participants in the T2DM+/P+ group as compared to the T2DM−/P+ and T2DM−/P− (control) groups ($p = 0.043$ and $p < 0.001$, respectively). Furthermore, a statistically significantly higher mean PRF weight was observed in the T2DM−/P− group (3.20 ±0.78 g), followed by the T2DM+/P+ group (2.16 ±0.73 g), and the T2DM−/P+ group (2.84 ±0.83 g) with the lowest value. These preliminary findings may serve as a basis for further multicenter studies with larger sample sizes.

Ethics approval and consent to participate

The study protocol (#FV1PE21122020R) was approved by the Institutional Research and Development Committee (IRDC) and the Institutional Human Ethics Committee (IHEC) at Saraswati Dental College and Hospital, Lucknow, India. Written informed consent was obtained from all the participants.

Data availability

All the data is included in the manuscript. Any further information may be obtained from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

The authors confirm that they used artificial intelligence (AI)-assisted technology solely for language refinement and to improve the clarity of writing. No AI assistance was employed in the generation of scientific content, data analysis or interpretation.

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References

1. Chen TL, Xu EL, Lu HJ, et al. The influence of diabetes enhanced inflammation on cell apoptosis and periodontitis. *Adv Biosci Biotechnol*. 2012;3(6A):712–719. doi:10.4236/abb.2012.326092
2. Basith Khan MA, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes – Global Burden of Disease and forecasted trends. *J Epidemiol Glob Health*. 2020;10(1):107–111. doi:10.2991/jegh.k.191028.001
3. Goyal R, Singhal M, Jialal I. *Type 2 Diabetes*. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2023. PMID:30020625.
4. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. *Australas Med J*. 2014;7(1):45–48. doi:10.4066/AMJ.2013.1979
5. Wu YY, Xiao E, Graves DT. Diabetes mellitus related bone metabolism and periodontal disease. *Int J Oral Sci*. 2015;7(2):63–72. doi:10.1038/ijos.2015.2
6. Maiti S, Akhtar S, Upadhyay AK, Mohanty SK. Socioeconomic inequality in awareness, treatment and control of diabetes among adults in India: Evidence from National Family Health Survey of India (NFHS), 2019–2021. *Sci Rep*. 2023;13(1):2971. doi:10.1038/s41598-023-29978-y
7. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol 2000*. 2014;64(1):57–80. doi:10.1111/prd.12002
8. Bains VK, Mahendra J, Mahendra L, Mittal M, Valli G. Markers, pathways, and current evidence for periodontitis-associated insulin resistance: A narrative review. *J Int Soc Prev Community Dent*. 2022;12(5):475–487. doi:10.4103/jispcd.JISPCD_92_22
9. Sanz M, Ceriello A, Buysschaert M, et al. Scientific evidence on the links between periodontal diseases and diabetes: Consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International Diabetes Federation and the European Federation of Periodontology. *Diabetes Res Clin Pract*. 2018;137:231–241. doi:10.1016/j.diabres.2017.12.001
10. Kartika RW, Alwi I, Suyatna FD, et al. The role of VEGF, PDGF and IL-6 on diabetic foot ulcer after platelet rich fibrin + hyaluronic therapy. *Heliyon*. 2021;7(9):e07934. doi:10.1016/j.heliyon.2021.e07934
11. Egierska D, Perszke M, Mazur M, Duś-Ilnicka I. Platelet-rich plasma and platelet-rich fibrin in oral surgery: A narrative review. *Dent Med Probl*. 2023;60(1):177–186. doi:10.17219/dmp/147298
12. Gunjiganur Vemanaradhya G, Rahman S, Adi DM, Machetty L. Impact of glutaraldehyde cross-linking on the advanced platelet-rich fibrin membrane: Macroscopic, conventional light microscopic and scanning electron microscopic analysis. *Dent Med Probl*. 2023;60(1):79–86. doi:10.17219/dmp/146271
13. Kardos D, Simon M, Vácz G, et al. The composition of hyperacute serum and platelet-rich plasma is markedly different despite the similar production method. *Int J Mol Sci*. 2019;20(3):721. doi:10.3390/ijms20030721
14. Agrawal AA. Evolution, current status and advances in application of platelet concentrate in periodontics and implantology. *World J Clin Cases*. 2017;5(5):159–171. doi:10.12998/wjcc.v5.i5.159
15. Gupta S, Jain A, Gupta M, et al. Influence of periodontitis and diabetes on structure and cytokine content of platelet-rich fibrin. *Oral Dis*. 2023;29(8):3620–3629. doi:10.1111/odi.14275
16. Pavlovic V, Ciric M, Jovanovic V, Trandafilovic M, Stojanovic P. Platelet-rich fibrin: Basics of biological actions and protocol modifications. *Open Med (Wars)*. 2021;16(1):446–454. doi:10.1515/med-2021-0259
17. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. In vitro release of growth factors from platelet-rich fibrin (PRF): A proposal to optimize the clinical applications of PRF. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108(1):56–61. doi:10.1016/j.tripleo.2009.02.004
18. Nair MR, Thomas R, Shah R, Gowda I, Gowda TM. Comparison of the angiogenic efficacy of conventional leukocyte- and platelet-rich fibrin versus low-speed advanced platelet-rich fibrin: An in vitro chorioallantoic membrane assay study. *Dent Med Probl*. 2024;61(1):77–83. doi:10.17219/dmp/150655
19. Bhattacharya HS, Gummaluri SS, Rani A, Verma S, Bhattacharya P, Rayashettypura Gurushanth SM. Additional benefits of titanium platelet-rich fibrin (T-PRF) with a coronally advanced flap (CAF) for recession coverage: A case series. *Dent Med Probl*. 2023;60(2):279-285. doi:10.17219/dmp/141919
20. Devanoorkar A, Kathariya R, Guttiganur N, Gopalakrishnan D, Bagchi P. Resistin: A potential biomarker for periodontitis influenced diabetes mellitus and diabetes induced periodontitis. *Dis Markers*. 2014;2014:930206. doi:10.1155/2014/930206
21. Kamil MA, Peeran SW, Basheer SN, Elhassan A, Alam MN, Thiruneervannan M. Role of resistin in various diseases with special emphasis on periodontal and periapical inflammation – a review. *J Pharm Bioallied Sci*. 2023;15(Suppl 1):S31–S35. doi:10.4103/jpbs.jpbs_655_22
22. Joshi A, Maddipati S, Chatterjee A, Lihala R, Gupta A. Gingival crevicular fluid resistin levels in chronic periodontitis with type 2 diabetes before and after non-surgical periodontal therapy: A clinico-biochemical study. *Indian J Dent Res*. 2019;30(1):47–51. doi:10.4103/ijdr.IJDR_215_17
23. Patel SP, Raju PA. Resistin in serum and gingival crevicular fluid as a marker of periodontal inflammation and its correlation with single-nucleotide polymorphism in human resistin gene at -420. *Contemp Clin Dent*. 2013;4(2):192–197. doi:10.4103/0976-237X.114878
24. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;45(Suppl 20):S162–S170. doi:10.1111/jcpe.12946
25. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(Suppl 1):S159–S172. doi:10.1002/JPER.18-0006
26. Chapple IL, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;45(Suppl 20):S68–S77. doi:10.1111/jcpe.12940
27. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes – 2021. *Diabetes Care*. 2021;44(Suppl 1):S15–S33. doi:10.2337/dc21-S002
28. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e56–e60. doi:10.1016/j.tripleo.2005.07.011
29. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551. doi:10.3109/00016356309011240
30. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121–135. doi:10.3109/00016356408993968
31. Mohan M, Jhingran R, Bains VK, et al. Impact of scaling and root planing on C-reactive protein levels in gingival crevicular fluid and serum in chronic periodontitis patients with or without diabetes mellitus. *J Periodontal Implant Sci*. 2014;44(4):158–168. doi:10.5051/jpis.2014.44.4.158
32. Bains VK, Mahendra J, Mahendra L, Mittal M, Gunam V. Quantitative association of platelet-rich fibrin (PRF) and hyperacute serum (HAS) with glycemic control (HbA1c) in chronic periodontitis patients: An ex vivo study. *J Pharm Bioallied Sci*. 2023;15(Suppl 1):S601–S607. doi:10.4103/jpbs.jpbs_428_22
33. Dohan Ehrenfest DM, Pinto NR, Pereda A, et al. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. *Platelets*. 2018;29(2):171–184. doi:10.1080/09537104.2017.1293812

34. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response. *J Periodontol*. 2017;88(1):112–121. doi:10.1902/jop.2016.160443
35. Aleidi SM, Shayeb E, Bzour J, et al. Serum level of insulin-like growth factor-I in type 2 diabetic patients: Impact of obesity. *Horm Mol Biol Clin Investig*. 2019;39(1). doi:10.1515/hmbci-2019-0015
36. Gao RY, Hsu BG, Wu DA, Hou JS, Chen MC. Serum fibroblast growth factor 21 levels are positively associated with metabolic syndrome in patients with type 2 diabetes. *Int J Endocrinol*. 2019;2019:5163245. doi:10.1155/2019/5163245
37. Singh M, Bains VK, Jhingran R, et al. Prevalence of periodontal disease in type 2 diabetes mellitus patients: A cross-sectional study. *Contemp Clin Dent*. 2019;10(2):349–357. doi:10.4103/ccd.ccd_652_18
38. Ummadisetty T, Chava VK, Reddy Bhumanapalli VR. Diabetes and periodontitis: How well are the patients aware about an established relation? *J Indian Soc Periodontol*. 2016;20(4):472–475. doi:10.4103/0972-124X.184035
39. Yao X, Zhang J, Zhang X, et al. Age at diagnosis, diabetes duration and the risk of cardiovascular disease in patients with diabetes mellitus: A cross-sectional study. *Front Endocrinol (Lausanne)*. 2023;14:1131395. doi:10.3389/fendo.2023.1131395
40. Alahmari MM, AlShaiban HM, Mahmood SE. Prevalence and associated factors for periodontal disease among type I and II diabetes mellitus patients: A cross-sectional study. *Healthcare (Basel)*. 2023;11(6):796. doi:10.3390/healthcare11060796
41. Narayan S, Malaiappan S. In vitro quantitative comparison of platelet-rich fibrin and plasma rich in growth factor with age, gender, blood parameters. *J Evol Med Dent Sci*. 2020;9(36):2620–2624. doi:10.14260/jemds/2020/570
42. Das N, Janardhana Amaranath BJ. Quantitative evaluation of modified advanced platelet-rich fibrin buffy coat among diabetic patients and tobacco smokers with chronic periodontitis. *J Indian Soc Periodontol*. 2022;26(1):24–31. doi:10.4103/jisp.jisp_498_20
43. Jianpeampoolpol B, Phuminart S, Subbalekha K. Platelet-rich fibrin formation was delayed in plastic tubes. *J Adv Med Med Res*. 2016;14(9):1–9. doi:10.9734/BJMMR/2016/25169
44. Miron RJ, Pinto NR, Quirynen M, Ghanaati S. Standardization of relative centrifugal forces in studies related to platelet-rich fibrin. *J Periodontol*. 2019;90(8):817–820. doi:10.1002/JPER.18-0553
45. Miron R, Choukroun J, Ghanaati S. Controversies related to scientific report describing g-forces from studies on platelet-rich fibrin: necessity for standardization of relative centrifugal force values. *Int J Growth Factors Stem Cells Dent*. 2018;1(3):80.
46. Abooj J, Varma S, Suragimath G, Zope SA, Pisal A. Modification to the centrifugation protocol increases the competence of platelet-rich fibrin in controlled diabetic patients? A light microscopic study. *World J Dent*. 2020;11(1):65–68. doi:10.5005/jp-journals-10015-1693
47. Baran SS, Temmerman A, Salimov F, et al. The effects of photobiomodulation on leukocyte and platelet-rich fibrin as barrier membrane on bone regeneration: An experimental animal study. *Photobiomodul Photomed Laser Surg*. 2021;39(4):245–253. doi:10.1089/photob.2020.4943
48. Bains VK, Chandra H, Jamaluddin K, Bains R. Awareness among health care professionals regarding interrelationship between diabetes mellitus and periodontal diseases: A step towards interprofessional collaborative practice. *Asian J Oral Health Allied Sci*. 2020;10(1):10. doi:10.25259/ajohas_16_2020

Salivary biomarkers for the prediction of Grade B and Grade C periodontitis: A case–control study

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Abstract

Background. The expression of the Wingless-related integration site (WNT) signaling pathway molecules has been reported to be aberrant in periodontitis.

Objectives. This case–control study aimed to determine the salivary concentrations of sclerostin (SOST), Dickkopf-related protein 1 (DKK1) and WNT family member 5a (WNT5a) and their ability to distinguish systemically healthy patients with periodontitis – across different progression rates – from periodontally healthy controls.

Material and methods. A total of 90 individuals participated in this study (30 healthy controls, 30 Grade B periodontitis patients, and 30 Grade C periodontitis patients). Salivary samples were collected using the unstimulated saliva collection method. Subsequently, clinical periodontal parameters were recorded. The enzyme-linked immunosorbent assay (ELISA) was utilized to determine the salivary levels of SOST, DKK1 and WNT5a.

Results. The salivary levels of SOST and DKK1 were significantly higher in Grade B periodontitis patients (14.85 ±3.28 ng/mL and 3.65 ±1.11 ng/mL, respectively) and Grade C periodontitis patients (7.45 ±3.84 ng/mL and 1.68 ±0.46 ng/mL, respectively) than in healthy controls (4.37 ±0.97 ng/mL and 0.88 ±0.15 ng/mL, respectively). Notably, their levels were significantly higher in periodontitis Grade B than in Grade C ($p < 0.0001$). The WNT5a salivary levels were also considerably higher in Grade B periodontitis patients (6.58 ±3.28 ng/mL) and Grade C periodontitis patients (13.41 ±1.73 ng/mL) than in healthy controls (2.03 ±0.31 ng/ml). Unlike SOST and DKK1, WNT5a showed significantly higher levels in periodontitis Grade C as compared to Grade B ($p < 0.0001$). Additionally, the receiver operating characteristic (ROC) analysis demonstrated that SOST, DKK1 and WNT5a showed significant diagnostic accuracy in distinguishing periodontitis from periodontal health with the area under the curve (AUC) values of 0.96, 0.97 and 0.99, respectively, and also in distinguishing periodontitis Grade B from periodontitis Grade C with AUC of 0.94, 0.97 and 0.92, respectively.

Conclusions. The salivary levels of SOST, DKK1 and WNT5a demonstrated high diagnostic value in distinguishing generalized periodontitis from periodontal health. The WNT5a levels increased with disease progression, effectively differentiating Grades B and C. Similarly, SOST and DKK1 also distinguished these grades, with higher levels in Grade B than in Grade C.

Keywords: DKK1, periodontitis, grading, SOST, WNT5a

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Highlights

- Salivary SOST, DKK1 and WNT5a biomarkers are significantly elevated in periodontitis patients in comparison with healthy controls.
- The WNT5a levels increase with disease progression, effectively distinguishing Grade C (rapid progression) from Grade B periodontitis.
- The SOST and DKK1 levels are higher in Grade B than Grade C, indicating distinct biomarker expression patterns.
- All three biomarkers show excellent diagnostic accuracy (AUC > 0.90) for detecting periodontitis and differentiating disease grades.
- These salivary biomarkers provide a non-invasive approach for periodontitis diagnosis, grading and disease monitoring.

Introduction

Periodontitis ranks among the most prevalent chronic diseases globally and significantly affects oral health.[1] It is a biofilm-mediated inflammatory condition characterized by intricate interactions between immune-inflammatory pathways and alterations in the symbiotic ecosystem, ultimately resulting in inflammation-induced bone loss.[2] The regulation of bone metabolism is a complex process influenced by various signaling pathways, driven by numerous local and systemic variables, such as chemokines, cytokines, hormones, as well as biochemical stimuli.[3]

The Wingless-related integration site (WNT) pathway is a signal transduction cascade and one of the most evolutionarily conserved cell signaling systems, playing a central role in numerous fundamental biological processes, including cell proliferation, polarity and fate determination, functioning in both embryonic development and the maintenance and regeneration of mature cells.[4–6] The dysregulation of WNT signaling has been reported to be a significant contributor to various diseases, including bone and metabolic abnormalities.[7,8] The activation of the WNT pathway is initiated by interactions between WNT ligands and their receptors at the cell membrane, which trigger the expression of WNT target genes.[9] A minimum of 19 WNT protein ligands bind with Frizzled (Fz) receptors, initiating signaling cascades that can be broadly classified into 2 main branches – β-catenin-dependent (canonical) and β-catenin-independent (non-canonical) WNT signaling pathways.[10]

Sclerostin (SOST) and Dickkopf-related protein 1 (DKK1) act as antagonists for the β-catenin-dependent pathway by binding to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) on osteoblast surfaces. This interaction effectively blocks the WNT signaling pathway, ultimately leading to the inhibition of osteoblast proliferation and differentiation.[11,12] In mice with induced periodontitis, the deletion of the *SOST* gene led to reduced alveolar bone resorption, decreased expression of receptor activator for nuclear factor-kappa B ligand (RANKL) and increased expression of osteoprotegerin (OPG).[13] Similarly, the constitutive deletion of *DKK1* in osteocytes within a ligature-induced periodontitis model completely prevented alveolar bone loss.[14] Moreover, studies conducted in vivo have demonstrated that patients with periodontitis exhibit elevated levels of SOST and DKK1 in both gingival tissues and gingival crevicular fluid (GCF).[15–19]

WNT family member 5a (WNT5a), a component of the β-catenin-independent pathway, plays an important role in both the development of periodontal tissues and the pathogenesis of periodontitis.[20] WNT5a has the capacity to either activate or inhibit the β-catenin-dependent pathway.[21] WNT5a activates proinflammatory signaling pathways and amplifies the secretion of proinflammatory cytokines, contributing to the exacerbation of periodontal tissue destruction.[22,23] Additionally, it has been demonstrated that WNT5a facilitates RANKL-induced osteoclastogenesis, ultimately leading to enhanced osteoclast differentiation and increased bone resorption, thereby inhibiting bone formation.[24]

Established during the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, the new periodontitis classification utilizes a multi-dimensional approach for staging and grading.[25] Grading offers additional insights into the biological characteristics of the disease, incorporating a longitudinal assessment of periodontitis progression rates and an evaluation of the risk for future disease advancement.[26] The limitations of conventional diagnostic methods have prompted the exploration of biomarker integration into the new classification system. This approach is expected to facilitate the identification of patients during active phases of periodontitis and to improve the accuracy of monitoring disease progression and treatment response.[26]

Saliva collection is straightforward, rapid and non-invasive, and it requires less manpower and fewer materials as compared to other diagnostic bodily fluids. Additionally, whole saliva provides a pooled sample from all periodontal sites, enabling a comprehensive assessment of an individual's disease status or risk rather than focusing on specific sites or teeth.[27] Given these advantages, saliva-based diagnostics holds considerable promise for the detection of periodontal diseases and the prediction of treatment outcomes, potentially advancing future diagnostic approaches in periodontology.[28]

Despite prior evidence highlighting the roles of SOST, DKK1 and WNT5a in periodontal tissue deterioration, there is a notable lack of studies investigating the salivary levels of WNT5a and DKK1 in patients with periodontitis, as well as a scarcity of research measuring the SOST levels in saliva. Therefore, this study aimed to evaluate the salivary concentrations of SOST, DKK1 and WNT5a with the objective of determining their efficacy in differentiating between Grades B and C periodontitis.

Material and methods

Study design and settings

This case–control study involved the analysis of patients who visited the dental clinics at the Department of Periodontics, College of Dentistry, University of Baghdad, Iraq, between January and May 2024. Ninety systemically healthy participants were included. All participants who met the established eligibility criteria were verbally informed about the study and asked to sign an informed consent form. Salivary samples were collected from all participants. Then, the patients received a full-mouth periodontal examination to determine their periodontal status. The study was approved by the ethics committee of the College of Dentistry, University of Baghdad, in accordance with the Declaration of Helsinki and its subsequent amendments concerning human research (reference No. 855, project No. 855623, December 12, 2023). The research was evaluated and validated in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Eligibility

During the preliminary screening phase, each participant underwent an assessment to determine their eligibility for inclusion in the study based on the following criteria: adults aged >18 years; systemically healthy; with ≥20 permanent teeth (excluding third molars); and without any systemic medical condition that could affect the periodontal status, particularly any disorders associated with bone health (rheumatoid arthritis, bleeding disorders, diabetes mellitus, recent bone fractures, ankylosing spondylitis, osteoporosis, and immunosuppressive therapy). The exclusion criteria were as follows: antibiotic use within the past 3 months; extensive periodontal therapy within the past 3 months or currently undergoing active periodontal treatment; the presence of dental implants; the presence of orthodontic appliances; pregnant or lactating women; post-menopausal women; patients with any oral lesions unrelated to periodontitis, including but not limited to aphthous ulcers, lichen planus, and others. Moreover, individuals presenting a waist-to-height ratio (WHtR) ≥0.5,[29] as well as those with a body mass index (BMI) ≥30 kg/m^2, were excluded from the study.

Study groups

Based on the periodontal status, the eligible individuals were categorized into healthy controls and periodontitis patients. The periodontitis group was further subdivided into Grade B and Grade C. The categorization was carried out based on the latest classification of periodontal diseases and conditions, while also integrating the guidelines from the British Society of Periodontology (BSP) regarding the practical implementation of this classification system in clinical settings.[26,30] Participants with no clinical attachment loss (CAL), bleeding on probing (BoP) <10% and probing pocket depth (PPD) ≤3 mm were grouped as healthy controls. For the periodontitis groups, participants were identified based on the presence of interdental CAL at two or more non-adjacent teeth, or buccal/oral CAL in conjunction with PPD > 3 mm at two or more teeth. All periodontitis cases were classified as generalized (involving ≥30% of teeth) and unstable, defined by PPD ≥ 5 mm or PPD ≥ 4 mm with BoP. Patients were further categorized as Grade B or Grade C according to the ratio of radiographic bone loss at the most affected site to the patient's age. A ratio of 0.5–1.0 was classified as Grade B, indicating moderate disease progression, whereas a ratio >1.0 was classified as Grade C, indicating rapid progression. Our study adopted the revised approach, which uses a 0.5–1.0 ratio to determine grades while excluding risk factors such as diabetes and smoking. This method aligns with recent literature advocating a simplified classification based solely on clinical measurements for grade assessment.[30] Regarding the smoking status, individuals who smoked ≥10 cigarettes per day for the past 5 years were classified as smokers, whereas those who had never smoked or had ceased smoking at least 2 years prior to the study were classified as non-smokers.[31]

Clinical examination

A single calibrated examiner performed all clinical assessments. The following periodontal parameters were evaluated: plaque index (PI)[32]; BoP[33]; PPD; and CAL at 6 sites per tooth (except PI, which was recorded at 4 sites per tooth), excluding third molars, using UNC-15 periodontal probes (Hu-Friedy, Chicago, USA). The plaque index and BoP were recorded dichotomously (present/absent) at 4 or 6 sites per tooth, respectively. The percentage of sites with plaque or bleeding was then calculated for each patient, and these percentages are presented as mean ± standard deviation ($M \pm SD$). Radiographic imaging was performed to confirm the diagnosis, focusing on the most affected tooth, defined as the tooth exhibiting the greatest interdental CAL. In addition, BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2), and WHtR was calculated as waist circumference in centimeters divided by height in centimeters.

Calibration was performed with an expert periodontist for diagnosis and the recording of clinical parameters prior to study commencement. A minimum interval of 2 h was maintained between consecutive measurements. These calibration sessions were repeated at different time points until an acceptable level of agreement was achieved. This included κ values >0.75 for PI and BoP, and intraclass correlation coefficients (*ICCs*) >0.90 for PPD and CAL.

Sample size

Using the first 15 samples from each group, a pilot study was conducted, and the salivary levels of the selected biomarkers were determined using the enzyme-linked immunosorbent assay (ELISA). Subsequently, the sample size was calculated according to the formula provided by Sharma et al. for case–control studies (Equation 1)[34]:

$$n = (r + 1/r) \times SD^2 \times (Z_\beta + Z_{\alpha/2})^2/d^2 \quad (1)$$

where:
n – sample size per group;
r – allocation ratio (n_2/n_1);
SD – standard deviation;
$Z_{\alpha/2}$ – critical value for the significance level;
Z_β – critical value for power; and
d – minimal clinically significant difference to detect.

Accordingly, a sample size of 30 was estimated for each group (30 for the healthy control group, 30 for the Grade B periodontitis group, and 30 for the Grade C periodontitis group), yielding a 2:1 ratio of cases to controls.

Salivary sample collection and analysis

Saliva samples were collected from the participants between 9:00 a.m. and 1:00 p.m., prior to any oral examination. Whole unstimulated saliva was obtained using the passive drooling method. The participants were instructed to abstain from eating, drinking, smoking, and oral hygiene practices for at least 1 h before collection. Approximately 10 min before sampling, they rinsed their mouths with water for 30 s, and then expectorated into sterile tubes while seated upright. A total of 3 mL of unstimulated saliva was collected and transferred into polypropylene tubes. The samples were centrifuged at 3,000 rpm for 5 min and subsequently stored at –20°C until analysis by ELISA.

The samples were allowed to thaw at room temperature. The protein levels in saliva were then quantified using commercially available ELISA kits (Cloud-Clone Corp., Katy, USA), according to the manufacturer's instructions. A standard curve was generated by plotting the optical density (OD) values for SOST, DKK1 and WNT5a, and regression equations were applied to convert these values into concentrations expressed in nanograms per milliliter (ng/mL).

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, v. 27.0 (IBM Corp., Armonk, USA), while graphical representations were generated using GraphPad Prism 10 (GraphPad Software, San Diego, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. For non-normally distributed data, non-parametric statistical methods were applied.

Differences in age, WHtR, BMI, cigarettes per day, PI, and BoP were analyzed using the one-way analysis of variance (ANOVA) followed by the Games–Howell post hoc tests, as these variables were normally distributed. In contrast, the Kruskal–Wallis test followed by Bonferroni-adjusted post hoc comparisons was used for the salivary biomarker levels, PPD, CAL, and the number of sites with PPD ≥ 4 mm, as well as the number of teeth with PPD ≥ 4 mm, due to their non-normal distribution. The χ^2 test was used to assess associations between categorical variables, including gender, the smoking status and the educational level, across the 3 study groups. Moreover, for comparisons between smokers and non-smokers within each group, the Mann–Whitney U test was used.

Spearman's correlation coefficient was applied to evaluate relationships between the salivary biomarker levels and other relevant variables. The receiver operating characteristic (ROC) analysis was performed to assess the diagnostic performance of SOST, DKK1 and WNT5a in distinguishing healthy and periodontitis groups, with the area under the curve (AUC) values used to determine sensitivity and specificity. Multiple linear regression analysis was conducted to examine the influence of various factors on the salivary biomarker levels across all groups. The level of statistical significance was set at 5% ($p < 0.05$).

Results

In the current study, 320 subjects were screened for eligibility. A total of 90 participants were recruited, after excluding individuals for several reasons, as detailed in Fig. 1. Subsequently, the participants were divided into 3 groups (30 healthy controls, 30 Grade B periodontitis patients, and 30 Grade C periodontitis patients).

Demographic variables

The demographic characteristics of the study groups are shown in Table 1. There were no differences in the mean age, gender, WHtR, and BMI among the study groups ($p > 0.05$). However, statistically significant differences among groups were observed in the mean number of teeth and the educational level of the study participants ($p < 0.001$). Additionally, no significant differences were observed in the distribution of smokers across the groups ($p > 0.05$).

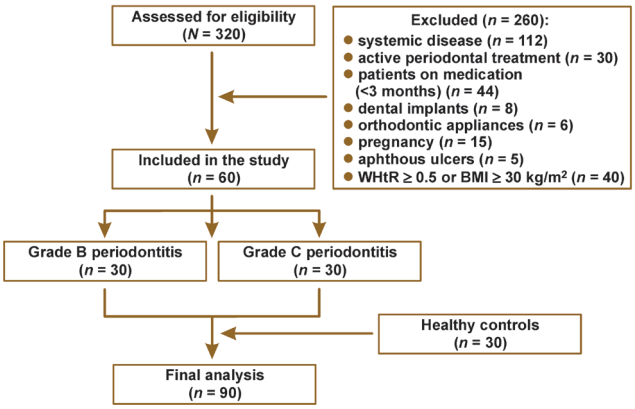

Fig. 1. Flowchart of the study
WHtR – waist-to-height ratio; BMI – body mass index.

Clinical periodontal parameters

Concerning the assessed clinical periodontal parameters (PI, BoP, PPD, CAL, and the number of sites with PPD ≥ 4 mm), all of them were significantly higher in both Grade B and Grade C periodontitis groups as compared to the control group ($p < 0.0001$). Additionally, the Grade C group exhibited significantly higher PI and CAL when compared to Grade B, as illustrated in Table 2.

Salivary levels of SOST, DKK1 and WNT5a

The salivary levels of all biomarkers across the groups are presented in Table 3. The concentrations of SOST, DKK1 and WNT5a were significantly higher in both periodontitis groups as compared to the healthy control group. Intergroup comparisons revealed significantly higher salivary levels of both SOST and DKK1 in the Grade B periodontitis group when compared to Grade C ($p < 0.0001$), whereas the salivary levels of WNT5a were significantly higher in Grade C than in Grade B ($p < 0.0001$), as illustrated in Fig. 2. Further analysis revealed that the salivary biomarker levels exhibited no significant differences between smokers and non-smokers across all groups ($p > 0.05$), as shown in Table 4 and Fig. 3.

Table 1. Demographic characteristics of the study groups

Characteristics		Groups			*p*-value
		healthy controls	Grade B periodontitis	Grade C periodontitis	
Age [years]		39.03 ±9.92	44.03 ±10.56	43.60 ±11.29	0.135[a]
Gender	M	20 (66.67)	23 (76.67)	21 (70.00)	0.770[b]
	F	10 (33.33)	7 (23.33)	9 (30.00)	
WHtR		0.46 ±0.02	0.47 ±0.01	0.46 ±0.02	0.142[a]
BMI [kg/m^2]		24.24 ±3.32	25.66 ±2.77	25.88 ±2.37	0.058[a]
Smoking status	smokers	10 (33.33)	13 (43.33)	12 (40.00)	0.797[b]
	non-smokers	20 (66.67)	17 (56.67)	18 (60.00)	
Cigarettes/day		28.00 ±10.06	32.31 ±13.01	33.75 ±12.27	0.520[a]
Number of teeth		27.2 ±1.5	23.4 ±3.0	23.9 ±2.7	<0.001*[c]
Educational level	no education	0 (0.00)	6 (20.00)	6 (20.00)	<0.001*[b]
	primary education	2 (6.67)	9 (30.00)	11 (36.67)	
	secondary education	4 (13.33)	11 (36.67)	10 (33.33)	
	higher education	24 (80.00)	4 (13.33)	3 (10.00)	

Data presented as mean ± standard deviation (*M ±SD*) or as number (percentage) (*n* (%)).
M – male; F – female; * statistically significant ([a] ANOVA; [b] χ^2 test; [c] Kruskal–Wallis test).

Table 2. Clinical parameters among the study groups

Parameters	Groups			*p*-value
	healthy controls	Grade B periodontitis	Grade C periodontitis	
PI [%]	20.23 ±9.96	47.13 ±16.44[a]	64.12 ±14.62[ab]	<0.001*[†]
BoP [%]	4.98 ±2.00	48.88 ±18.94[a]	57.72 ±17.75[a]	<0.001*[†]
PPD [mm]	1.90 ±0.76	4.40 ±0.47[a]	4.79 ±0.74[a]	<0.001*[‡]
CAL [mm]	0.00 ±0.00	3.07 ±0.89[a]	4.36 ±0.35[ab]	<0.001*[‡]
Number of sites with PPD ≥ 4 mm	0.0 ±0.0	9.77 ±8.96[a]	22.67 ±21.59[a]	<0.001*[‡]

Data presented as *M ±SD*.
PI – plaque index; BoP – bleeding on probing; PPD – probing pocket depth; CAL – clinical attachment loss; * statistically significant ([†] ANOVA with the Games–Howell post hoc test; [‡] Kruskal–Wallis test with the Bonferroni post hoc test; [a] significantly different from the control group; [b] significantly different from the Grade B periodontitis group).

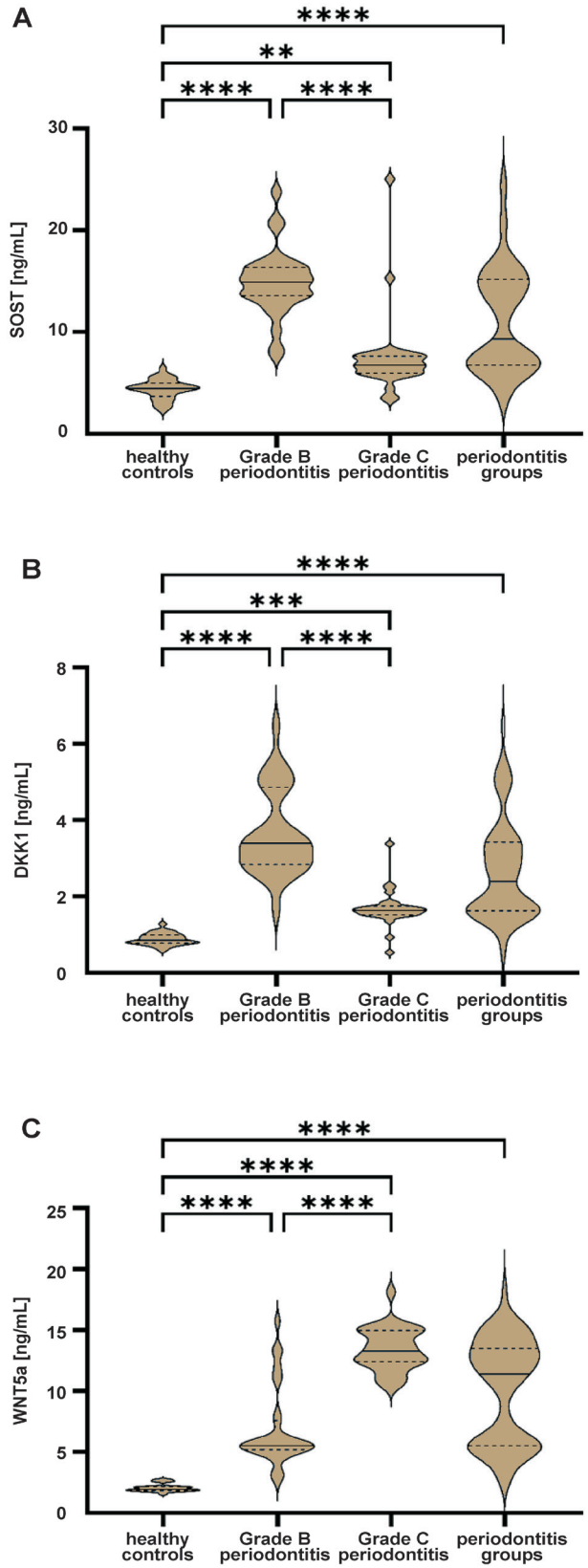

Fig. 2. Violin plots for the salivary concentrations of biomarkers among the study groups

A – SOST; B – DKK1; C – WNT5a.
Solid lines represent median (*Me*) and dashed lines represent interquartile range (*IQR*).
** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ (Kruskal–Wallis test and the Bonferroni post hoc test).

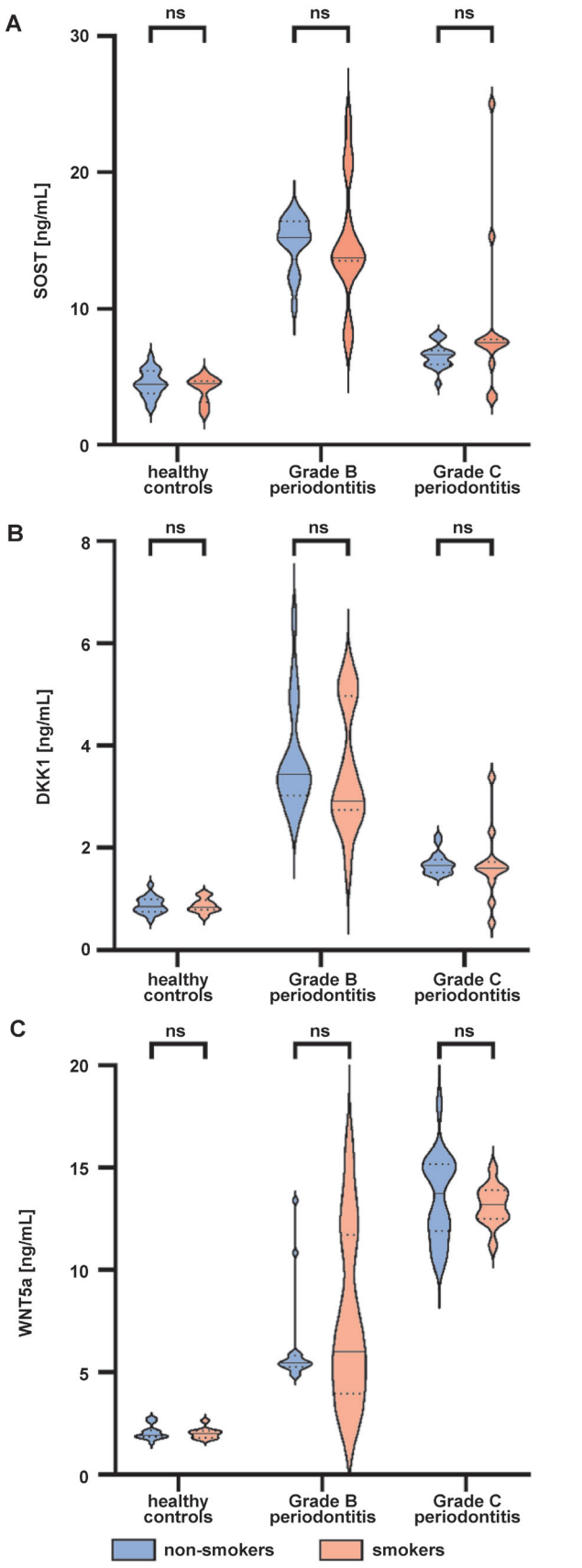

Fig. 3. Violin plots for the salivary concentrations of biomarkers in smokers and non-smokers across all groups

A – SOST; B – DKK1; C – WNT5a.
Solid lines represent *Me* and dashed lines represent *IQR*.
ns – non-significant ($p > 0.05$) (multiple Mann–Whitney *U* test).

Table 3. Comparison of the salivary levels of biomarkers [ng/mL] among the study groups

Biomarkers	Groups			
	healthy controls	Grade B periodontitis	Grade C periodontitis	periodontitis groups
SOST	4.47 (4.37 ±0.97)	14.91 (14.85 ±3.28)	6.75 (7.45 ±3.84)	9.30 (11.15 ±5.14)
DKK1	0.85 (0.88 ±0.15)	3.40 (3.65 ±1.11)	1.64 (1.68 ±0.46)	2.40 (2.66 ±1.30)
WNT5a	2.00 (2.03 ±0.31)	5.53 (6.58 ±3.28)	13.25 (13.41 ±1.73)	11.40 (10.11 ±0.31)

Data presented as median (*Me*) (*M* ±*SD*).
SOST – sclerostin; DKK1 – Dickkopf-related protein 1; WNT5a – Wingless-related integration site (WNT) family member 5a.

Table 4. Comparison of the salivary levels of biomarkers [ng/mL] in smokers and non-smokers among the study groups

Biomarkers	Healthy controls		Grade B periodontitis		Grade C periodontitis	
	smokers (n = 10)	non-smokers (n = 20)	smokers (n = 13)	non-smokers (n = 17)	smokers (n = 12)	non-smokers (n = 18)
SOST	4.52 (4.09 ±0.96)	4.47 (4.50 ±0.97)	13.74 (14.86 ±4.56)	15.21 (14.85 ±1.97)	7.50 (8.84 ±5.88)	6.60 (6.50 ±0.89)
DKK1	0.85 (0.88 ±0.14)	0.85 (0.88 ±0.16)	2.91 (3.51 ±1.21)	3.44 (3.75 ±1.06)	1.61 (1.66 ±0.70)	1.67 (1.70 ±0.21)
WNT5a	2.01 (2.03 ±0.26)	1.93 (2.07 ±0.34)	6.00 (7.61 ±4.22)	5.45 (6.20 ±2.29)	13.22 (13.17 ±0.97)	13.75 (13.56 ±2.11)

Data presented as *Me* (*M* ±*SD*).

Correlation between the salivary biomarker levels and other variables

Table 5 presents the coefficients of correlation between the salivary biomarkers (SOST, DKK1 and WNT5a) and clinical and demographic variables, as well as their interrelationships. All 3 biomarkers showed statistically significant correlations with PI, BoP, PPD, CAL, and the number of sites with PPD ≥ 4 mm.

SOST was positively correlated with age and WHtR ($p < 0.05$), while DKK1 showed positive correlations with both WHtR and BMI ($p < 0.05$). In contrast, the number of teeth was negatively correlated with all salivary biomarkers ($p < 0.0001$).

Furthermore, the 3 tested biomarkers demonstrated significant positive correlations between each other ($p < 0.001$).

ROC analysis for diagnostic accuracy

The diagnostic potential of salivary biomarkers to distinguish periodontal health from periodontitis and its grades was evaluated using the ROC analysis, as presented in Table 6 and Fig. 4. All biomarkers (SOST, DKK1 and WNT5a) demonstrated excellent discriminatory ability between periodontal health and periodontitis, with the AUC values of 0.96, 0.97 and 0.99, respectively.

Table 5. Correlations between salivary biomarkers and other variables

Variables	Salivary biomarkers		
	SOST	DKK1	WNT5a
Age	r = 0.229*	r = 0.158	r = 0.199
WHtR	r = 0.213*	r = 0.219*	r = 0.051
BMI	r = 0.669	r = 0.217*	r = 0.174
Cigarettes/day	r = 0.080	r = 0.029	r = 0.096
Number of teeth	r = –0.485****	r = –0.497****	r = –0.405****
PI	r = 0.452***	r = 0.431***	r = 0.477***
BoP	r = 0.536***	r = 0.543***	r = 0.681***
PPD	r = 0.506****	r = 0.574****	r = 0.744****
CAL	r = 0.450***	r = 0.467***	r = 0.781***
Number of sites with PPD ≥ 4 mm	r = 0.505****	r = 0.547****	r = 0.758****
SOST	–	r = 0.836***	r = 0.488***
DKK1	–	–	r = 0.477***

r – Spearman's correlation coefficient; * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$.

Table 6. Receiver operating characteristic (ROC) analysis for salivary biomarkers across all groups.

ROC analysis	Biomarkers	AUC	Sensitivity [%]	Specificity [%]	Cut-off point	95% *CI*	*p*-value
Healthy controls vs. periodontitis	SOST	0.96	93.33	96.67	5.805	0.92–1.00	<0.0001*
	DKK1	0.97	96.67	100.00	1.315	0.94–1.00	<0.0001*
	WNT5a	0.99	98.33	10.000	2.975	0.99–1.00	<0.0001*
Healthy controls vs. Grade B periodontitis	SOST	1.00	100.00	96.67	6.100	1.00–1.00	<0.0001*
	DKK1	1.00	100.00	100.00	1.470	1.00–1.00	<0.0001*
	WNT5a	0.99	96.67	100.00	2.975	0.99–1.00	<0.0001*
Healthy controls vs. Grade C periodontitis	SOST	0.92	90.00	93.33	5.605	0.84–1.00	<0.0001*
	DKK1	0.95	93.33	100.00	1.315	0.89–1.00	<0.0001*
	WNT5a	1.00	100.00	100.00	6.545	1.00–1.00	<0.0001*
Grade B periodontitis vs. Grade C periodontitis	SOST	0.94	96.67	93.33	8.230	0.87–1.00	<0.0001*
	DKK1	0.97	96.67	96.67	2.395	0.93–1.00	<0.0001*
	WNT5a	0.92	83.33	93.33	10.870	0.84–0.99	<0.0001*

CI – confidence interval; * statistically significant.

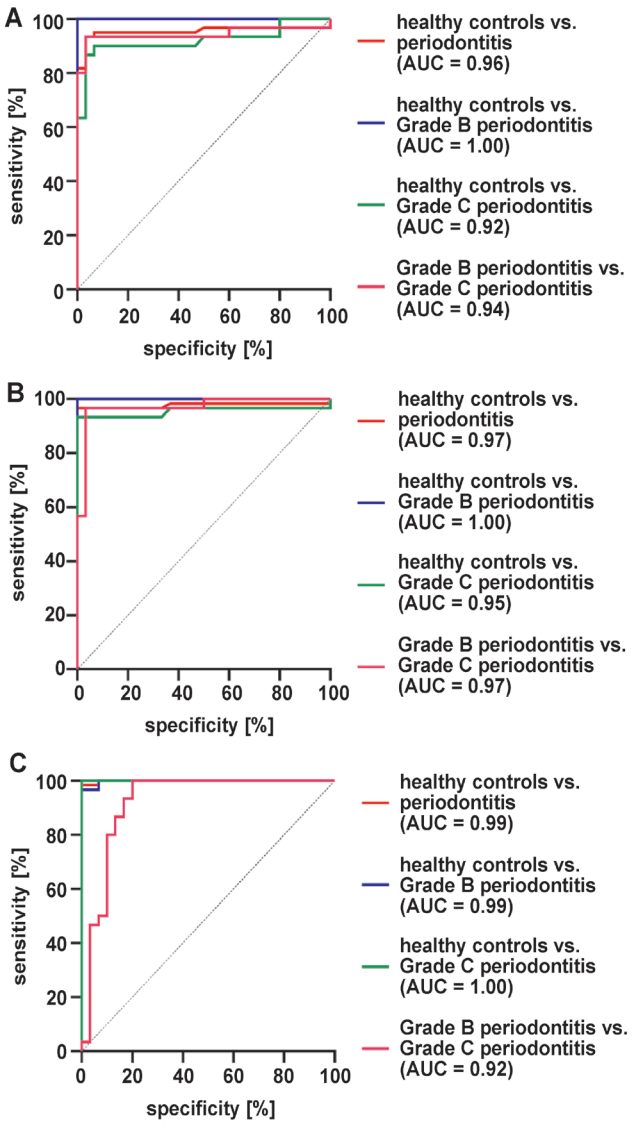

Fig. 4. Receiver operating characteristic (ROC) curves for salivary biomarkers

A – SOST; B – DKK1; C – WNT5a. AUC – area under the curve.

DKK1 demonstrated the highest sensitivity and specificity (100% each) for distinguishing the healthy group from Grade B periodontitis, with a cut-off value of 1.470. This was followed by WNT5a (96.69% sensitivity and 100.00% specificity; cut-off: 2.975) and SOST (100.00% sensitivity and 96.69% specificity; cut-off: 6.100).

For distinguishing healthy controls from Grade C periodontitis, WNT5a demonstrated the highest sensitivity and specificity (100% each). This was followed by DKK1 (93.33% sensitivity and 100.00% specificity) and SOST (90.00% sensitivity and 93.33% specificity).

Further analysis showed that all biomarkers (SOST, DKK1 and WNT5a) demonstrated excellent ability to discriminate between periodontitis grade B and grade C, with the AUC values of 0.94, 0.97 and 0.92, respectively.

Multiple linear regression

Multiple linear regression analysis revealed that SOST was significantly negatively associated with CAL ($B = -2.29$; $p < 0.001$) and the number of teeth ($B = -0.385$; $p = 0.048$). Similarly, DKK1 showed significant negative associations with PI ($B = -0.021$; $p = 0.005$), CAL ($B = -0.495$; $p < 0.001$), and the number of teeth ($B = -0.107$; $p = 0.024$). Both SOST and DKK1 were positively associated with periodontitis ($B = 14.433$; $p < 0.001$ and $B = 3.573$; $p < 0.001$, respectively). Furthermore, WNT5a demonstrated significant positive associations with PI, CAL, the number of teeth, and periodontitis, as presented in Table 7.

Discussion

The current study demonstrated that the salivary levels of the selected biomarkers involved in the WNT signaling pathway (SOST, DKK1 and WNT5a) were significantly elevated in systemically healthy individuals with periodontitis

Table 7. Multiple linear regression models for the salivary biomarker levels

Multiple linear regression models	SOST			DKK1			WNT5a		
	B	*SE*	*p*-value	*B*	*SE*	*p*-value	*B*	*SE*	*p*-value
Model I		$R^2 = 0.600$			$R^2 = 0.638$			$R^2 = 0.707$	
Age	0.062	0.040	0.124	0.001	0.010	0.879	0.002	0.033	0.944
WHtR	9.465	21.860	0.666	5.950	5.322	0.267	−10.781	18.109	0.553
BMI	−0.001	0.140	0.997	−0.001	0.034	0.986	−0.002	0.116	0.852
Number of teeth	−0.385	0.191	0.048*	−0.107	0.047	0.024*	0.506	0.158	0.002*
PI	−0.033	0.030	0.273	−0.021	0.007	0.005*	0.005	0.025	0.027
BoP	−0.025	0.030	0.419	−0.001	0.007	0.942	−0.029	0.025	0.259
PPD	0.346	0.489	0.418	0.091	0.119	0.445	−0.110	0.405	0.786
CAL	−2.290	0.460	<0.001*	−0.495	0.112	<0.001*	1.571	0.381	<0.001*
Periodontitis	14.433	2.420	<0.001*	3.573	0.588	<0.001*	3.998	2.000	0.049*
Model II		$R^2 = 0.567$			$R^2 = 0.629$			$R^2 = 0.700$	
Number of teeth	−0.479	0.169	0.006*	−0.110	0.041	0.009*	0.555	0.140	<0.001*
PI	–	–	–	−0.021	0.006	0.001*	0.043	0.021	0.047*
CAL	−2.454	0.388	<0.001*	−0.483	0.100	<0.001*	1.530	0.341	<0.001*
Periodontitis	14.205	1.537	<0.001*	3.931	0.381	<0.001*	2.817	1.295	0.032*

B – unstandardized regression coefficient; *SE* – standard error; R^2 – coefficient of determination; * statistically significant.

as compared to those with a healthy periodontium. These biomarkers not only indicated the presence of periodontitis, but also exhibited high sensitivity in distinguishing between different rates of disease progression (periodontitis Grade B and Grade C). This highlights their potential utility in the non-invasive diagnosis and grading of periodontitis, thereby contributing to more precise and targeted treatment strategies.

The inherent limitations of traditional diagnostic methods have prompted investigation into the integration of biomarkers into the new periodontitis classification system, particularly for disease grading, thereby enhancing the data obtained from standard clinical measures.[26] The selection of saliva as a diagnostic medium in this study is based on its unique properties and considerable potential for disease detection and monitoring.[35] As an easily obtainable biofluid that can be collected without specialized clinical facilities or advanced technical expertise, it represents a valuable medium for both research and diagnostic applications. Numerous salivary biomarkers have demonstrated a remarkable ability to differentiate periodontitis from periodontal health.[36–40] However, to date, no single biomarker has been identified that can reliably diagnose periodontal disease.[41] Therefore, the present study aimed to identify promising salivary biomarkers that could serve as a basis for monitoring periodontal disease progression and provide a foundation for future research.

Over recent decades, the WNT signaling pathway has attracted considerable scientific interest due to its pivotal role in regulating various cellular processes, including bone metabolism.[42] Evidence indicates that the WNT signaling pathway – particularly the WNT/β-catenin pathway – is involved not only in regulating osteoblast differentiation and bone formation, but also in controlling genes essential for osteoclast maturation and bone resorption. Among the key regulators (agonists and antagonists) of the WNT pathway associated with periodontitis are SOST, DKK1 and WNT5a.[43]

It is well established that periodontitis is associated with elevated levels of local and systemic proinflammatory cytokines.[44,45] In this context, the expression of SOST and DKK1 has been reported to be upregulated by tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and transforming growth factor-beta 1 (TGF-β1) in several diseases, including periodontitis,[46–49] suggesting their potential role in its pathogenesis. Baek et al. demonstrated that TNF-α stimulates the production of SOST in MLO-Y4 osteocytes.[50] It was shown that nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) directly binds to the NF-κB binding sites on the mouse SOST promoter, thereby enhancing SOST production through NF-κB activation.[50] Additionally, a study using an experimental periodontitis model in osteocyte-specific DKK1-deletion mice demonstrated increased volume and density of the alveolar process.[14] Notably, substantial variations were observed in the infiltration of inflammatory cells and proinflammatory cytokines within periodontal tissues, indicating that the ablation of DKK1 in osteocytes reduced the inflammatory response.[14] Moreover, SOST and DKK1, primarily expressed by osteocytes, have been shown to increase RANKL expression while simultaneously decreasing the OPG levels.[51,52] Since the RANKL/OPG ratio is critical in the pathogenesis of periodontitis,[53] it is worth noting that SOST and DKK1 may promote osteoclast formation in a RANKL-dependent manner, which further underscores their role in disease progression.

In the present study, the salivary levels of SOST and DKK1 were significantly increased in the periodontitis groups (Grade B and C) as compared to the healthy group. Additionally, both biomarkers showed significant positive correlations with PI, BoP and CAL ($p < 0.001$), as well as with PPD and the number of sites with PPD ≥ 4 mm ($p < 0.0001$). To the best of our knowledge, no previous studies have evaluated the salivary levels of SOST or DKK1 in periodontitis patients with different rates of disease progression in comparison with individuals with a healthy periodontium. However, SOST has been detected in saliva. A previous study assessed the salivary SOST levels before and after non-surgical periodontal treatment.[54] Although no significant changes were observed post-treatment, the baseline SOST levels were reported to be 1.6-fold lower in the chronic periodontitis group.[54]

Our findings are in agreement with a previous study reporting increased mRNA expression levels of SOST and DKK1 in the periodontal tissues of systemically healthy individuals with chronic periodontitis as compared to periodontally healthy participants.[18] Furthermore, the same study observed elevated serum SOST levels in the periodontitis group, whereas the DKK1 levels did not differ significantly between the periodontitis and non-periodontitis groups.[18] Despite differences in study design and analytical methods, these findings may suggest that saliva more accurately reflects changes in the DKK1 levels than serum in systemically healthy individuals with periodontitis. This may be explained by the fact that saliva contains the components of GCF, in which DKK1 has been detected in periodontitis patients.[19,55] However, further studies are required to elucidate the exact mechanisms underlying the elevation of DKK1 in saliva. Numerous studies have assessed the SOST levels in GCF. A study conducted by Balli et al. demonstrated that the SOST levels were markedly elevated in patients with chronic periodontitis as compared to healthy individuals.[17] Additionally, the SOST levels showed a marked decrease following non-surgical periodontal treatment.[17] Likewise, Chatzopoulos et al. reported that the SOST levels were significantly higher in both the generalized moderate and severe chronic periodontitis groups as compared to the healthy group.[16] Moreover, a recent study showed that the GCF levels of SOST were elevated in patients with periodontitis Stage III Grade B and Stage III Grade C as compared to gingivally healthy individuals.[56] However, no significant differences were observed between the 2 grades. This lack of distinction was attributed to similarity in the bone resorption mechanisms involving the WNT pathway, as well as to the fact that GCF samples were collected from the sites with the highest CAL in both groups.[56]

Unexpectedly, we observed significantly higher salivary SOST and DKK1 levels in Grade B periodontitis than in Grade C ($p < 0.0001$). This discrepancy may be attributed to differences in disease severity between the study groups. This is supported by the multiple linear regression models for SOST and DKK1, which indicated that higher CAL was associated with lower levels of both biomarkers when controlling for other variables (Table 7). These contrasting findings may be explained by the regulatory mechanisms operating at more advanced stages of periodontitis, potentially leading to reduced expression of these biomarkers, or by individual variability in biological responses to periodontal destruction.[57,58] In light of these considerations, further research is warranted to elucidate the specific mechanisms underlying the dynamics of these biomarkers.

WNT5a has been shown to modulate the production of inflammatory cytokines and exacerbate the deterioration of the periodontium. The inhibition of WNT5a using small interfering RNA (siRNA) or a neutralizing antibody markedly reduced the expression of IL-1β, monocyte chemotactic protein-1 (MCP-1) and matrix metalloproteinase-2 (MMP-2) in macrophages infected with *Porphyromonas gingivalis* (Pg).[23] Another study demonstrated that WNT5a expression in human gingival epithelial cells (HGECs) was significantly increased following 12 h of stimulation with Pg-derived lipopolysaccharide (LPS) at concentrations of 0.1 and 1.0 μg/mL,[22] suggesting a proinflammatory role for WNT5a in gingival tissues during periodontitis. Additionally, WNT5a expressed by osteoblasts binds to a co-receptor complex involving receptor tyrosine kinase-like orphan receptor 2 (Ror2) and a Frizzled receptor (Ror2/Fz complex) on osteoclast precursors. This activates non-canonical WNT signaling, leading to the upregulation of RANKL expression. Consequently, the sensitivity of osteoclast precursors to RANKL increases, thereby promoting osteoclast differentiation.[24]

One study evaluated the WNT5a levels in serum, and found no significant differences between periodontitis and non-periodontitis patients.[59] Chatzopoulos et al. reported that in GCF, the WNT5a levels were comparable among healthy and generalized moderate and severe chronic periodontitis groups.[16] However, they also observed that the total WNT5a protein levels were substantially higher at diseased sites than at healthy sites among periodontitis patients.[16]

In the present study, the salivary WNT5a levels were significantly elevated in the periodontitis groups as compared to the healthy group ($p < 0.0001$). Notably, the WNT5a levels were significantly higher in Grade C periodontitis than in Grade B ($p < 0.0001$). This finding may be explained by the greater number of sites with PPD ≥ 4 mm in the Grade C group as compared to the Grade B group. Although this difference was not statistically significant, it may still be considered clinically relevant. Moreover, WNT5a showed significant positive correlations with PI, BOP and CAL ($p < 0.001$), as well as with PPD and the number of sites with PPD ≥ 4 mm ($p < 0.0001$), suggesting that the WNT5a levels increase with disease progression. Although no previous studies have evaluated WNT5a in saliva, these findings are consistent with those of Chatzopoulos et al., who associated increased GCF WNT5a levels with more severe periodontal destruction.[16]

In the present investigation, smoking had no significant effect on the salivary levels of SOST, DKK1 and WNT5a across all study groups ($p > 0.05$). This finding may be attributed to the relatively small number of smokers included in the sample. At present, there is limited evidence regarding the impact of smoking on the salivary levels of these biomarkers. However, one study reported that the SOST and DKK1 levels were markedly elevated in the periodontal tissues of smokers, regardless of the diabetes mellitus status.[60] In contrast, another study found no significant differences in the GCF levels of SOST and WNT5a between smokers and non-smokers.[61] Despite these inconsistent findings, the results of the present study suggest that, irrespective of the smoking status, the evaluated salivary biomarkers demonstrate significant potential to differentiate periodontitis from periodontal health across different rates of disease progression.

The primary strength of this study lies in providing novel insights into the salivary levels of SOST, DKK1 and WNT5a in patients with generalized periodontitis. Moreover, the study groups were matched for age, gender, WHtR, and BMI. Notably, differences in the expression of *WNT* genes in human marrow stromal cells between young and aged individuals have been reported in the literature.[62] In addition, genetic variations in the WNT/β-catenin pathway have been associated with metabolic conditions, including obesity.[63] By controlling for these variables, we minimized their potential confounding effects on the WNT levels, as supported by the multiple linear regression models (Table 7).

However, this study has several limitations. Due to its cross-sectional design, the long-term effects of altered SOST, DKK1 and WNT5a levels on periodontal tissues cannot be determined. Further biochemical studies are needed to clarify the precise role of these biomarkers in periodontitis. Additionally, including patients with Grade A periodontitis as a comparative group could provide further insight into the biomarker levels at lower rates of disease progression. Finally, the generalizability of these findings may be limited to the specific study conditions. Therefore, further research across diverse populations and settings, including the incorporation of systemic risk factors such as diabetes, is necessary to validate and extend these findings.

Conclusions

Our results indicate that the salivary levels of SOST, DKK1 and WNT5a have significant diagnostic potential for distinguishing periodontal health from periodontitis. WNT5a demonstrated a strong ability to differentiate between Grades B and C, showing a progressive increase with disease severity. In contrast, SOST and DKK1 also exhibited significant diagnostic value, but followed a different pattern, with levels increasing at moderate progression rates (Grade B) and decreasing at higher progression rates (Grade C). These findings highlight the potential utility of these biomarkers in the diagnosis and monitoring of periodontitis.

Ethics approval and consent to participate

The study was approved by the ethics committee of the College of Dentistry, University of Baghdad, Iraq, in accordance with the Declaration of Helsinki and its subsequent amendments concerning human research (reference No. 855, project No. 855623, December 12, 2023). Written informed consent was obtained from all the participants.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Dentino A, Lee S, Mailhot J, Hefti AF. Principles of periodontology. *Periodontol 2000*. 2013;61(1):16–53. doi:10.1111/j.1600-0757.2011.00397.x
2. Hajishengallis G, Korostoff JM. Revisiting the Page & Schroeder model: The good, the bad and the unknowns in the periodontal host response 40 years later. *Periodontol 2000*. 2017;75(1):116–151. doi:10.1111/prd.12181
3. Crockett JC, Mellis DJ, Scott DI, Helfrich MH. New knowledge on critical osteoclast formation and activation pathways from study of rare genetic diseases of osteoclasts: Focus on the RANK/RANKL axis. *Osteoporos Int*. 2011;22(1):1–20. doi:10.1007/s00198-010-1272-8
4. Eisenmann DM. Wnt signaling. *WormBook*. 2005:1–17. doi:10.1895/wormbook.1.7.1
5. Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus. *Science*. 2000;287(5458):1606–1609. doi:10.1126/science.287.5458.1606
6. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol*. 1998;14:59–88. doi:10.1146/annurev.cellbio.14.1.59
7. Miao CG, Yang YY, He X, et al. Wnt signaling pathway in rheumatoid arthritis, with special emphasis on the different roles in synovial inflammation and bone remodeling. *Cell Signal*. 2013;25(10):2069–2078. doi:10.1016/j.cellsig.2013.04.002
8. Ackers I, Malgor R. Interrelationship of canonical and non-canonical Wnt signalling pathways in chronic metabolic diseases. *Diab Vasc Dis Res*. 2018;15(1):3–13. doi:10.1177/1479164117738442
9. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest*. 2006;116(5):1202–1209. doi:10.1172/jci28551
10. Kikuchi A, Yamamoto H, Sato A. Selective activation mechanisms of Wnt signaling pathways. *Trends Cell Biol*. 2009;19(3):119–129. doi:10.1016/j.tcb.2009.01.003

11. Rossini M, Gatti D, Adami S. Involvement of WNT/β-catenin signaling in the treatment of osteoporosis. *Calcif Tissue Int*. 2013;93(2):121–132. doi:10.1007/s00223-013-9749-z
12. Lerner UH, Ohlsson C. The WNT system: Background and its role in bone. *J Intern Med*. 2015;277(6):630–649. doi:10.1111/joim.12368
13. Yang X, Han X, Shu R, et al. Effect of sclerostin removal in vivo on experimental periodontitis in mice. *J Oral Sci*. 2016;58(2):271–276. doi:10.2334/josnusd.15-0690
14. Goes P, Dutra C, Lösser L, Hofbauer LC, Rauner M, Thiele S. Loss of Dkk-1 in osteocytes mitigates alveolar bone loss in mice with periodontitis. *Front Immunol*. 2019;10:2924. doi:10.3389/fimmu.2019.02924
15. Sankardas PA, Lavu V, Lakakula BV, Rao SR. Differential expression of periostin, sclerostin, receptor activator of nuclear factor-κB, and receptor activator of nuclear factor-κB ligand genes in severe chronic periodontitis. *J Investig Clin Dent*. 2019;10(1):e12369. doi:10.1111/jicd.12369
16. Chatzopoulos GS, Mansky KC, Lunos S, Costalonga M, Wolff LF. Sclerostin and WNT-5a gingival protein levels in chronic periodontitis and health. *J Periodontal Res*. 2019;54(5):555–565. doi:10.1111/jre.12659
17. Balli U, Aydogdu A, Dede FO, Turer CC, Guven B. Gingival crevicular fluid levels of sclerostin, osteoprotegerin, and receptor activator of nuclear factor-κB ligand in periodontitis. *J Periodontol*. 2015;86(12):1396–1404. doi:10.1902/jop.2015.150270
18. Napimoga MH, Nametala C, Da Silva FL, et al. Involvement of the Wnt-β-catenin signalling antagonists, sclerostin and Dickkopf-related protein 1, in chronic periodontitis. *J Clin Periodontol*. 2014;41(6):550–557. doi:10.1111/jcpe.12245
19. Romero-Sánchez C, Giraldo S, Heredia-P AM, et al. Association of serum and crevicular fluid Dickkopf-1 levels with disease activity and periodontitis in patients with early rheumatoid arthritis. *Curr Rheumatol Rev*. 2022;18(2):124–135. doi:10.2174/1573397117666211116105118
20. Wei X, Liu Q, Guo S, Wu Y. Role of Wnt5a in periodontal tissue development, maintenance, and periodontitis: Implications for periodontal regeneration (Review). *Mol Med Rep*. 2021;23(3):167. doi:10.3892/mmr.2020.11806
21. Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol*. 2006;4(4):e115. doi:10.1371/journal.pbio.0040115
22. Maekawa T, Kulwattanaporn P, Hosur K, et al. Differential expression and roles of secreted Frizzled-related protein 5 and the Wingless homolog Wnt5a in periodontitis. *J Dent Res*. 2017;96(5):571–577. doi:10.1177/0022034516687248
23. Zhang Q, Liu J, Ma L, Bai N, Xu H. Wnt5a is involved in LOX-1 and TLR4 induced host inflammatory response in peri-implantitis. *J Periodontal Res*. 2020;55(2):199–208. doi:10.1111/jre.12702
24. Maeda K, Kobayashi Y, Udagawa N, et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat Med*. 2012;18(3):405–412. doi:10.1038/nm.2653
25. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89 (Suppl 1):S173–S182. doi:10.1002/JPER.17-0721
26. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89 (Suppl 1):S159–S172. doi:10.1002/JPER.18-0006
27. Miller CS, Foley JD, Bailey AL, et al. Current developments in salivary diagnostics. *Biomark Med*. 2010;4(1):171–189. doi:10.2217/bmm.09.68
28. Ji S, Choi Y. Point-of-care diagnosis of periodontitis using saliva: Technically feasible but still a challenge. *Front Cell Infect Microbiol*. 2015;5:65. doi:10.3389/fcimb.2015.00065
29. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. *Nutr Res Rev*. 2010;23(2):247–269. doi:10.1017/S0954422410000144
30. Dietrich T, Ower P, Tank M, et al.; British Society of Periodontology. Periodontal diagnosis in the context of the 2017 classification system of periodontal diseases and conditions – implementation in clinical practice. *Br Dent J*. 2019;226(1):16–22. doi:10.1038/sj.bdj.2019.3
31. Altıngöz SM, Kurgan Ş, Önder C, et al. Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: A cross-sectional study. *J Periodontol*. 2021;92(9):1274–1285. doi:10.1002/JPER.20-0406
32. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol*. 1972;43(1):38. doi:10.1902/jop.1972.43.1.38
33. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J*. 1975;25(4):229–235. PMID:1058834.
34. Sharma SK, Mudgal SK, Thakur K, Gaur R. How to calculate sample size for observational and experimental nursing research studies. *Natl J Physiol Pharm Pharmacol*. 2020;10(1):1–8. https://www.njppp.com/fulltext/28-1567942207.pdf. Accessed February 4, 2024.
35. Zhang Y, Sun J, Lin CC, Abemayor E, Wang MB, Wong DTW. The emerging landscape of salivary diagnostics. *Periodontol 2000*. 2016;70(1):38–52. doi:10.1111/prd.12099
36. Arias-Bujanda N, Regueira-Iglesias A, Balsa-Castro C, Nibali L, Donos N, Tomás I. Accuracy of single molecular biomarkers in saliva for the diagnosis of periodontitis: A systematic review and meta-analysis. *J Clin Periodontol*. 2020;47(1):2–18. doi:10.1111/jcpe.13202
37. Mohammed A, Fadhil R, Mahmood MS, Al-Waeli HA. Diagnostic biomarkers for periodontitis (observational case–control study). *J Bagh Coll Dent*. 2024;36(2):11–19. doi:10.26477/jbcd.v36i2.3673
38. Harbood OM, Abbas RF. Salivary fractalkine differentiating periodontitis from periodontally healthy subjects. *Int J Dent*. 2024;2024:3007148. doi:10.1155/2024/3007148
39. Sorsa T, Alassiri S, Grigoriadis A, et al. Active MMP-8 (aMMP-8) as a grading and staging biomarker in the periodontitis classification. *Diagnostics (Basel)*. 2020;10(2):61. doi:10.3390/diagnostics10020061
40. Durgapal S, Shetty M. Effect of non-surgical periodontal therapy on the salivary levels of IL-18 and IL-35 in patients with periodontitis. *Dent Med Probl*. 2025;62(1):41–48. doi:10.17219/dmp/169387
41. Zhang Y, Kang N, Xue F, et al. Evaluation of salivary biomarkers for the diagnosis of periodontitis. *BMC Oral Health*. 2021;21(1):266. doi:10.1186/s12903-021-01600-5
42. Vlashi R, Zhang X, Wu M, Chen G. Wnt signaling: Essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders. *Genes Dis*. 2022;10(4):1291–1317. doi:10.1016/j.gendis.2022.07.011
43. Chatzopoulos GS, Koidou VP, Wolff LF. Expression of Wnt signaling agonists and antagonists in periodontitis and healthy subjects, before and after non-surgical periodontal treatment: A systematic review. *J Periodontal Res*. 2022;57(4):698–710. doi:10.1111/jre.13029
44. Page RC, Kornman KS. The pathogenesis of human periodontitis: An introduction. *Periodontol 2000*. 1997;14:9–11. doi:10.1111/j.1600-0757.1997.tb00189.x
45. Raheem ZJ, Abdulbaqi HR. Diagnostic potential of salivary interleukin-1β and IL-10 for distinguishing periodontal health from periodontitis and stable from unstable periodontitis: A case–control study. *Int J Dent*. 2024;2024:8006278. doi:10.1155/2024/8006278
46. Manokawinchoke J, Limjeerajarus N, Limjeerajarus C, Sastravaha P, Everts V, Pavasant P. Mechanical force-induced TGFB1 increases expression of SOST/POSTN by hPDL cells. *J Dent Res*. 2015;94(7):983–989. doi:10.1177/0022034515581372
47. Kim JH, Kim AR, Choi YH, et al. Tumor necrosis factor-α antagonist diminishes osteocytic RANKL and sclerostin expression in diabetes rats with periodontitis. *PLoS One*. 2017;12(12):e0189702. doi:10.1371/journal.pone.0189702
48. Diarra D, Stolina M, Polzer K, et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat Med*. 2007;13(2):156–163. doi:10.1038/nm1538
49. Hardy R, Juarez M, Naylor A, et al. Synovial DKK1 expression is regulated by local glucocorticoid metabolism in inflammatory arthritis. *Arthritis Res Ther*. 2012;14(5):R226. doi:10.1186/ar4065
50. Baek K, Hwang HR, Park HJ, et al. TNF-α upregulates sclerostin expression in obese mice fed a high-fat diet. *J Cell Physiol*. 2014;229(5):640–650. doi:10.1002/jcp.24487
51. Wijenayaka AR, Kogawa M, Lim HP, Bonewald LF, Findlay DM, Atkins GJ. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS One*. 2011;6(10):e25900. doi:10.1371/journal.pone.0025900
52. Fujita K, Janz S. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. *Mol Cancer*. 2007;6:71. doi:10.1186/1476-4598-6-71

53. Al-Ghurabi BH, Mohssen SM. Salivary level of RANKL and OPG in chronic periodontitis. *J Bagh Coll Dent*. 2015;27(1):189–194. https://www.jbcd.uobaghdad.edu.iq/index.php/jbcd/article/view/657/pdf_319. Accessed June 25, 2024.
54. Beiler TF, De Mello Neto JM, Alves JC, Hamlet S, Ipe D, Da Silva Figueredo CM. Impact of non-surgical periodontal treatment on salivary expression of cytokines related to bone metabolism. *Odontology*. 2020;108(4):646–652. doi:10.1007/s10266-020-00502-2
55. Ozden FO, Demir E, Lutfioglu M, Acarel EE, Bilgici B, Atmaca A. Effects of periodontal and bisphosphonate treatment on the gingival crevicular levels of sclerostin and Dickkopf-1 in postmenopausal osteoporosis with and without periodontitis. *J Periodontal Res*. 2022;57(4):849–858. doi:10.1111/jre.13023
56. Ashifa N, Viswanathan K, Srinivasan S, Kumar S, Sundaram R, Pavithran VK. Assessment of sclerostin levels in the gingival crevicular fluid of patients with periodontitis: A clinico-biochemical cross-sectional study. *J Adv Periodontol Implant Dent*. 2023;15(1):3–9. doi:10.34172/japid.2023.009
57. Chen Y, Hu Y. Wnt signaling activation in gingival epithelial cells and macrophages of experimental periodontitis. *Dent J (Basel)*. 2023;11(5):129. doi:10.3390/dj11050129
58. Mahmood AA, Abbas RF, Hussein HM. Novel association between single-nucleotide polymorphisms of *IKKβ* at rs17875746 and rs12676482 and periodontitis. *Dent Med Probl*. 2023;60(4):627–634. doi:10.17219/dmp/170879
59. Schulz J, Knappe C, Graetz C, et al. Secreted Frizzled-related protein 5 serum levels in human periodontitis – a nested case–control study. *J Clin Periodontol*. 2019;46(5):522–528. doi:10.1111/jcpe.13087
60. Miranda TS, Napimoga MH, Feres M, et al. Antagonists of Wnt/β-catenin signalling in the periodontitis associated with type 2 diabetes and smoking. *J Clin Periodontol*. 2018;45(3):293–302. doi:10.1111/jcpe.12854
61. Chatzopoulos GS, Costalonga M, Mansky KC, Wolff LF. WNT-5a and SOST levels in gingival crevicular fluid depend on the inflammatory and osteoclastogenic activities of periodontal tissues. *Medicina (Kaunas)*. 2021;57(8):788. doi:10.3390/medicina57080788
62. Shen L, Zhou S, Glowacki J. Effects of age and gender on WNT gene expression in human bone marrow stromal cells. *J Cell Biochem*. 2009;106(2):337–343. doi:10.1002/jcb.22010
63. Chen N, Wang J. Wnt/β-catenin signaling and obesity. *Front Physiol*. 2018;9:792. doi:10.3389/fphys.2018.00792

Original paper

Comparative analysis of dental students' preferences and satisfaction with procedures and outcomes using resin 3D-printing and conventional methods for provisional restoration fabrication

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Abstract

Background. The integration of computer-aided design/computer-aided manufacturing (CAD/CAM) into dental curricula has enabled students to learn crown preparation using both CAD/CAM technology and the conventional methods, as well as to compare the accuracy of onlay designs and the overall quality of restorations.

Objectives. The aim of the present study was to investigate the impact of a comprehensive CAD/CAM exercise on second-year dental students' perceptions of the digital workflow for provisional indirect restoration fabrication as compared to the conventional provisional restoration fabrication techniques.

Material and methods. The inclusion criteria comprised second-year dental students who completed both the practical activity and the survey. Students who did not complete either the practical activity or the survey were excluded. The exercise simulated a clinical scenario in a pre-clinical simulation laboratory, using a typodont model. Students prepared 2 first molar teeth for a non-metallic onlay restoration – tooth 36, using the conventional method, and tooth 46, using a three-dimensional (3D) printer. A seven-item questionnaire was developed to evaluate areas of interest, including technique preference, satisfaction with the fit of the restoration, its anatomy, interproximal contacts, the intaglio surface, occlusal adjustments, and the ease of polishing.

Results. A total of 125 students (52.74%) completed the proposed activity. Among the variables examined, only the interproximal contacts of the 3D-printed onlays demonstrated statistical significance ($p = 0.0178$). Significant differences between the conventional and 3D-printing methods were observed across several survey parameters, including preference, fit, anatomy, interproximal contacts (within each cohort), the intaglio surface, and occlusal aspects ($p < 0.001$).

Conclusions. The CAD/CAM provisional indirect restorations were rated better by second-year students than the provisional restorations fabricated using the conventional method.

Keywords: CAD/CAM, practice management, dental students, technology, digital dentistry

Highlights

- Computer-aided design/computer-aided manufacturing (CAD/CAM) in dental curricula helps to prepare dental students for their future practice, providing the most up-to-date treatment for patients.
- Onlay preparation using CAD/CAM significantly influences dental students' perceptions of the digital workflow for restorations.
- Evaluating how CAD/CAM exercises influence students' perceptions of the digital workflow compared with the conventional techniques is essential for improving dental education.

Introduction

Rehabilitation and esthetics are important parameters widely analyzed in contemporary dentistry.[1–3] These factors, combined with technological advances, have increased the precision and acceptance of dental treatment.[4,5] The emergence of digital dentistry, including computer-aided design/computer-aided manufacturing (CAD/CAM) technology, reflects this evolution.[6,7] In this context, CAD/CAM composite restorative materials have become increasingly accessible for subtractive fabrication procedures.[8]

In modern dentistry, provisional indirect restorations play a crucial role in protecting the prepared teeth while maintaining function and esthetics during treatment. However, the conventional fabrication methods are often time-consuming and highly dependent on the operator's skill. With the advent of three-dimensional (3D) printing, there has been a shift toward more efficient and precise restorative solutions. In parallel, the implementation of CAD/CAM technology in dental curricula has been increasingly reported across institutions, with studies demonstrating that many dental schools recognize the importance of incorporating this technology into their educational programs.[4,9,10] A survey revealed that 76% of dental schools in the United States have integrated CAD/CAM into their preclinical courses, while 58% also provide clinical experience related to CAD/CAM restorations.[11] This transition toward digital dentistry is essential, as it prepares students for the demands of contemporary dental practice, where CAD/CAM technology is increasingly adopted.[10,12]

Despite the increasing presence and use of CAD/CAM technology, a notable gap remains in students' knowledge and confidence regarding these systems. A national survey reported that many predoctoral dental students demonstrated limited understanding of CAD/CAM technology, restoration design, and the materials used in these procedures.[7] This lack of familiarity is concerning, particularly because CAD/CAM systems play a central role in the fabrication of indirect restorations, which are among the most commonly performed dental restorations.[13,14] Furthermore, although students are exposed to CAD/CAM technology during training, their practical experience and proficiency with these systems vary considerably, emphasizing the need for more comprehensive instruction and hands-on practice.[15,16]

Research has shown that students who engage with CAD/CAM technology tend to perform better indirect restoration preparations than those trained exclusively with the conventional methods.[17,18] The use of CAD/CAM improves the accuracy of restoration design and enhances the overall quality of restorations produced by students.[19] In addition, this technology enables immediate feedback and assessment, which are important components of the learning process.[18] As dental schools continue to adapt their curricula to incorporate CAD/CAM training, it is essential to address the educational gaps identified in students' knowledge and skills to maximize the benefits of this technology.[9,10]

Although previous studies have investigated the mechanical PROPERTIES of 3D-printed and conventionally fabricated restorations, limited attention has been given to dental students' perspectives regarding these techniques, particularly in relation to the ease of use, efficiency and clinical outcomes. Understanding students' preferences and satisfaction is important for guiding future curricular development and improving clinical training. Therefore, the aim of this study was to investigate and compare the satisfaction and preferences of second-year dental students through a survey and practical activity involving provisional onlays fabricated using CAD/CAM and the conventional techniques. The hypothesis was that CAD/CAM exercises would positively influence students' performance and perceptions.

Material and methods

This study was submitted to the Ethics Committee of The Ohio State University, Columbus, USA, received an exemption approval (No. 20220799), and was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. The second-year dental curriculum at the College of Dentistry includes fixed restorative

and prosthodontic dentistry. During their preclinical education, students are introduced to digital impression acquisition (scanning) and computer-aided design (CAD), while computer-aided manufacturing (CAM) is currently being implemented into the curriculum. As part of efforts to enhance the educational program, a comprehensive CAD/CAM exercise was incorporated into the 4th course of the restorative curriculum for second-year dental students.

Sample selection and eligibility criteria

The study sample consisted of dental students enrolled in the 4th course of the restorative curriculum during 2022 and 2023. Participation was voluntary, and all second-year dental students enrolled in the operative dentistry course were eligible to participate. Students were required to complete the proposed activity and respond to the survey.

The inclusion criteria were enrollment as a second-year student at the College of Dentistry and the completion of both the activity and the survey. The exclusion criteria consisted of failure to complete either the activity or the survey.

Activity proposed

The exercise simulated a clinical scenario in a preclinical simulation laboratory, using a typodont mannequin. Dental students prepared 2 ivorine molar teeth for a non-metallic onlay restoration – tooth 36 (mandibular left first molar) and tooth 46 (mandibular right first molar) (Nissin Dental Products, Kyoto, Japan). The prepared teeth were mounted in the mannequin throughout the simulation.

A digital impression of tooth 46 was obtained using the TRIOS 3 intraoral scanner (3Shape, Copenhagen, Denmark). With the support of blended learning resources, tutorial videos and faculty guidance, students completed the CAD design of their onlays, using the 3Shape Design Studio software. The designed restorations were exported in the Standard Tessellation Language (STL) format and imported into the AnyCubic Workshop software (Anycubic Technology, Shenzhen, China) for slicing and 3D printing preparation. Using the Photon M2 DLP 3D printer (Anycubic), students fabricated individualized 3D-printed resin restorations corresponding to their prepared teeth. The restorations were subsequently processed and light-cured in a 3D-printing laboratory. The simulation concluded with students seating, adjusting (the occlusal, proximal and intaglio surfaces) and cementing the 3D-printed restorations with the use of the Temp-Bond™ NE provisional cement (KaVo Kerr, Brea, USA). The hands-on exercise was complemented by CAD/CAM lectures and a collaborative session with the College of Engineering, providing a multidisciplinary educational approach.

In the second part of the exercise, students fabricated a provisional onlay, using the direct technique with the Provisa® Plus bis-acryl material (Benco Dental, Pittston, USA), which was molded in an external surface form (ESF) fabricated from vacuum-formed polypropylene sheets over stone casts. This procedure was performed on the contralateral typodont tooth 36, which had been prepared using the same design as tooth 46. Students then polished, finished and adjusted the occlusion of the provisional restorations.

The consistency between both exercises allowed students to gain firsthand experience with both the conventional direct bis-acryl technique and the CAD/CAM workflow for the fabrication of indirect resin provisional restorations.

For both activities (3D-printed and resin provisional restorations), 6 parameters were evaluated: (1) crown fit; (2) anatomy; (3) interproximal contacts; (4) the intaglio surface, (5) occlusion; and (6) polishability. The evaluation criteria for all parameters were presented during the class activities to guide students toward achieving a standardized outcome.

Survey applied

This study aimed to investigate the impact of the proposed exercise on students' perceptions of the digital workflow for provisional indirect restoration fabrication in comparison with the conventional techniques, given the importance of CAD/CAM dentistry in the predoctoral academic setting. To address these objectives, a survey was administered to the intervention group in both cohorts (second-year dental students) after the completion of the comprehensive CAD/CAM exercise to evaluate their perceptions of the quality of restorations obtained through the 2 workflows.[4] The survey was anonymous and clearly stated that responses would not affect students' academic evaluation or interfere with their educational experience.

Seven survey questions were designed to evaluate the areas of interest: the comparison of technique preference; satisfaction with the restoration fit; satisfaction with the restoration anatomy; satisfaction with interproximal adjustment; satisfaction with intaglio surface adjustment; satisfaction with occlusal adjustment; and satisfaction with restoration polishability. The responses were recorded using a Likert scale ranging from 1 (lowest) to 10 (highest). The questions were as follows:

– (Question 1A) Please rate your preference for the 3D-printed provisional indirect restoration fabrication technique;
– (Question 1B) Please rate your preference for the conventional resin provisional restoration fabrication technique (ESF + Provisa);
– (Question 2A) Please rate your satisfaction with the fit of the 3D-printed provisional indirect restoration;

– (Question 2B) Please rate your satisfaction with the fit of the conventional resin provisional restoration (ESF + Provisa);
– (Question 3A) Please rate your satisfaction with the anatomy of the 3D-printed provisional indirect restoration;
– (Question 3B) Please rate your satisfaction with the anatomy of the conventional resin provisional restoration (ESF + Provisa);
– (Question 4A) Please rate your satisfaction with the interproximal adjustment required for the 3D-printed provisional indirect restoration;
– (Question 4B) Please rate your satisfaction with the interproximal adjustment required for the conventional resin provisional restoration (ESF + Provisa);
– (Question 5A) Please rate your satisfaction with the intaglio surface adjustment required for the 3D-printed provisional indirect restoration;
– (Question 5B) Please rate your satisfaction with the intaglio surface adjustment required for the conventional resin provisional restoration (ESF + Provisa);
– (Question 6A) Please rate your satisfaction with the occlusal adjustment required for the 3D-printed provisional indirect restoration;
– (Question 6B) Please rate your satisfaction with the occlusal adjustment required for the conventional resin provisional restoration (ESF + Provisa);
– (Question 7A) Please rate your satisfaction with the polishability of the 3D-printed provisional indirect restoration; and
– (Question 7B) Please rate your satisfaction with the polishability of the conventional resin provisional restoration (ESF + Provisa).

All scores provided by the students were recorded. Two authors (LMN and GVOF) evaluated both the activities and the submitted responses during the post-intervention phase. The post-intervention assessment consisted of evaluating the provisional restorations fabricated after the demonstration and analyzing the responses obtained from the survey. The authors classified the responses according to their reliability using the following scale: 1 = unreliable or missing response; 2 = somewhat reliable; 3 = moderately reliable; and 4 = highly reliable response.

Cohort groups

The invitation to participate in this study was sent to 118 second-year dental students (55 males and 63 females) in the 1st cohort and 119 students (55 males and 64 females) in the 2nd cohort, totaling 237 participants. The age range/mean age was 20–38/22 years and 19–41/22 years, respectively.

Following the instructional steps demonstrated by the faculty, students designed and printed 237 provisional indirect restorations and fabricated 237 conventional resin provisional restorations fitted to their prepared typodont teeth.

Statistical analysis

The collected data was organized in a spreadsheet for statistical analysis. Data normality was assessed using the Kolmogorov–Smirnov test. Subsequently, paired *t* tests were performed to compare the 3D-printing and conventional methods for each survey question. Descriptive analysis was also conducted. Logistic regression analysis was used to determine whether differences between cohorts were significant enough to justify separate analyses, aiming to identify potential cohort-related disparities. Statistical analyses were performed using the R software, v. 3.6.1 (R Core Team, 2021; https://www.r-project.org), with a significance level set at 0.05.

Results

Of the 118 students from the 1st cohort, 77 responded to the questionnaire (65.25%), whereas in the 2nd cohort, 48 of the 119 students responded (40.34%). Overall, 125 of the 237 invited dental students completed the survey, corresponding to a response rate of 52.74%. The collected results were summarized using means (*M*) and standard deviations (*SD*), and are presented in Table 1.

A logistic regression model was initially employed, with cohort used as the outcome variable (Table 2). Among the variables analyzed, only the interproximal contact parameter of the 3D-printed indirect restorations demonstrated statistical significance (p = 0.0178), indicating a notable difference between cohorts for this specific variable. Importantly, diagnostic assessments revealed no evidence of collinearity within the model.

Table 1. Summary of the statistics with regard to both cohorts and the overall results

Parameter		Overall (N = 125)	Cohort 1 (n = 77)	Cohort 2 (n = 48)
Technique preference	resin	5.57 ±2.37	5.90 ±2.44	5.04 ±2.18
	3D-printed	8.44 ±1.45	8.45 ±1.45	8.42 ±1.47
Fit	resin	6.45 ±2.22	6.47 ±2.33	6.42 ±2.06
	3D-printed	8.22 ±1.58	8.22 ±1.49	8.21 ±1.71
Anatomy	resin	7.18 ±2.00	7.43 ±1.86	6.77 ±2.15
	3D-printed	7.98 ±1.56	8.00 ±1.55	7.96 ±1.60
Interproximal contacts	resin	4.58 ±2.36	4.66 ±2.34	4.44 ±2.41
	3D-printed	8.57 ±1.77	8.95 ±1.27	7.98 ±2.24
Intaglio surface	resin	6.90 ±2.39	7.25 ±2.40	6.33 ±2.27
	3D-printed	8.52 ±1.70	8.68 ±1.58	8.26 ±1.87
Occlusion	resin	6.21 ±2.32	6.25 ±2.30	6.15 ±2.37
	3D-printed	7.67 ±1.92	7.73 ±1.74	7.58 ±2.18
Polishability	resin	7.34 ±2.22	7.58 ±2.16	6.96 ±2.29
	3D-printed	7.19 ±2.08	7.35 ±2.04	6.94 ±2.14
Cohort	1	77 (61.6)	77 (100.0)	0 (0.0)
	2	48 (38.4)	0 (0.0)	48 (100.0)

Data presented as mean ± standard deviation (*M* ±*SD*) or as number (percentage) (*n* (%)).

Table 2. Logistic regression analysis comparing the results between the cohorts

Parameter		Estimate	*SE*	Statistic	*p*-value
(Intercept)		2.354	1.699	1.386	0.166
Technique preference	resin	−0.152	0.138	−1.100	0.272
	3D-printed	−0.074	0.188	−0.395	0.693
Fit	resin	0.200	0.138	1.449	0.147
	3D-printed	0.320	0.242	1.326	0.185
Anatomy	resin	−0.172	0.161	−1.067	0.286
	3D-printed	−0.030	0.192	−0.159	0.874
Interproximal contacts	resin	0.002	0.134	0.016	0.988
	3D-printed	−0.439	0.185	−2.371	0.018*
Intaglio surface	resin	−0.200	0.132	−1.515	0.130
	3D-printed	0.019	0.157	0.120	0.905
Occlusion	resin	0.138	0.126	1.098	0.272
	3D-printed	0.108	0.144	0.753	0.452
Polishability	resin	0.041	0.133	0.309	0.757
	3D-printed	−0.121	0.125	−0.967	0.334

SE – standard error; * statistically significant.

All groups demonstrated normal distribution. Therefore, paired *t* tests were considered appropriate for comparing the resin and 3D-printing methods across the survey questions, as this is a parametric statistical test. Since the logistic regression analysis identified a significant association between the "interproximal contacts_3D" variable and the cohort status, separate *t* tests were performed within each cohort for this parameter (Table 3). The *t* test results revealed significant differences between the resin and 3D-printing methods across several survey parameters. Significant differences were observed for technique preference, fit, anatomy, interproximal contacts (analyzed separately within each cohort), the intaglio surface, and occlusion ($p < 0.001$), indicating distinct perceptions and outcomes between the 2 fabrication methods. However, no statistically significant difference was found between the resin and 3D-printing methods regarding polishability ($p = 0.5540$), suggesting similar performance for this parameter across cohorts.

Table 3. Statistical comparison of the clinical evaluation criteria between resin-based and 3D-printing procedures

Question	Cohort	Estimated difference (resin−3D)	*p*-value
Technique preference	combined	−2.8720	<0.001*
Fit	combined	−1.7500	<0.001*
Anatomy	combined	−0.7903	<0.001*
Interproximal contacts	1	−4.3158	<0.001*
Interproximal contacts	2	−3.5417	<0.001*
Intaglio surface	combined	−1.6371	<0.001*
Occlusion	combined	−1.4640	<0.001*
Polishability	combined	0.1613	0.554

The 'Estimated difference' column reflects the mean difference (resin score minus 3D score); negative values indicate superior performance with the 3D-printing method; interproximal contacts are analyzed individually by cohort. * statistically significant.

Students' scores for the Part A questions (3D-printed activity) are presented in Table 1. The average authors' reliability scores for these questions were 3.02 (LMN) and 3.31 (GVOF), indicating moderately reliable student responses. For the Part B questions, the mean reliability scores were 3.44 (LMN) and 3.10 (GVOF), which also reflected a moderate level of reliability in the responses submitted by students.

Discussion

The aim of this study was to investigate the impact of a comprehensive CAD/CAM exercise on second-year dental students' perceptions of the digital workflow for provisional indirect restoration fabrication in comparison with the conventional techniques. The findings demonstrated that, according to students' perceptions, the digital workflow achieved better overall outcomes than traditional methods.

The integration of CAD/CAM technology into dental education has become increasingly important, particularly in indirect restoration preparation. This technology not only enhances the learning experience of dental students, but also aligns with the evolving demands of modern dental practice. CAD/CAM systems enable the digital design and fabrication of dental restorations, including crowns, bridges, onlays, and inlays.[4] Since the 2010s, several studies have reported on the impact of CAD/CAM technology on academic performance and educational outcomes. Dehghan et al. described the introduction of CAD/CAM technology at the University of Tennessee College of Dentistry, the first dental school in the United States to integrate it into the fourth-year curriculum.[20] The authors concluded that CAD/CAM technology served as a valuable educational tool for dental students, offering cost-effective improvement alongside exceptional patient care.[20]

In a study by Lam et al., the preferences and perceptions of final-year dental students regarding intraoral scanning and the conventional impression-making techniques were investigated.[21] Data collected through an online questionnaire showed that although many students preferred intraoral scanning, some continued to favor conventional impression-making because of its perceived efficiency. Both groups recognized the advantages of intraoral scanning, particularly in defect identification and infection control. Notably, the "pro-scanning" group reported that intraoral scanning required less chairside assistance, was easier for beginners to learn, and allowed more efficient use of scanner software. Regression analysis indicated that students tended to prefer techniques they perceived as more efficient, leading the authors to conclude that, despite its advantages, conventional impression-making remained preferable for some students.[21]

In contrast, the present study evaluated dental students' perceptions of the digital workflow for provisional restoration fabrication in 2 cohorts participating in a comprehensive CAD/CAM exercise. The exercise included CAD design, 3D printing and provisional onlay seating, and survey responses were used to compare preferences between the 3D-printed and conventionally fabricated resin restorations. In the first stage of the analysis, a logistic regression model was applied to assess differences between the cohorts. Among the evaluated variables, "interproximal contacts_3D" was the only statistically significant factor ($p = 0.0178$), indicating differences in interproximal perceptions between the cohorts. Importantly, no collinearity issues were detected, supporting the robustness of the regression model.

Moreover, significant differences were observed between the resin and 3D-printing methods across several parameters, including the overall preference, the restoration fit, anatomy, interproximal adjustment (within each cohort), intaglio adjustment, and occlusal adjustment ($p < 0.001$), as well as factors that may influence color, translucency or whiteness.[22] These findings indicate distinct perceptions and outcomes associated with each fabrication method. The results further underscore the importance of integrating CAD/CAM exercises into dental curricula, as students demonstrated a preference for 3D printing in multiple aspects of the provisional fabrication process. However, no significant differences were identified between the 2 methods regarding polishability ($p = 0.5540$), suggesting comparable performance in this domain across cohorts.

Research indicates that CAD/CAM provisional restorations exhibit superior mechanical properties, particularly in terms of fracture resistance and flexural strength. It is important to highlight that 3D-printable materials may also display varying mechanical properties, which can directly influence the outcomes obtained.[23–25] Pantea et al. demonstrated that both 3D-printed and CAD/CAM-milled provisional prostheses outperformed conventionally fabricated restorations by achieving higher fracture resistance values.[26] Similarly, Alam et al. reported that CAD/CAM provisional crowns exhibited enhanced physical properties, supporting their indication for long-term provisional restorations.[27] Furthermore, a systematic review conducted by Jain et al. concluded that CAD/CAM technology improved the overall quality of provisional crowns, demonstrating superior mechanical and physical properties in comparison with conventional alternatives.[28] These enhanced properties suggest that CAD/CAM techniques may be better suited for environments subjected to mechanical stress, thereby contributing to the longevity and stability of provisional restorations.

Regarding fit, studies have consistently shown that CAD/CAM provisional restorations provide superior marginal and internal adaptation compared with manually fabricated ones. Abdullah et al. reported that CAD/CAM-fabricated crowns exhibited smaller marginal gaps than directly fabricated restorations, thereby reducing the potential for bacterial contamination and improving the protection of the underlying tooth structure.[29] These findings are supported by additional studies published in 2017[30] and 2019,[31] which demonstrated that CAD/CAM crowns achieved superior internal fit when evaluated using different measurement techniques. Reduced marginal discrepancies are critical for minimizing microleakage and enhancing the overall clinical performance of provisional restorations, which is fundamental to successful restorative dentistry.

Moreover, the benefits of using of CAD/CAM technology extend to color stability and esthetic outcomes. Rayyan et al. reported that CAD/CAM interim restorations demonstrated superior color stability and marginal quality, representing a significant advantage for esthetic restorations.[32] In addition, advances in CAD/CAM materials, including highly biocompatible resins, suggest that CAD/CAM-fabricated restorations are capable of fulfilling both functional and esthetic requirements more effectively than conventional materials.[33]

Despite the well-documented advantages of CAD/CAM techniques, the conventional methods remain widely used because of their simplicity and ease of repair. As noted by Pantea et al., direct techniques utilizing composite resins may facilitate immediate chairside adjustments and repairs when necessary.[26] Nevertheless, the growing body of evidence suggests that the long-term benefits of CAD/CAM provisional restorations, including enhanced durability, superior fit and improved esthetic outcomes, make them a more favorable option for contemporary dental practice.

A study by Douglas et al. examined students' performance in crown design using CAD/CAM vs. conventional wax methods.[17] While students demonstrated a strong preference for CAD/CAM, their morphological evaluations were comparable between the 2 techniques, suggesting that CAD/CAM, despite its appeal, may not offer a definitive advantage in terms of anatomical accuracy.[17] In line with these findings, the present study found no significant difference between resin and 3D-printed provisional onlays regarding anatomical fidelity and polishability. These outcomes suggest that while CAD/CAM offers numerous advantages in workflow efficiency and student engagement, its ability to produce superior morphology remains limited. Together, these studies underscore the need for a balanced approach in dental education, recognizing both the advantages and constraints of the emerging technologies like CAD/CAM.

Nassani et al. demonstrated that all groups showed a high level of interest in digital dentistry,[4] a finding consistent with another study.[34] As a result, more than 60% of the students contributed to the sample size in their research.[4] A similar trend was observed in the present study, with an average participation rate of 52.74% (65.25% in the 1st

cohort and 40.34% in the 2nd cohort). This variation raises questions regarding the consistency of students' actual interest. However, the appropriate and timely integration of digital technologies into predoctoral education remains essential for optimizing the learning environment.[35]

The present findings also support previous research. Another study by Nassani et al. reported satisfaction among dental professionals regarding the marginal accuracy and anatomical detail of CAD/CAM restorations,[36] while Refeis et al.'s study demonstrated high student satisfaction with the fit and adaptation of CAD/CAM crowns.[37]

Study limitations and insights

The fact that the 2 cohorts were significantly different with regard to the "interproximal contact_3D" variable, together with the single-institution design, a small convenience sample and a low response rate, may introduce bias into the results. In addition, the study relied exclusively on subjective outcome measures based on Likert-scale questions, without incorporating qualitative data or the assessment of clinical competence. As such, it reflects only students' perceptions of the exercise and does not evaluate actual performance or meaningful learning outcomes.

Furthermore, 2 teeth were prepared (36 and 46) instead of a single standardized tooth, which may have improved comparative control by reducing the confounding variables, such as side preference and differences in preparation quality. The use of typodont teeth for provisional restorations also limits the direct extrapolation of the findings to clinical settings involving definitive restorations. In addition, the difference in the materials used for the 2 types of temporary crowns may have had a significant influence on the results obtained. Finally, the questionnaire employed was not validated, as no content validity assessment or confirmatory factor analysis (CFA) was performed.

These limitations should be taken into account when interpreting the results and in the design of future studies on CAD/CAM integration into dental education.

Future research recommendations

Future research should investigate the long-term effects of CAD/CAM exercises on students' learning outcomes and clinical performance. In addition, evaluating the complexity of design software and the chairside efficiency associated with each technique would provide a more comprehensive understanding of the role of digital technology in dental education, thereby enabling educators to further refine curricula in accordance with the ongoing technological advancements. Furthermore, comparing the results obtained in this study with those of experienced dental professionals may help identify potential differences between students and clinicians regarding the perception and application of CAD/CAM workflows.

Conclusions

This study demonstrated that the CAD/CAM exercise achieved more favorable outcomes than the conventional restoration fabrication techniques, significantly influencing dental students' perceptions of digital workflows for provisional restorations and supporting the proposed positive hypothesis. Students showed a preference for 3D-printed restorations, particularly regarding fit and marginal adaptation, in agreement with the findings reported in the existing literature. The integration of such exercises into dental curricula may help prepare future dentists with the knowledge and skills required to navigate the evolving field of digital dentistry. Nevertheless, further research is needed to investigate long-term educational outcomes and broader clinical applications.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Ohio State University, Columbus, USA (No. of approval: 20220799). Written informed consent was obtained from all the participants.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Kahn S, Rodrigues WJdPR, Dias AT, Resende R, Fernandes JCH, Fernandes GVdO. Crown lengthening surgery in the esthetic area for optimized results: A review with surgical recommendations. *Surgeries*. 2024;5(4):1043–1055. doi:10.3390/surgeries5040084
2. De Siqueira GRC, Tavares JR, Pedrosa RF, De Siqueira RAC, Fernandes GVdO. Immediate implant with provisionalization and soft tissue grafting after 4-year follow-up. *Clin Adv Periodontics*. 2022;12(1):32–38. doi:10.1002/cap.10162
3. Cunha J, Fernandes GVdO, Fernandes JCH, Lopes PC, Rio R. The interference of age and gender on smile characterization analyzed on six parameters: A clinical-photographic pilot study. *Medicina (Kaunas)*. 2023;59(3):595. doi:10.3390/medicina59030595

4. Nassani LM, Bencharit S, Schumacher F, Lu WE, Resende R, Fernandes GVdO. The impact of technology teaching in the dental predoctoral curriculum on students' perception of digital dentistry. *Dent J (Basel)*. 2024;12(3):75. doi:10.3390/dj12030075
5. Kahn S, Kahn MB, Zimmermman D, Dias AT, Fernandes GVO. Developing a periodontal surgical guide to reach better performance in crown-lengthening procedures: A case report. *Perio Clínica*. 2022;22(2):47–59.
6. Brito Leitão CI, Fernandes GVdO, Pereira Azevedo LP, Araújo FM, Donato H, Maia Correia AR. Clinical performance of monolithic CAD/CAM tooth-supported zirconia restorations: Systematic review and meta-analysis. *J Prosthodont Res*. 2022;66(3):374–384. doi:10.2186/jpr.JPR_D_21_00081
7. Fernandes GVdO, Nassani LM. The implementation of CAD/CAM technology at schools of dentistry: A short communication. *Env Dent J*. 2023;5(1):1–3. doi:10.12944/EDJ.05.01.03
8. Ling L, Ma Y, Malyala R. A novel CAD/CAM resin composite block with high mechanical properties. *Dent Mat*. 2021;37(7):1150–1155. doi:10.1016/j.dental.2021.03.006
9. Islam MS, Al-Fakhri A, Rahman M. Computer aided design/computer aided manufacturing (CAD/CAM) technology in the undergraduate dental programs in the MENA region. *Eur J Dent Educ*. 2023;28(1):142–147. doi:10.1111/eje.12930
10. Fischer K, Metz M, Sekula M, Katwal D, Hannigan D, Williams T. Effect of educational intervention on computer-aided-design and computer-aided-manufacturing technology to preclinical dental students. *Eur J Dent Educ*. 2023;28(1):320–327. doi:10.1111/eje.12953
11. Ishida Y, Kuwajima Y, Kobayashi T, et al. Current implementation of digital dentistry for removable prosthodontics in US dental schools. *Int J Dent*. 2022;2022:7331185. doi:10.1155/2022/7331185
12. Vlagos K, Salazar D, Reale A, et al. Implementation of a single-tooth pre-doctoral CAD/CAM dentistry curriculum at UIC: History, description, SWOT analysis, and quantitative evaluation. *Educ Sci*. 2021;11(6):301. doi:10.3390/educsci11060301
13. Alhamed FJ, Neiva GF, Bak SY, Karl E, Inglehart MR. Pre-doctoral dental students' computer-aided design/computer-aided manufacturing-related education, knowledge, attitudes and behavior: A national survey. *J Dent Educ*. 2022;87(4):562–571. doi:10.1002/jdd.13144
14. Alhussain BS, Almawh AA, AlMuhanna AS, et al. Dentists' perception about chair-side CAD/CAM; a cross-sectional study in Riyadh, Saudi Arabia. *Arch Pharm Pract*. 2022;13(1):46–52. doi:10.51847/YYj7R47uCt
15. Callan RS, Palladino CL, Furness AR, Bundy EL, Ange BL. Effectiveness and feasibility of utilizing E4D technology as a teaching tool in a preclinical dental education environment. *J Dent Educ*. 2014;78(10):1416–1423. doi:10.1002/j.0022-0337.2014.78.10.tb05815.x
16. Schwindling FS, Deisenhofer UK, Porsche M, Rammelsberg P, Kappel S, Stober T. Establishing CAD/CAM in preclinical dental education: Evaluation of a hands-on module. *J Dent Educ*. 2015;79(10):1215–1221. doi:10.1002/j.0022-0337.2015.79.10.tb06015.x
17. Douglas RD, Hopp CD, Augustin MA. Dental students' preferences and performance in crown design: Conventional wax-added versus CAD. *J Dent Educ*. 2014;78(12):1663–1672. doi:10.1002/j.0022-0337.2014.78.12.tb05845.x
18. Mays KA, Crisp HA, Vos P. Utilizing CAD/CAM to measure total occlusal convergence of preclinical dental students' crown preparations. *J Dent Educ*. 2016;80(1):100–107. doi:10.1002/j.0022-0337.2016.80.1.tb06063.x
19. Siegel SC, Kramer SB, Deranek KM. Lean methods applied to CAD/CAM pedagogy in the dental simulation laboratory. *J Dent Educ*. 2019;83(9):1081–1091. doi:10.21815/JDE.019.100
20. Dehghan M, Simon JF, Harrison J. Integrating the CEREC technology at UT College of Dentistry. *J Tenn Dent Assoc*. 2012;92(1):19–21. PMID:22870548.
21. Lam WYH, Mak KCK, Maghami E, Molinero-Mourelle P. Dental students' preference and perception on intraoral scanning and impression making. *BMC Med Educ*. 2021;21(1):501. doi:10.1186/s12909-021-02894-3
22. Dal Piva AMdO, Verhoeff H, da Rosa LS, Pereira GKR, Kleverlaan CJ, Tribst JPM. Optical properties of advanced lithium disilicate. *Dent Med Probl*. 2025;62(4):691–699. doi:10.17219/dmp/188369
23. Paradowska-Stolarz AM, Wieckiewicz M, Mikulewicz M, et al. Comparison of the tensile modulus of three 3D-printable materials used in dentistry. *Dent Med Probl*. 2023;60(3):505–511. doi:10.17219/dmp/166070
24. Paradowska-Stolarz A, Wieckiewicz M, Kozakiewicz M, Jurczyszyn K. Mechanical properties, fractal dimension, and texture analysis of selected 3D-printed resins used in dentistry that underwent the compression test. *Polymers (Basel)*. 2023;15(7):1772. doi:10.3390/polym15071772
25. Meissner H, Vacquier M, Kresse-Walczak K, Boening K. Mechanical, optical and surface properties of 3D-printed and conventionally processed polyamide 12. *Dent Med Probl*. 2024;61(5):729–738. doi:10.17219/dmp/186712
26. Pantea M, Ciocoiu R, Greabu M, et al. Compressive and flexural strength of 3D-printed and conventional resins designated for interim fixed dental prostheses: An in vitro comparison. *Materials (Basel)*. 2022;15(9):3075. doi:10.3390/ma15093075
27. Alam M, Chugh A, Kumar A, Rathee M, Jain P. Comparative evaluation of fracture resistance of anterior provisional restorations fabricated using conventional and digital techniques – an in vitro study. *J Indian Prosthodont Soc*. 2022;22(4):361–367. doi:10.4103/jips.jips_547_21
28. Jain S, Sayed ME, Shetty M, et al. Physical and mechanical properties of 3D-printed provisional crowns and fixed dental prosthesis resins compared to CAD/CAM milled and conventional provisional resins: A systematic review and meta-analysis. *Polymers (Basel)*. 2022;14(13):2691. doi:10.3390/polym14132691
29. Abdullah AO, Tsitrou E, Pollington S. Comparative in vitro evaluation of CAD/CAM vs conventional provisional crowns. *J Appl Oral Sci*. 2016;24(3):258–263. doi:10.1590/1678-775720150451
30. Lee WS, Lee DH, Lee KB. Evaluation of internal fit of interim crown fabricated with CAD/CAM milling and 3D printing system. *J Adv Prosthodont*. 2017;9(4):265–270. doi:10.4047/jap.2017.9.4.265
31. Peng CC, Chung KH, Ramos Jr. V. Assessment of the adaptation of interim crowns using different measurement techniques. *J Prosthodont*. 2019;29(1):87–93. doi:10.1111/jopr.13122
32. Rayyan MM, Aboushelib M, Sayed NM, Ibrahim A, Jimbo R. Comparison of interim restorations fabricated by CAD/CAM with those fabricated manually. *J Prosthet Dent*. 2015;114(3):414–419. doi:10.1016/j.prosdent.2015.03.007
33. Benli M, Eker-Gümüş B, Kahraman Y, Huck O, Özcan M. Can polylactic acid be a CAD/CAM material for provisional crown restorations in terms of fit and fracture strength? *Dent Mat J*. 2021;40(3):772–780. doi:10.4012/dmj.2020-232
34. Sharab L, Adel M, Abualsoud R, et al. Perception, awareness, and attitude toward digital dentistry among pre-dental students: An observational survey. *Bull Natl Res Cent*. 2022;46:246. doi:10.1186/s42269-022-00937-3
35. Afshari FS, Sukotjo C, Alfaro MF, et al. Integration of digital dentistry into a predoctoral implant program: Program description, rationale, and utilization trends. *J Dent Educ*. 2017;81:986–994. doi:10.21815/JDE.017.050
36. Nassani MZ, Ibraheem S, Shamsy E, Darwish M, Faden A, Kujan O. A survey of dentists' perception of chair-side CAD/CAM technology. *Healthcare (Basel)*. 2021;9(1):68. doi:10.3390/healthcare9010068
37. Reifeis PE, Kirkup ML, Willis LH, Browning WD. Introducing CAD/CAM into a predoctoral dental curriculum: A case study. *J Dent Educ*. 2014;78(10):1432–1441. PMID:25281677.

Original paper

Papilla characteristics and gingival exhibit in the interdental and gingival smile line: An age- and gender-based evaluation

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Abstract

Background. The interdental papillae, constrained within the complex configuration of interproximal embrasures, play a significant role in dental esthetics.

Objectives. The aim of the present study was to evaluate papilla characteristics and gingival exhibit in the interdental smile line (ISL) and the gingival smile line (GSL) with reference to age and gender.

Material and methods. A total of 120 periodontally healthy patients aged 20–60 years were equally divided into 4 groups: group I – male patients aged 20–40 years; group II – female patients aged 20–40 years; group III – male patients aged 41–60 years; and group IV – female patients aged 41–60 years. The maxillary anterior teeth and the corresponding gingival tissues were evaluated for papilla height (PH), gingival thickness (GT), gingival exhibit in ISL (visible interdental papillae on smiling – VIG) and GSL (visible midfacial gingiva on smiling – VMG), and the papilla presence index (PPI) scores through both clinical examination and digital photographic analysis.

Results. The overall mean values of PH, VIG and VMG were higher in groups II and IV, whereas GT showed lower values in these groups. The VIG values for groups I and II were 1.87 ±1.83 mm and 3.47 ±1.88 mm, respectively, while groups III and IV demonstrated values of 1.05 ±1.70 mm and 2.73 ±2.40 mm, respectively. Age-based comparisons revealed reduced values for all evaluated parameters in the older age groups. The PPI scores demonstrated a predominance of PPI-1 in groups I, II and III, whereas group IV predominantly exhibited PPI-2 scores. Regarding the smile line characteristics, a low GSL (LGSL) was predominantly observed across all groups irrespective of gender, while a low ISL (LISL) was more common in groups I and III, and a high ISL (HISL) predominated in groups II and IV.

Conclusions. Gender-related variability was evident in the parameters of PH, VIG and VMG, which demonstrated considerably higher mean values in groups II and IV (females), whereas GT exhibited lower values in these groups. Age-related changes were observed in PH, VIG, VMG, and GT, with a notable reduction in the mean values among the older age groups.

Keywords: gingival thickness, esthetics, papilla height, gingival smile line, interdental smile line

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Highlights

- Females exhibited greater papillary and gingival display, whereas males demonstrated a thicker gingival biotype.
- Age-related reductions were observed in papilla height (PH), gingival thickness (GT) and gingival display.
- A low gingival smile line (LGSL) was consistently evident across all age groups.
- Males predominantly showed a low interdental smile line (LISL), while females exhibited a high interdental smile line (HISL).

Introduction

A smile transcends cultural and linguistic boundaries, and is universally recognized as a form of personal greeting. Individuals who smile are often perceived as happier, more pleasant and more trustworthy, reflecting an overall positive personality. The esthetic impact of an attractive smile extends beyond appearance, influencing an individual's social, physical and psychological well-being.[1–3]

The increasing emphasis on smile esthetics has grown alongside greater public awareness, further amplified by media influence and digital editing platforms that allow patients to visualize and seek the desired esthetic outcomes with ease. In parallel, advancements in periodontal, orthodontic, restorative, and implant therapies have equipped clinicians with a wide range of treatment modalities to meet patients' expectations for an attractive smile. Nevertheless, each patient presents unique characteristics and esthetic preferences, requiring clinicians to approach smile esthetics through an interdisciplinary perspective to achieve optimal effects. A multidisciplinary assessment and treatment approach facilitates comprehensive rehabilitation aimed at enhancing esthetics, particularly in cases with multifactorial underlying causes.[4]

The principal components of an attractive smile include the lip framework, the maxillary anterior teeth and gingival scaffold, all of which should be proportionate and harmonious with one another and with the surrounding facial structures.[5–7] The parabolic contour of the gingiva is primarily determined by the position of the gingival margin, which exhibits a defined spatial orientation in both the anteroposterior and apicocoronal dimensions.[8] The display of the teeth and gingival scaffold through the lip framework constitutes the "white" and "pink" components of the smile, both of which are essential elements of smile esthetics.

In addition, several parameters are known to influence smile characteristics, including the smile line, the smile arc, lip curvature, the gingival zenith position, gingival display, and tooth display at rest and during smiling. The interdental papillae, constrained within the complex anatomy of the interproximal embrasures, also play a significant role in esthetics.[9–11] Although each parameter may be evaluated individually, all components must function in harmony to create the integrity required for an optimal final esthetic effect. Furthermore, these smile characteristics may vary among different populations, highlighting the importance of considering ethnicity as a relevant factor in the smile assessment.

For a more detailed assessment of smile esthetics, Hochman et al. proposed the classification of smiles into the interdental smile line (ISL) and the gingival smile line (GSL), based on the gingival tissue display of the interdental and midfacial gingiva during smiling.[12] Preliminary studies evaluating tooth and gingival display in relation to ISL and GSL demonstrated that most participants exhibited a high ISL and a low GSL, suggesting a greater esthetic significance of the interdental papillae as compared to the midfacial gingiva.[13]

In addition, Cardaropoli et al. introduced the papilla presence index (PPI), a classification system based on the positional relationship among the interdental papilla, the cementoenamel junction (CEJ) and the adjacent teeth.[14] However, the association between PPI and smile esthetics has not been extensively investigated, and the available literature on this topic remains limited.

Although substantial evidence exists regarding the factors influencing smile esthetics, there is still a paucity of literature exploring the interrelationship among these parameters. Understanding these associations may provide valuable clinical guidance for periodontal and restorative procedures in the maxillary esthetic zone. Given the close relationship between smile esthetics and gingival as well as interdental papillary display, the present study was designed to evaluate papilla presence and gingival exhibit in ISL and GSL with reference to age and gender.

Material and methods

A total of 120 periodontally healthy patients aged 20–60 years were recruited from the outpatient clinics of the Department of Periodontics and Implant Dentistry of Ranjeet Deshmukh Dental College and Research Centre, Nagpur, India. The study protocol and procedures were thoroughly explained to all participants, and written informed consent was obtained from each patient prior to enrollment. The study protocol adhered to the principles of the Declaration of Helsinki, and was reviewed and approved by the Institutional Ethics Committee. The study was also registered with the Clinical Trials Registry – India.

Sample size

A sample size of approx. 106 participants was estimated to provide a 90% confidence level, a 7.5% margin of error and an effect size of 0.31, based on the study conducted by Kolte et al., in which gingival exhibit and lip dimensions in the maxillary anterior region were evaluated and categorized according to gender.[2] Considering these parameters, a final sample size of 120 participants was determined for the present study.

Eligibility criteria

Participants were enrolled in the clinical trial after fulfilling the predefined eligibility criteria. The inclusion criteria comprised: (a) fully erupted maxillary anterior teeth; (b) properly aligned teeth; (c) the absence of anatomical or pathological abnormalities[15,16]; (d) clinically healthy periodontal tissues, as determined by the plaque index (PI)[17] and the gingival index (GI)[18] scores ranging from 0 to 1; and (e) the absence of dental restorations in the evaluated teeth. The exclusion criteria were as follows: (a) a history of periodontal surgical therapy; (b) previous orthodontic treatment; (c) the presence of systemic diseases, such as diabetes mellitus or hypertension; (d) the use of medications known to influence gingival tissues, including nifedipine, cyclosporine and phenytoin; and (e) facial asymmetry.

Grouping

A total of 120 periodontally healthy study participants who fulfilled the inclusion criteria were divided in to 4 groups: group I – 30 male patients aged 20–40 years; group II – 30 female patients aged 20–40 years; group III – 30 male patients aged 41–60 years; and group IV – 30 female patients aged 41–60 years.

Outcome variables

The primary outcome variables included the assessment of the PPI scores and gingival exhibit in ISL and GSL. The secondary outcome variables comprised the correlation of papilla height (PH) and gingival thickness (GT) with age and gender, as well as the association of gingival display with age, gender, ISL, and GSL within the study population.

Procedures

At the initial visit, all patients were evaluated for eligibility according to the study inclusion criteria, and standardized frontal digital photographs were obtained using a Canon camera equipped with a Canon EF 50 mm f/1.8 STM lens and built-in flash (Canon, Tokyo, Japan). The camera was positioned at a right angle to the patient to ensure image standardization. To maintain consistency, patients were seated at a distance of approx. 3 feet to avoid intrusion into personal space. Photographs were captured both at physiological rest and during smiling. The images were subsequently analyzed using Adobe Photoshop (Adobe, San Jose, USA) under a resolution of 1,680 × 1,050 pixels and an enlargement ratio of 1:1.2. Initial examinations and recording of study parameters were performed by 2 calibrated clinicians (APK and SPM), who underwent calibration prior to the commencement of the study. The following parameters were recorded:

(1) ISL: The interdental smile line was assessed based on the visibility of the interdental papillae during smiling and was categorized into 3 groups: high ISL (HISL), when the interdental papillae of the maxillary anterior teeth were visible; low ISL (LISL), when no interdental papillary display was observed; and Cupid's bow ISL (CB-ISL), when the central interdental papilla was not visible, but the papillae associated with the lateral incisors and/or more distal teeth were visible. All measurements and classifications were performed using standardized digital photographs (Fig. 1).

(2) GSL: The gingival smile line was assessed based on the visibility of the midfacial gingiva during smiling and was categorized into 3 groups: high GSL (HGSL), when the midfacial gingiva of the maxillary anterior teeth was visible; low GSL (LGSL), when no midfacial gingival display was observed; and Cupid's bow GSL (CB-GSL), when the midfacial gingiva of the central incisors was not visible, but gingival display was present in the lateral incisors and more distal teeth. All measurements and classifications were performed using standardized digital photographs (Fig. 1).



Fig. 1. Interdental smile line (ISL) (visible interdental papillae on smiling – VIG) and gingival smile line GSL (visible midfacial gingiva on smiling – VMG)

(3) Visible interdental papillae on smiling in ISL (VIG): Interdental papillary display on the right and left sides was recorded as the distance between the lower vermillion border and the tip of the interdental papilla. The amount of interdental papilla exhibited in the interdental smile line (ISL) was measured on frontal smiling photographs, from the tip of the interdental papillae of the maxillary anterior teeth to the lower vermillion border of the maxillary lip at the corresponding interdental positions on both the right and left sides. The mean value of these measurements was calculated for each patient. All measurements were performed using standardized digital photographs (Fig. 2A, Fig. 5).

(4) Visible midfacial gingiva on smiling in GSL (VMG): Midfacial gingival display on the right and left sides was recorded as the distance from the gingival zenith point of the respective teeth to the lower vermillion border of the maxillary lip at the midfacial positions. The mean value of these recordings was calculated for each patient. The measurement was done on photographs (Fig. 2B, Fig. 5).

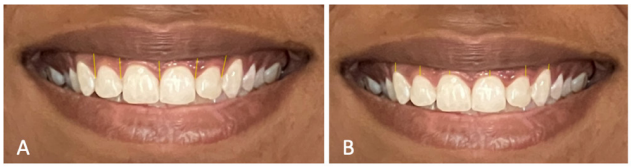

Fig. 2. Interdental papillary exhibit on smiling in the interdental smile line (ISL) (A) and midfacial gingival exhibit on smiling in the gingival smile line (GSL) (B)

(5) GT: Gingival thickness was evaluated for each individual tooth following the topical application of anesthetic gel, and measured at the midpoint between the gingival margin and the mucogingival junction (MGJ), using an endodontic spreader with a rubber stopper. The mean value obtained from all maxillary anterior teeth was recorded as the final score. All measurements were performed clinically.

(6) PH: Papilla height was measured using a periodontal probe from an imaginary line drawn from the gingival zenith of the adjacent teeth to the tip of the papilla. All measurements were performed clinically. (Fig. 3, Fig. 5).

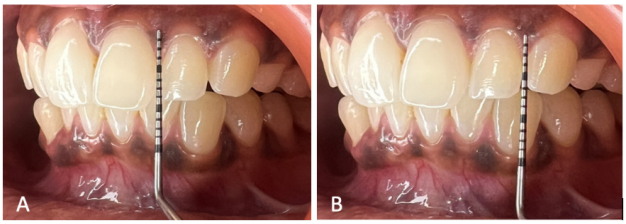

Fig. 3. Papilla height (PH) measured from the gingival zenith of the teeth to tip of the papilla

(7) PPI: The presence (PPI-1) or absence (PPI-2) of the papilla was determined based on the positional relationship between the interdental papilla, CEJ and the adjacent teeth, as described by Cardaropoli et al.[13] All measurements were performed clinically. (Fig. 4, Fig. 5).

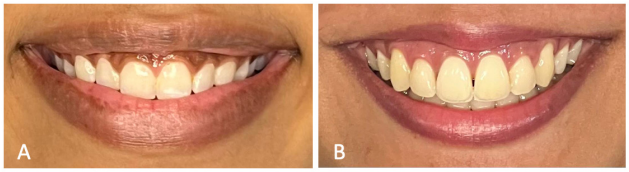

Fig. 4. Papilla presence index (PPI)
A – PPI-1 (the presence of the papilla); B – PPI-2 (the absence of the papilla).

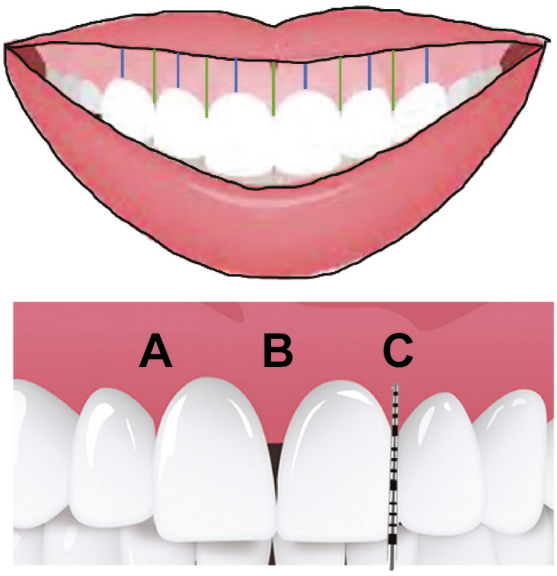

Fig. 5. Schematic illustration of the evaluated parameters
Top: green line – visible interdental papillae on smiling (VIG); blue line – visible midfacial gingiva on smiling (VMG).
Bottom: A – PPI-1; B – PPI-2; C – papilla height (PH)

Statistical analysis

All measurements were continuous variables and were expressed as mean and standard deviation ($M \pm SD$) for each group. Categorical parameters, such as PH, GT, VIG, and VMG were numerically represented in relation to age and gender, while the comparisons of the types of ISL and GSL, as well as the PPI scores across the study groups were performed using descriptive statistics, and the results were presented as percentages.

The paired sample t test was used to assess mean differences in PH, GT, VIG, and VMG with respect to age and gender, with two-sided p-values applied to determine statistical significance. The χ^2 test was used to evaluate the association of ISL, GSL and PPI with the study groups, including different age and gender categories. Data analysis was performed using IBM SPSS Statistics for Windows, v. 29.0 (IBM Corp., Armonk, USA).

Results

The study included 120 periodontally healthy patients divided into 4 groups according to age and gender, providing a total of 720 maxillary anterior teeth and 600 interdental papillae for evaluation, along with the corresponding midfacial gingival displays. Both ISL and GSL served as reference parameters for the assessment of gingival display.

All clinical and photographic measurements were performed by 2 calibrated examiners, who demonstrated excellent inter-observer agreement for the evaluated parameters in both male and female participants.

Table 1 presents the mean values PH, GT, VIG, and VMG across all study groups, along with the intragroup comparisons. The mean PH values for groups I and II were 3.13 ±0.63 mm and 3.91 ± 0.48 mm, respectively, whereas groups III and IV demonstrated values of 2.96 ±0.60 mm and 3.55 ±0.48 mm, respectively. Comparisons based on gender within the corresponding age groups revealed statistically significant differences ($p < 0.001$). However, when comparisons were performed within the same gender across different age groups (groups I vs. III and groups II vs. IV), the former comparison was not significant, whereas the latter showed statistical significance ($p = 0.004$). Significant differences were also observed in the mean GT values among groups I, II, III, and IV. Similarly, comparisons between groups I and III and between groups II and IV demonstrated statistically significant differences ($p = 0.039$ and $p = 0.002$, respectively), indicating age-related changes in GT among both male and female participants. The mean VIG values for groups I, II, III, and IV were 1.87 ±1.83 mm, 3.47 ±1.88 mm, 1.05 ±1.70 mm, and 2.73 ±2.40 mm, respectively. The comparisons of this parameter revealed statistically significant differences ($p = 0.001$). Among male participants (groups I and III), the VIG values differed significantly between age groups, whereas no significant differences were observed among female participants (groups II and IV). For the VMG parameter, a significant difference was identified between groups I and II, with mean values of 0.31 ±0.64 mm and 0.81 ±1.01 mm, respectively. The remaining comparisons for this parameter did not demonstrate statistically significant differences, despite variations in the mean values.

Table 2 presents the ISL and GSL categories, and the PPI scores across all study groups, along with their respective comparisons. Regarding ISL, the predominant smile line categories for groups I, II, III, and IV were LISL, HISL, LISL, and HISL, with frequencies of 46.7%, 73.3%, 60.0%, and 66.7%, respectively. Intragroup comparison revealed a statistically significant difference between groups III and IV ($p = 0.024$). For GSL, the most predominant category in all 4 groups was LGSL, with frequencies of 76.7%, 46.7%, 93.3%, and 63.3% for groups I, II, III, and IV, respectively. In group I, the second most prevalent category was CB-GSL, accounting for 13.3%, whereas in group II, HGSL was the second most common category. The comparison of the GSL categories between groups II and IV demonstrated a statistically significant difference ($p = 0.029$). For PPI, PPI-1 was the most predominant score across all 4 groups, with a higher prevalence observed among male participants (groups I and III).

Discussion

The maxillary anterior segment, owing to its prominence and visibility, has gained considerable importance in smile esthetics, where the harmonious display of the

Table 1. Mean values of papilla height (PH), gingival thickness (GT), visible interdental papillae on smiling (VIG) and visible midfacial gingiva on smiling (VMG) across all study groups, along with the intragroup comparisons

Parameter	Group I	Group II	*p*-value	Group III	Group IV	*p*-value	Group I	Group III	*p*-value	Group II	Group IV	*p*-value
PH [mm]	3.13±0.63	3.91±0.48	<0.001*	2.96±0.60	3.55±0.48	<0.001*	3.13±0.63	2.96±0.60	0.349	3.91±0.48	3.55±0.48	0.004*
GT [mm]	1.92±0.28	1.03±0.17	<0.001*	1.62±0.70	0.90±0.13	<0.001*	1.92±0.28	1.62±0.70	0.039*	1.03±0.17	0.90±0.13	0.002*
VIG [mm]	1.87±1.83	3.47±1.88	0.001*	1.05±1.70	2.73±2.40	0.001*	1.87±1.83	1.05±1.70	0.043*	3.47±1.88	2.73±2.40	0.140
VMG [mm]	0.31±0.64	0.81±1.01	0.011*	0.18±0.82	0.60±1.16	0.125	0.31±0.64	0.18±0.82	0.573	0.81±1.01	0.60±1.16	0.402

Data presented as mean ± standard deviation (*M ±SD*).
Groups: I – male patients aged 20–40 years; II – female patients aged 20–40 years; III – male patients aged 41–60 years; IV – female patients aged 41–60 years.
* statistically significant ($p < 0.05$).

Table 2. Categories of the interdental smile line (ISL) and the gingival smile line (GSL), and the papilla presence index (PPI) scores across all study groups, along with their respective comparisons

Smile line/PPI		Group I ($n = 30$)	Group II ($n = 30$)	*p*-value	Group III ($n = 30$)	Group IV ($n = 30$)	*p*-value	Group I ($n = 30$)	Group III ($n = 30$)	*p*-value	Group II ($n = 30$)	Group IV ($n = 30$)	*p*-value
ISL	HISL	6 (20.0)	22 (73.3)	0.983	10 (33.3)	20 (66.7)	0.024*	6 (20.0)	10 (33.3)	0.467	22 (73.3)	20 (66.7)	0.723
	LISL	14 (46.7)	4 (13.3)		18 (60.0)	10 (33.3)		14 (46.7)	18 (60.0)		4 (13.3)	10 (33.3)	
	CB-ISL	10 (33.3)	4 (13.3)		2 (6.7)	0 (0.0)		10 (33.3)	2 (6.7)		4 (13.3)	0 (0.00)	
GSL	HGSL	3 (10.0)	10 (33.3)	0.292	1 (3.3)	5 (16.7)	0.871	3 (10.0)	1 (3.3)	0.137	10 (33.3)	5 (16.7)	0.029*
	LGSL	23 (76.7)	14 (46.7)		28 (93.3)	19 (63.3)		23 (76.7)	28 (93.3)		14 (46.7)	19 (63.3)	
	CB-GSL	4 (13.3)	6 (20.0)		1 (3.3)	6 (20.0)		4 (13.3)	1 (3.3)		6 (20.0)	6 (20.0)	
PPI	PPI-1	28 (93.3)	27 (90.0)	0.626	18 (60.0)	14 (46.7)	0.232	28 (93.3)	18 (60.0)	0.765	27 (90.0)	14 (46.7)	0.464
	PPI-2	2 (6.7)	3 (10.0)		12 (40.0)	16 (53.3)		2 (6.7)	12 (40.0)		3 (10.0)	16 (53.3)	

Data presented as number (percentage) (*n* (%)).
Groups: I – male patients aged 20–40 years; II – female patients aged 20–40 years; III – male patients aged 41–60 years; IV – female patients aged 41–60 years.
HISL – high ISL; LISL – low ISL; CB-ISL – Cupid's bow ISL; HGSL – high GSL; LGSL – low GSL; CB-GSL – Cupid's bow GSL; * statistically significant ($p < 0.05$).

teeth and gingival structures has the potential to enhance the overall attractiveness of the smile. Efforts to identify and evaluate intraoral and extraoral characteristics, such as the gingival zenith position,[8] gingival display,[19] tooth and gingival display,[20] facial and dental proportions,[21] maxillary lip dimensions,[2] and the nasolabial angle,[5] have been incorporated to varying extents into restorative and periodontal procedures aimed at improving esthetic outcomes.

The concepts of ISL and GSL, together with their role in defining smile characteristics and their relationship with peri- and intraoral features, warrant further investigation to establish harmonious relationships that contribute to the design of an esthetic smile. Furthermore, evaluating these parameters in relation to age and gender variability may provide clinically relevant information for procedures involving the maxillary anterior region. In addition, recent studies have utilized three-dimensional (3D) facial scans through linear and angular measurements, demonstrating no significant differences between 3D facial scanning and cone-beam computed tomography (CBCT) soft tissue volumetric assessment, thereby supporting its use as an alternative non-radiographic method.[22]

The observations of the present study indicate notable gender-related differences in the evaluated parameters. Papilla height, VIG in ISL, and VMG in GSL demonstrated considerably higher mean values in groups II and IV, whereas GT exhibited lower values in these groups. These findings may reflect the generally recognized biological differences between genders, with males typically exhibiting more robust anatomical structures than females. Papilla height, measured from an imaginary line drawn from the gingival zenith of the adjacent teeth to the tip of the papilla, is of particular relevance, as the present study is among the few investigations to clinically evaluate this parameter. The observed gender-related variability, favoring females in groups II and IV as compared to males in groups I and III, may be associated with triangular crown morphology and greater apicocoronal dimensions of the interproximal areas.[9,23] However, in both of these previous studies, PH was not directly measured and was assessed only in terms of papilla presence or absence. In addition, the PH values decreased with advancing age irrespective of gender, as observed in groups III and IV, and these findings are consistent with those reported by Chow et al.[24]

The display of teeth and gingival tissues during smiling, particularly the interdental and midfacial gingiva, has long attracted clinical interest, as these structures are considered essential components of an esthetic smile. In the present investigation, the parameters of VIG and VMG were quantitatively evaluated, and the findings demonstrated substantial differences between groups I and II as well as between groups III and IV, with female participants exhibiting greater gingival display both at the midfacial and interdental levels. The VIG values were considerably higher than the VMG values, which may be attributed to the more coronal position of the interdental papillae, a feature specifically represented in ISL. Although females in groups II and IV demonstrated greater VMG values than males in groups I and III within GSL, the extent of this display was markedly lower when compared with the gingival display observed in ISL. These observations are consistent with the findings reported by Kolte et al.,[2] although the gingival display values described in their study were greater for both genders than those observed in the present investigation. This discrepancy may be attributed to differences in the measurement protocol, as Kolte et al. recorded measurements separately for each category of ISL and GSL, whereas the present study assessed gingival display cumulatively, irrespective of smile category. Another possible explanation for the greater VIG and VMG observed among females is the presence of a fuller smile pattern, in which the lip musculature undergoes greater stretching and reduction in fullness and length, thereby resulting in increased gingival display.[25]

An important strength of the present study is that all parameters were evaluated in 2 distinct age groups for both male and female participants, enabling the assessment of changes associated with the aging process. The evaluated parameters – PH, VIG and VMG – demonstrated notable age-related alterations, with overall reductions in their mean values observed with advancing age. The reduction in PH was particularly pronounced among females in groups II and IV as compared to males in groups I and III.

Gingival thickness is an important parameter in periodontal and implant therapy, as it serves as a valuable source of autogenous tissue for the management of gingival recession and dehiscence defects. Owing to its site-specific and individual variability, as well as its relevance in therapeutic procedures, GT has attracted considerable clinical interest. In the present study, GT was evaluated using the direct, predictive, and relatively simple measurement method described by Stein et al.,[26] which identifies a reference point located midway between the gingival margin and MGJ, directly over the alveolar crest. The findings demonstrated substantially greater GT values in groups I and III as compared to groups II and IV. These observations are consistent with previous investigations,[9,27] and may be attributed to the generally more robust anatomical characteristics observed in males, as well as age-related epithelial thinning and reduced keratinization.

In the assessment of GSL, the most predominant category was LGSL, irrespective of gender. In contrast, for ISL, the predominant category was LISL in groups I and III, and HISL in groups II and IV, indicating a clear gender-related variability. These findings are particularly important, as they highlight the greater sensitivity of ISL in identifying interdental gingival display, which appears to be more prominently expressed than midfacial gingival display.

The present investigation is among the first studies to evaluate PPI, and the findings demonstrated a predominance of PPI-1 scores among the study participants. This observation may be attributed to the inclusion of only periodontally healthy individuals, most of whom exhibited interproximal areas completely filled by the interdental papillae, whereas only a limited number demonstrated PPI-2 scores. Comparisons between groups I and II and groups III and IV revealed a reduction in the proportion of participants with PPI-1 scores and a corresponding increase in PPI-2 scores in the older age groups. Similarly, comparisons between groups I and III and groups II and IV demonstrated greater differences among older female participants than among males, possibly reflecting the more delicate gingival tissues in females and their greater susceptibility to age-related alterations. In addition, the greater GT values observed in groups I and III may explain the predominance of PPI-1 scores in these groups, as thicker gingival tissues may be more resistant to structural changes.

Limitations

Several limitations of the present study should be acknowledged. First, as the clinical trial was conducted on a homogeneous population belonging to a specific racial and ethnic demographic, the findings may be more representative of the Asian population, and potential variations among other ethnic groups warrant further investigation to broaden the applicability of the results. Second, clinical measurements were performed using a periodontal probe, which may have introduced minor inaccuracies, particularly over convex and concave tooth surfaces. Third, factors such as mouth breathing and Angle's classification, both of which may influence smile characteristics, were not included in the analysis. Finally, age-related maxillary remodeling and variations in lip and nasal support, which may also affect smile esthetics, were not evaluated in the present study.

Conclusions

The observations obtained from the present study may have important clinical implications and can be applied, in conjunction with specific treatment objectives, to assist clinicians in achieving an esthetically pleasing and functionally appropriate smile. Gender-related variability in intraoral characteristics was evident in the parameters of PH, VIG and VMG, which demonstrated considerably higher mean values in groups II and IV, whereas GT exhibited lower values in these groups. These findings also appeared to influence PPI scores, with PPI-1 predominating among participants in groups I and III. Age-related changes were observed across all evaluated parameters, including PH, GT, VIG, and VMG, with a notable reduction in the mean values associated with advancing age. The predominant presence of LGSL within the GSL categories, irrespective of gender, together with the predominance of LISL in groups I and III, and HISL in groups II and IV, not only demonstrated gender-related variability, but also highlighted the sensitivity of ISL in identifying interdental gingival display.

Trial registration

This clinical study was registered with the Clinical Trials Registry – India (CTRI/2023/06/053423).

Ethics approval and consent to participate

The trial was approved by the Institutional Ethics Committee at Ranjeet Deshmukh Dental College and Research Centre, Nagpur, India (IEC/VSPMDCRC/2/2023). Written informed consent was obtained from all participants.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Van der Geld P, Oosterveld P, Van Heck G, Kuijpers-Jagtman AM. Smile attractiveness. Self-perception and influence on personality. *Angle Orthod*. 2007;77(5):759–765. doi:10.2319/082606-349
2. Kolte AP, Kolte RA, Trivedi DS, Deshpande NM. Association of gingival exhibit with lip dimensions, intercommissural width, and gingival and interdental smile lines – a gender-based evaluation. *Int J Esthet Dent*. 2022;17(4):436–447. PMID:36426615.
3. Afroz S, Rathi S, Rajput G, Rahman SA. Dental esthetics and its impact on psycho-social well-being and dental self confidence: A campus based survey of North Indian university students. *J Indian Prosthodont Soc*. 2013;13(4):455–460. doi:10.1007/s13191-012-0247-1
4. Zawawi KH, Malki GA, Al-Zahrani MS, Alkhiary YM. Effect of lip position and gingival display on smile and esthetics as perceived by college students with different educational backgrounds. *Clin Cosmet Investig Dent*. 2013;5:77–80. doi:10.2147/CCIDE.S53359
5. Kolte AP, Kolte RA, Samarth GK. Association of the nasolabial angle and lip length with the gingival and interdental smile line – a gender based evaluation. *J Esthet Restor Dent*. 2021;33(3):503–509. doi:10.1111/jerd.12657
6. Garber DA, Salama MA. The aesthetic smile: Diagnosis and treatment. *Periodontol 2000*. 1996;11:18–28. doi:10.1111/j.1600-0757.1996.tb00179.x

7. Chrapla P, Paradowska-Stolarz A, Skoskiewicz-Malinowska K. Subjective and objective evaluation of the symmetry of maxillary incisors among residents of southwest Poland. *Symmetry*. 2022;14(6):1257. doi:10.3390/sym14061257
8. Kolte AP, Kolte RA, Ahuja C. Assessment of gingival zenith position and its level relative to age and gender in maxillary anterior teeth. *Quintessence Int*. 2018;49(9):761–768. doi:10.3290/j.qi.a41009
9. Kolte RA, Kolte AP, Purohit AR. Gender-based evaluation of interproximal dimensions. Influence on interdental papillae and gingival thickness in maxillary anterior teeth. *Int J Esthet Dent*. 2023;18(2):128–140. PMID:37166768.
10. Hochman MN, Chu SJ, Da Silva BP, Tarnow DP. Layperson's esthetic preference to the presence or absence of the interdental papillae in the low smile line: A web-based study. *J Esthet Restor Dent*. 2019;31(2):113–117. doi:10.1111/jerd.12478
11. Lee DW, Kim CK, Park KH, Cho KS, Moon IS. Non-invasive method to measure the length of soft tissue from the top of the papilla to the crestal bone. *J Periodontol*. 2005;76(8):1311–1314. doi:10.1902/jop.2005.76.8.1311
12. Hochman MN, Chu SJ, Tarnow DP. Maxillary anterior papilla display during smiling: A clinical study of the interdental smile line. *Int J Periodontics Restorative Dent*. 2012;32(4):375–383. PMID:22577642.
13. Kolte RA, Kolte AP, Rathi P. Association of the gingival line angle with the gingival and interdental smile line: A gender based evaluation. *J Esthet Restor Dent*. 2019;31(6):601–607. doi:10.1111/jerd.12517
14. Cardaropoli D, Re S, Corrente G. The papilla presence index (PPI): A new system to assess interproximal papillary levels. *Int J Periodontics Restorative Dent*. 2004;24(5):488–492. doi:10.11607/prd.00.0596
15. Paradowska-Stolarz A, Kawala B. Dental anomalies in maxillary incisors and canines among patients with total cleft lip and palate. Appl Sci. 2023;13(11):6635. doi:10.3390/app13116635
16. Gupta V, Kaur H, Mishra D, Yadav VS, Kala S. Distribution of biopsied gingival lesions according to the proceedings from the 2017 World Workshop classification: A three-year retrospective study. *Dent Med Probl*. 2022;59(1):105–110. doi:10.17219/dmp/141555
17. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121–135. doi:10.3109/00016356408993968
18. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551. doi:10.3109/00016356309011240
19. Sepolia S, Sepolia G, Kaur R, Gautam DK, Jindal V, Gupta SC. Visibility of gingiva – an important determinant for an esthetic smile. *J Indian Soc Periodontol*. 2014;18(4):488–492. doi:10.4103/0972-124X.138703
20. Kolte A, Kolte R, Swami RK. Assessment of tooth and gingival display in maxillary anterior teeth and premolars relative to gingival and interdental smile lines. *Int J Esthet Dent*. 2020;15(2):184–194. PMID:32467947.
21. Hasanreisoglu U, Berksun S, Aras K, Arslan I. An analysis of maxillary anterior teeth: Facial and dental proportions. *J Prosthet Dent*. 2005;94(6):530–538. doi:10.1016/j.prosdent.2005.10.007
22. Perrotti G, Reda R, Rossi O, D'Apolito I, Testori T, Testarelli L. A radiation free alternative to CBCT volumetric rendering for soft tissue evaluation. *Braz Dent Sci*. 2023;26(1):e3726. doi:10.4322/bds.2023.e3726
23. Stappert CF, Tarnow DP, Tan JHP, Chu SJ. Proximal contact areas of the maxillary anterior dentition. *Int J Periodontics Restorative Dent*. 2010;30(5):471–477. PMID:20814600.
24. Chow YC, Eber RM, Tsao YP, Shotwell JL, Wang HL. Factors associated with the appearance of gingival papillae. *J Clin Periodontol*. 2010;37(8):719–727. doi:10.1111/j.1600-051X.2010.01594.x
25. Ahmad I. Anterior dental aesthetics: Dentofacial perspective. *Br Dent J*. 2005;199(2):81–88. doi:10.1038/sj.bdj.4812521
26. Stein JM, Lintel-Höping N, Hammächer C, Kasaj A, Tamm M, Hanisch O. The gingival biotype: Measurement of soft and hard tissue dimensions – a radiographic morphometric study. *J Clin Periodontol*. 2013;40(12):1132–1139. doi:10.1111/jcpe.12169
27. Vandana KL, Gupta I. The relation of gingival thickness to dynamics of gingival margin position pre- and post-surgically. *J Indian Soc Periodontol*. 2016;20(2):167–173. doi:10.4103/0972-124X.175173

Original paper

In vivo evaluation of alveolar ridge bone formation resulting from the administration of a natural propolis extract combined with a bovine xenograft

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Abstract

Background. Natural propolis is widely known to be an antibacterial agent with strong antioxidant and anti-inflammatory properties. In combination with a bovine xenograft, it is expected to promote bone formation in the alveolar ridge, following tooth extraction.

Objectives. The aim of the present study was to evaluate the effect of administering a propolis extract combined with a bovine xenograft on alveolar bone formation.

Material and methods. The lower incisor teeth of *Cavia cobaya* were extracted and the resulting alveolar sockets were filled with different materials based on the group assignment: polyethylene glycol (PEG); a propolis extract (PE); a bovine xenograft (BX); and the propolis extract–bovine xenograft composite (PE–BX). The alveolar sockets were examined after 7 and 30 days by means of immunohistochemical staining for the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), toll-like receptor 2 (TLR2), tumor necrosis factor-alpha (TNF-α), and type 1 collagen. The one-way analysis of variance (ANOVA) and post-hoc Tukey's test were used to compare the groups.

Results. The PE–BX group demonstrated promising results, with increased type I collagen levels and reduced expression of inflammatory markers (NF-κB, TLR2 and TNF-α) on days 7 and 30 ($p < 0.001$). Furthermore, in comparison with the BX group, this material demonstrated significantly higher type I collagen expression on days 7 and 30 ($p < 0.01$).

Conclusions. The composite of a natural propolis extract and a bovine xenograft enhances type I collagen expression and reduces the expression of inflammatory markers. Further research is warranted to explore its potential for alveolar bone preservation.

Keywords: propolis, medicine, bone formation, tooth extraction socket, alveolar socket healing

Highlights

- Propolis extract-based treatment significantly reduced key inflammatory markers (NF-κB, TLR2 and TNF-α) as compared to the control group.
- In these treatment groups, enhanced collagen production was also observed, especially at later healing stages.
- No significant difference was observed between the propolis-only and propolis extract–bovine xenograft (PE–BX) combination groups.
- The PE–BX composite shows promising potential for preserving alveolar bone after tooth extraction.
- Further research is needed to confirm and expand on these findings.

Introduction

Bone resorption following tooth extraction can result in significant complications for subsequent prosthetic rehabilitation, including removable and fixed dentures, as well as dental implants. Excessive force during extraction may trigger severe inflammation, leading to increased osteoclast activity and consequent alveolar bone resorption. Therefore, alveolar ridge preservation is essential to minimize post-extraction bone loss.[1,2] This principle should be applied in all cases of edentulous ridges to optimize the functional and esthetic outcomes of prosthetic treatment, particularly implant therapy.[3,4]

Natural propolis is widely recognized as an alternative therapeutic agent for the treatment of various diseases. Numerous studies have demonstrated that propolis exhibits antibacterial activity (particularly against Gram-positive bacteria), as well as strong antioxidant and anti-inflammatory properties.[5–8] Propolis contains resins and various bioactive compounds, including bioflavonoids, artepillin, apigenin, and caffeic acid phenethyl ester (CAPE), which are known for their anti-inflammatory, antioxidant, antibacterial, antiviral, and immunomodulatory effects, and have been shown to promote tissue healing.[9–12]

A study by Kresnoadi et al. investigated osteogenesis induced by a combination of propolis and a bovine bone graft, reporting higher expression of heat shock protein 70 (HSP 70) and osteocalcin, and increased osteoblast numbers as compared to the control group at days 3 and 7.[13] Further research by the same group demonstrated that this combination enhanced transforming growth factor-beta (TGF-β) expression and osteoblast numbers, while reducing interleukin-1β (IL-1β) levels and osteoclast production during alveolar bone regeneration.[14]

Under physiological conditions, bone undergoes continuous remodeling through the coordinated activities of osteoclasts and osteoblasts. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) plays a pivotal role in osteoclastogenesis, and is activated via the canonical pathway by receptor activator of nuclear factor kappa-B ligand (RANKL) and tumor necrosis factor-alpha (TNF-α). In contrast, toll-like receptors (TLRs) contribute to the maintenance of bone homeostasis by modulating inflammatory responses.[15,16] Type I collagen constitutes a major component of the organic bone matrix and is synthesized primarily by osteoblasts.[17] These markers are therefore important indicators in the evaluation of alveolar bone formation during socket healing.

This study aimed to develop an alternative material for alveolar ridge preservation based on a composite of a natural propolis extract and a bovine xenograft, with the objective of enhancing bone regeneration and reducing the risk of ridge resorption.

Materials and methods

This research was approved by the Ethical Clearance Commission at the Faculty of Dental Medicine of the Airlangga University, Surabaya, Indonesia (approval No. 014/HRCC FODM/III/2018).

The first preparation consisted of a natural propolis extract (PE) obtained from an apiary in East Java. The bovine xenograft (BX) used in this study was sourced from the tissue bank of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The PE–BX composite was prepared using polyethylene glycol (PEG) as a carrier. PEG 400 and PEG 4000 (CARBOWAX™; Dow, Jakarta, Indonesia) were mixed at a 1:1 ratio prior to incorporation with the active materials.

The study comprised 4 groups: control; PE; BX; and PE–BX. In each treatment group, the material contained 2% active ingredient, as recommended in a previous study by Yuli Prabowo et al.[18]

Healthy male guinea pigs (*Cavia cobaya*) were selected as the experimental subjects based on the following criteria: 3.5 months of age; body weight of 300–350 g; the absence of visible lesions; and intact sensory function. The animals were acclimatized for 1 week prior to the experiment, and were provided standard pellet feed and water ad libitum.

Anesthesia was induced by the intramuscular injection of ketamine at a dose of 20 mg per 300 g b.w. (Kepro, Deventer, the Netherlands). Following anesthesia, the lower incisor was extracted. The extraction socket was filled with the designated material according to the treatment

group, and subsequently closed using simple interrupted sutures.

On days 7 and 30, the animals were euthanized to allow the harvesting of the lower incisor tissue. The specimens were fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, USA) and decalcified using ethylenediaminetetraacetic acid (EDTA) (Titriplex® III; Merck, Darmstadt, Germany). The tissues then underwent dehydration, clearing, paraffin embedding, and deparaffinization. Subsequently, the samples were processed for immunohistochemical analysis to evaluate the expression of NF-κB, TLR2, TNF-α, and type I collagen, using specific monoclonal antibodies (Abcam, Cambridge, UK). The prepared slides were examined under a light microscope at magnifications of ×100, ×400 and ×1,000.

Observations were focused on the apical third of the extraction socket. Type I collagen expression was assessed in osteoblasts, whereas the expression of NF-κB, TLR2 and TNF-α was evaluated in osteoclasts. Positive cells were counted manually to minimize potential counting errors.

Statistical analysis

The one-way analysis of variance (ANOVA) and post-hoc Tukey's test were used to compare the groups (IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA)). The *p*-value applied in this study was 0.05.

Results

NF-κB expression

NF-κB expression in osteoclasts demonstrated a pattern similar to that of TLR2 and TNF-α on both day 7 and day 30. The control group exhibited the highest NF-κB expression, followed by the BX group, whereas the PE–BX composite group showed the lowest expression levels. All groups demonstrated a reduction in expression from day 7 to day 30. Statistical analysis revealed that the PE and PE–BX groups differed significantly from the control group ($p < 0.001$). The PE group also differed significantly from the BX group ($p < 0.001$), as did the BX group from the PE–BX group ($p < 0.001$). On day 30, a significant difference was observed between the PE and BX groups ($p < 0.01$). However, no significant differences were found between the PE and PE–BX groups (Fig. 1). Representative histological images of the apical third of the extraction socket are shown in Fig. 2. The yellow arrow indicates osteoclasts along the bone margin exhibiting positive NF-κB expression.

TLR2 expression

On day 7, the PE and PE–BX groups differed significantly from the control group ($p < 0.05$ and $p < 0.001$,

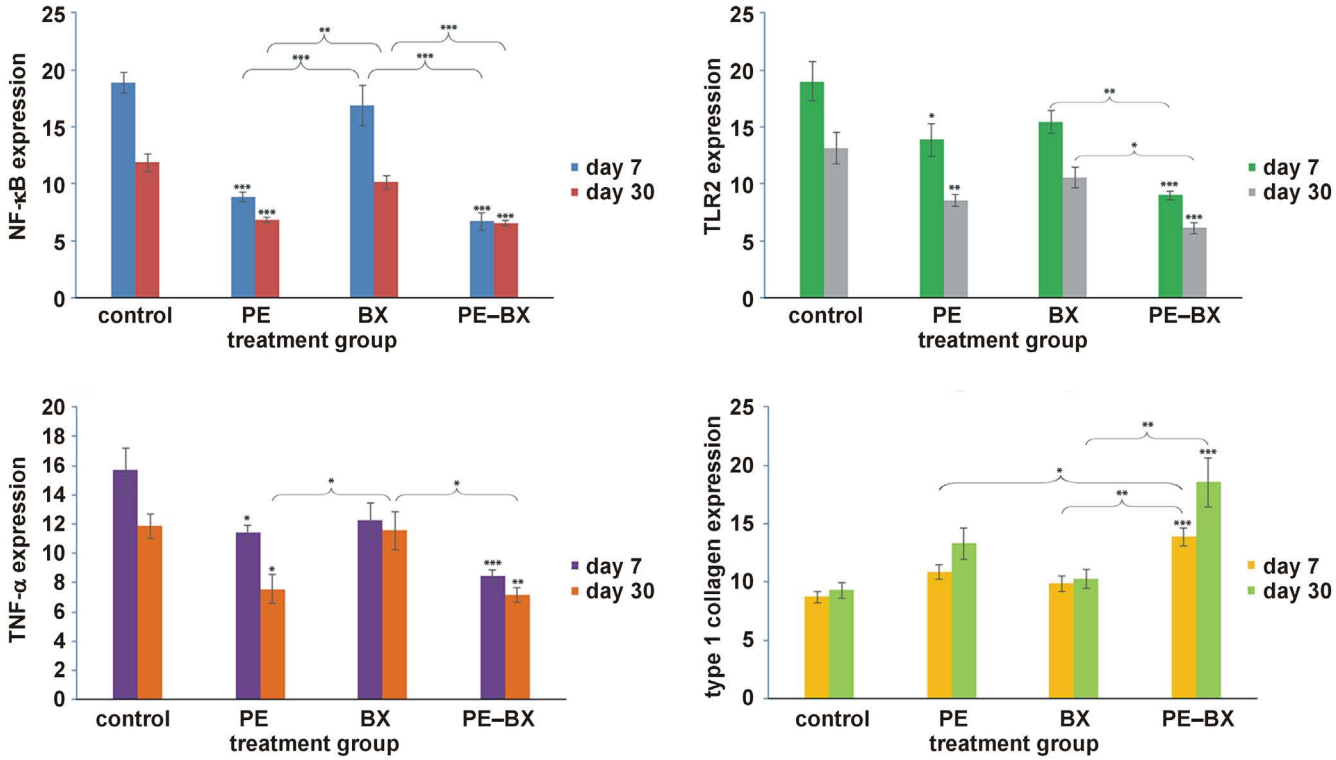

Fig. 1. Expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), toll-like receptor 2 (TLR2), tumor necrosis factor-alpha (TNF-α), and type 1 collagen for each group ($n = 7$) on day 7 and day 30

Data presented as mean ± standard error (*M* ±*SE*).
Groups according to the material used: control – polyethylene glycol (PEG); a propolis extract (PE); a bovine xenograft (BX); and the propolis extract–bovine xenograft composite (PE–BX). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

respectively). Similarly, on day 30, both the PE and PE–BX groups showed significant differences as compared to the control group ($p < 0.01$ and $p < 0.001$, respectively). The control group exhibited the highest TLR2 expression, whereas the PE–BX group demonstrated the lowest expression levels. On both day 7 and day 30, the PE–BX group differed significantly from the BX group ($p < 0.01$ and $p < 0.05$, respectively). However, no significant differences were observed between the control and BX groups (Fig. 1). Representative histological images of the apical third of the extraction socket are presented in Fig. 3. The yellow arrow indicates osteoclasts along the bone margin showing positive TLR2 expression.

TNF-α expression

On day 7, the PE and PE–BX groups differed significantly from the control group, with p-values of <0.05 and <0.001, respectively. A slight reduction in TNF-α expression was observed in the PE group on day 30. The PE–BX group also showed a significant difference as compared to the control group on day 30 ($p < 0.01$). Furthermore, the propolis-containing groups demonstrated significant differences in comparison with the BX group on day 30 (Fig. 1). Representative histological images of the apical third of the extraction socket are shown in Fig. 4. The yellow arrow indicates osteoclasts along the bone margin exhibiting positive TNF-α expression.

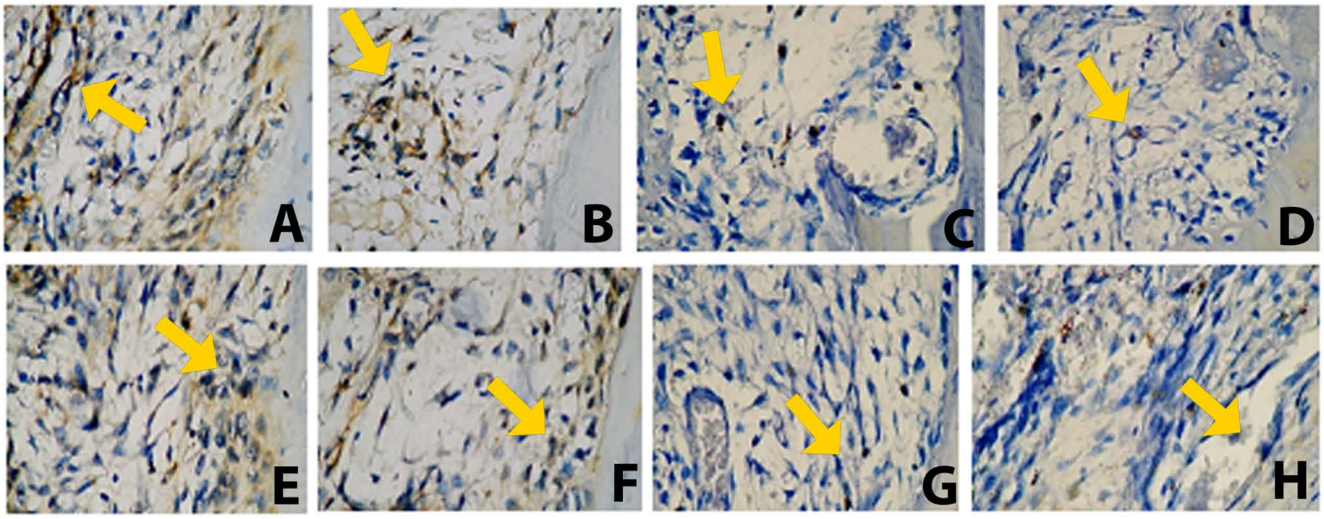

Fig. 2. Representative cross-sectional images of NF-κB staining in the apical third of the extraction socket obtained at 7 and 30 days post-extraction

A, E – control group; B, F – BX group; C, G – PE group; D, H – PE–BX group; upper pictures – day 7; lower pictures – day 30; ×400 magnification. The yellow arrow indicates osteoblasts demonstrating positive NF-κB expression.

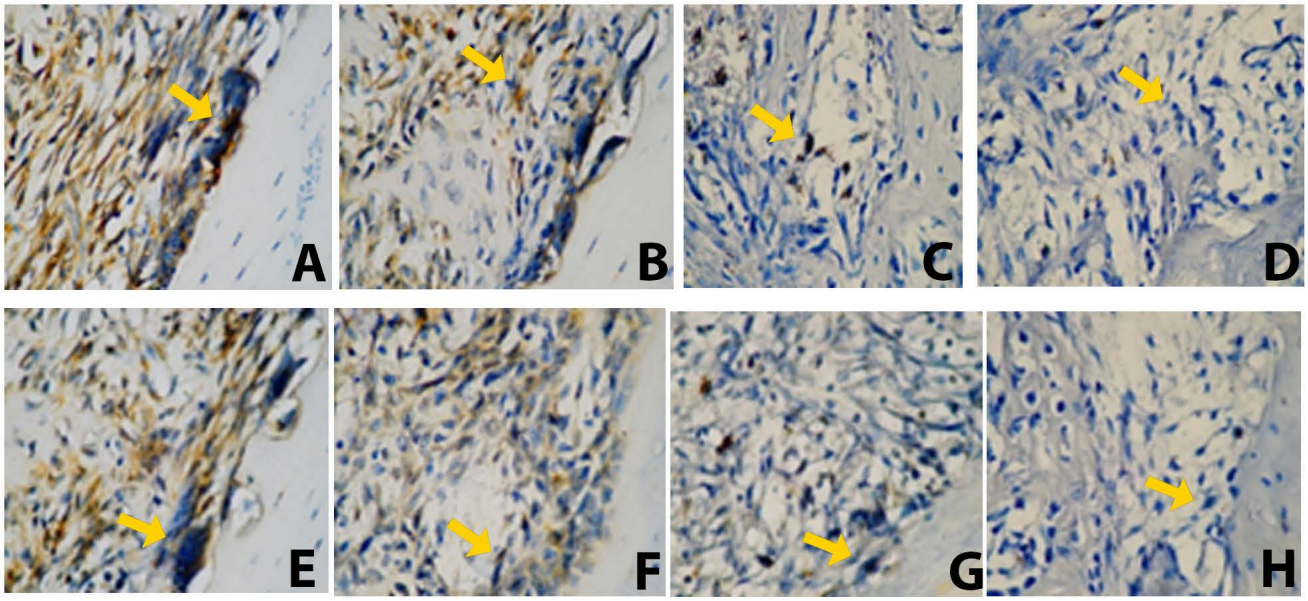

Fig. 3. Representative cross-sectional images of TLR2 staining in the apical third of the extraction socket obtained at 7 and 30 days post-extraction

A, E – control group; B, F – BX group; C, G – PE group; D, H – PE–BX group; upper pictures – day 7; lower pictures – day 30; ×400 magnification. The yellow arrow indicates osteoblasts demonstrating positive TLR2 expression.

Type 1 collagen expression

An increasing trend in type I collagen expression was observed in all groups from day 7 to day 30. However, only the PE–BX group showed a significant difference in comparison with the control group ($p < 0.001$). A significant difference was also identified between the PE and PE–BX groups at the early observation time point (day 7) ($p < 0.05$). Furthermore, the PE–BX group differed significantly from the BX group at both time points ($p < 0.01$) (Fig. 1). Representative histological images of the apical third of the extraction socket are presented in Fig. 5. The yellow arrow indicates osteoblasts along the bone margin exhibiting positive type I collagen expression.

Discussion

The topical application of the ethanol–propolis extract/bovine xenograft composite significantly enhanced bone healing in the tooth socket as compared to the control group. Overall, reduced expression levels of NF-κB, TLR2 and TNF-α were observed, along with increased

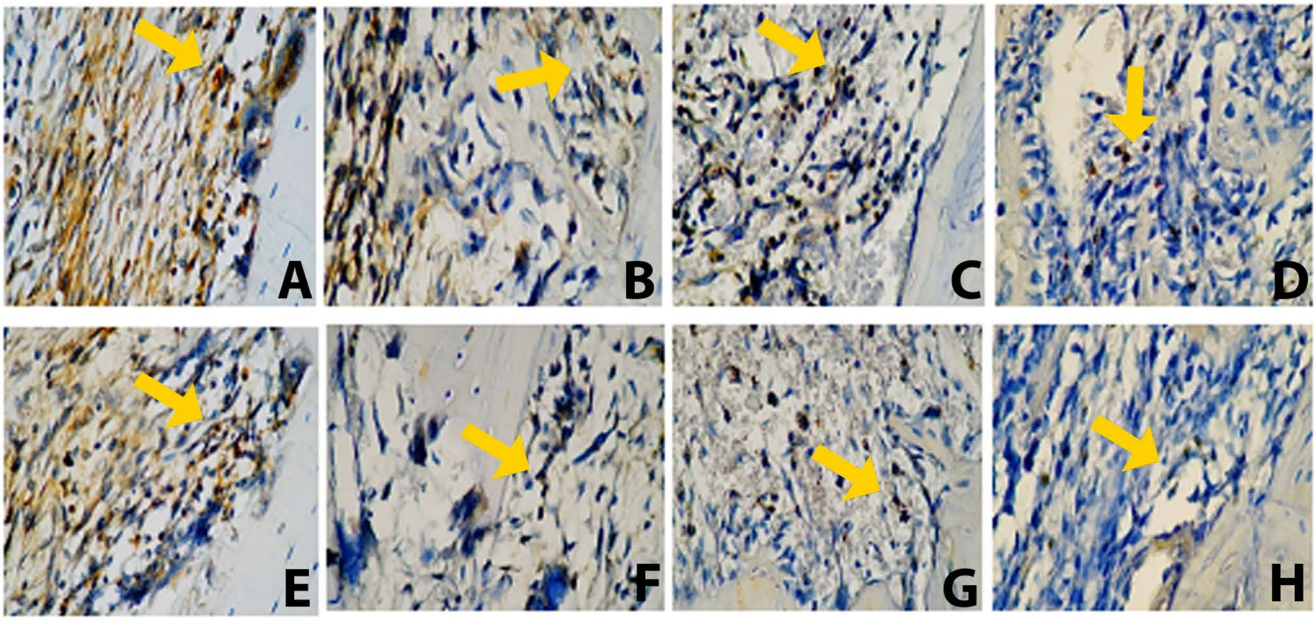

Fig. 4. Representative cross-sectional images of TNF-α staining in the apical third of the extraction socket obtained at 7 and 30 days post-extraction

A, E – control group; B, F – BX group; C, G – PE group; D, H – PE–BX group; upper pictures – day 7; lower pictures – day 30; ×400 magnification. The yellow arrow indicates osteoblasts demonstrating positive TNF-α expression.

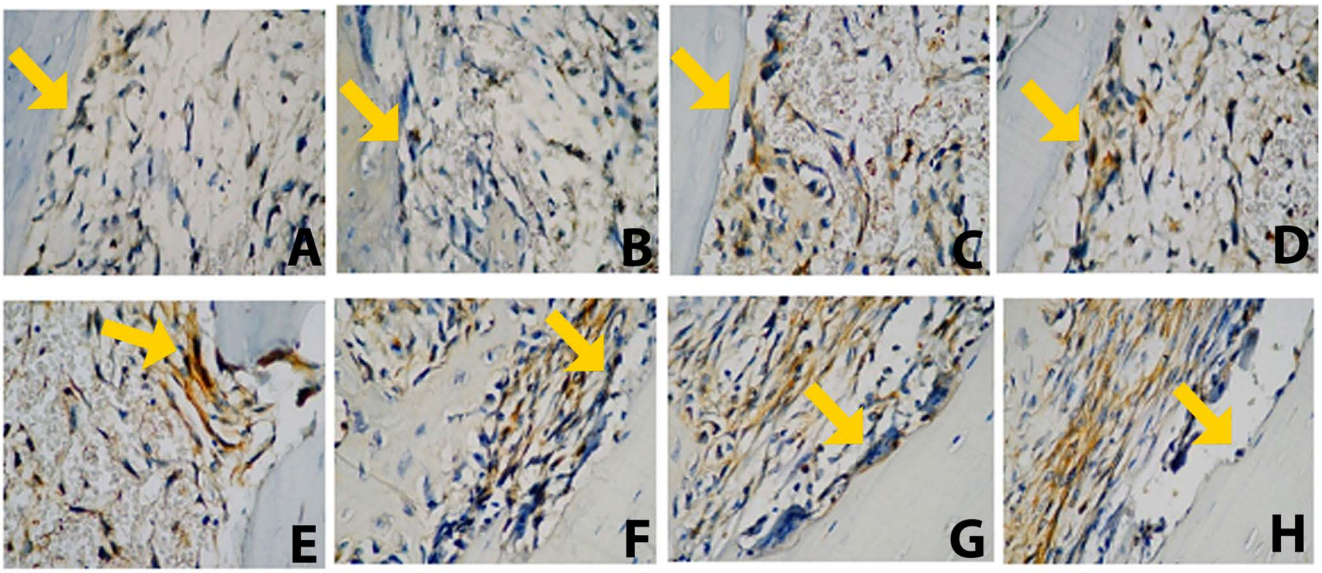

Fig. 5. Representative cross-sectional images of type 1 collagen staining in the apical third of the extraction socket obtained at 7 and 30 days post-extraction

A, E – control group; B, F – BX group; C, G – PE group; D, H – PE–BX group; upper pictures – day 7; lower pictures – day 30; ×400 magnification. The yellow arrow indicates osteoblasts demonstrating positive type 1 collagen expression.

expression of type I collagen, particularly in the propolis-based groups. No significant differences were found between the 2 propolis-based groups.

This study involved the random allocation of *Cavia cobaya* into 4 groups – a control group and 3 treatment groups. All animals included in this study were selected according to the specific criteria described in the Methods section.

A similar study by Hozzein et al. investigated the effects of topical propolis application on cutaneous wound healing in diabetic mice.[19] Topical propolis demonstrated remarkable wound-healing effects, even in streptozotocin-induced type 1 diabetic mice. The levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, and matrix metalloproteinase-9 (MMP-9)) were significantly reduced, approaching those observed in the non-diabetic control group. Furthermore, propolis promoted type I collagen expression via the transforming growth factor-beta 1 (TGF-β1)/Smad2/3 signaling pathway.[19]

The remarkable effects of the propolis and bovine bone graft combination applied to the extraction socket were investigated by Kresnoadi et al.[20] They reported that this combination increased bone morphogenetic protein-7 (BMP-7) expression and decreased nuclear factor of activated T cells 1 (NFATc1) expression in osteoblasts on days 7 and 14. Significant differences were observed between the combination group and the control group ($p < 0.05$).[20]

The propolis extract used in this study contained 2.56% caffeic acid, 1.55% apigenin, 1.28% flavonoids, 0.82% saponins, 1.03% quercetin, and 1.15% terpenoids. Halim et al. compared the composition of Indonesian propolis with its Brazilian counterpart.[21] Qualitative analyses showed that the Indonesian propolis contained substantial amounts of flavonoids and phenolic compounds, followed by tannins, essential oils, steroids and triterpenoids, as well as saponins, alkaloids, glycosides, and sugars.[21]

The principal components of propolis are flavonoids and phenolic acids, including CAPE, which may constitute up to 50% of its bioactive fraction. Flavonoids, one of the largest group of naturally occurring phenolic compounds, are present in nearly all flowering plants and include many of the most common pigments found in plants worldwide. However, the flavonoid profile of propolis differs slightly from that of floral flavonoids due to enzymatic modification and processing by bees during propolis production.

The results showed that the expression levels of NF-κB, TLR2 and TNF-α decreased in all groups by day 30. The PE–BX group exhibited the lowest expression levels, followed by the PE, BX, and control groups, indicating a reduction in inflammatory markers, particularly after socket filling with the PE–BX combination. This effect may be attributed to the complex chemical composition of propolis, which contains phenolic compounds such as cinnamic acid. According to Conti et al., cinnamic acid can downregulate TLR2, human leukocyte antigen – DR isotype (HLA-DR), and cluster of differentiation 80 (CD80) expression while upregulating TLR4 expression in human monocytes.[22]

Flavonoids (such as quercetin, galangin and pinocembrin), along with caffeic acid, benzoic acid and cinnamic acid, may act on microbial membranes or cell walls, causing structural and functional damage.[23] Parolia et al. characterized propolis as having antibacterial properties due to its ability to inhibit bacterial RNA polymerase.[24] In addition, it exhibits immunomodulatory, antioxidant and wound-healing activities, largely attributable to its capacity to neutralize free radicals. Moreover, propolis contains trace elements such as iron and zinc, which are essential for collagen synthesis. Aromatic compounds present in propolis, including caffeic acid, are known to possess antimicrobial, antibacterial, anti-inflammatory, immunomodulatory, and hepatoprotective properties.[24]

Type I collagen plays a crucial role in osteogenesis, as it is assembled into fibers that form the structural and mechanical scaffold of bone.[25] It is also the most abundant organic component of the bone matrix.

In general, the molecular mechanisms that may occur following propolis administration include: (1) reduced expression of RANKL and the inhibition of the macrophage colony-stimulating factor (M-CSF)-induced RANK signaling pathway, leading to suppressed differentiation of osteoclast precursor cells; and (2) the upregulation of osteoblast-related gene markers, including *Alp*, *Runx2*, *Osx*, *Col-1*, thereby promoting osteoblast proliferation, differentiation and mineralization.

Flavonoids and CAPE in propolis are known to decrease osteoclast numbers while increasing osteoblast populations. They also exhibit anti-inflammatory effects by reducing the levels of pro-inflammatory cytokines.[26] Similarly, Iqbal et al. demonstrated that a 40% propolis extract reduced osteoclast numbers in guinea pigs by day 14 after tooth extraction.[27] This effect is attributed to the high flavonoid content in the 40% propolis gel, which inhibits osteoclastogenesis while promoting osteoblast maturation and enhancing bone remodeling activity.[27]

The study demonstrated promising results for the presented combination material. However, it was limited by the observation period, which lasted only 30 days, rather than extending until the complete healing of the alveolar bone and the full resolution of the lesion.

Conclusions

The administration of a combination of a natural propolis extract and a bovine bone graft during the extraction socket healing process can enhance type I collagen expression while reducing the NF-κB, TLR2 and TNF-α levels, thereby promoting alveolar bone regeneration.

Ethics approval and consent to participate

This research was approved by the Ethical Clearance Commission at the Faculty of Dental Medicine of the Airlangga University, Surabaya, Indonesia (approval No. 014/HRCC FODM/III/2018).

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Alhourani MA, Kasem T, Hamadah O. Comparative study between using a tissue adhesive (N-BCA & OCA) and surgical sutures in free gingival graft surgery: A randomized controlled clinical trial. *Dent Med Probl*. 2022;59(2):241–248. doi:10.17219/dmp/135382
2. Kouhestani F, Rezai Rad M, Mohaghegh S, Motamedian SR. Effect of metformin on the behavior of dental pulp stem cells cultured on freeze-dried bone allografts. *Dent Med Probl*. 2021;58(3):343–349. doi:10.17219/dmp/131988
3. Darby I, Chen S, De Poi R. Ridge preservation: What is it and when should it be considered. *Aust Dent J*. 2008;53(1):11–21. doi:10.1111/j.1834-7819.2007.00008.x
4. Kalsi AS, Kalsi JS, Bassi S. Alveolar ridge preservation: Why, when and how. *Br Dent J*. 2019;227(4):264–274. doi:10.1038/s41415-019-0647-2
5. Martinotti S, Ranzato E. Propolis: A new frontier for wound healing? *Burns Trauma*. 2015;3:9. doi:10.1186/s41038-015-0010-z
6. Przybyłek I, Karpiński TM. Antibacterial properties of propolis. *Molecules*. 2019;24(11):2047. doi:10.3390/molecules24112047
7. Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. *Oxid Med Cell Longev*. 2018;2018:7074209. doi:10.1155/2018/7074209
8. Zulhendri F, Lesmana R, Tandean S, et al. Recent update on the anti-inflammatory activities of propolis. *Molecules*. 2022;27(23):8473. doi:10.3390/molecules27238473
9. Katircioğlu H, Mercan N. Antimicrobial activity and chemical compositions of Turkish propolis from different regions. *Afr J Biotechnol*. 2006;5(11):1151–1153. doi:10.4314/ajb.v5i11.43001
10. Widjiastuti I, Suardita K, Saraswati W. The expressions of NF-kb and TGFb-1 on odontoblast-like cells of human dental pulp injected with propolis extracts. *Dent J (Majalah Kedokteran Gigi)*. 2014;47(1):13–18. doi:10.20473/j.djmkg.v47.i1.p13-18
11. Magnavacca A, Sangiovanni E, Racagni G, Dell'Agli M. The antiviral and immunomodulatory activities of propolis: An update and future perspectives for respiratory diseases. *Med Res Rev*. 2022;42(2):897–945. doi:10.1002/med.21866
12. Yang J, Pi A, Yan L, et al. Research progress on therapeutic effect and mechanism of propolis on wound healing. *Evid Based Complement Alternat Med*. 2022;2022:5798941. doi:10.1155/2022/5798941
13. Kresnoadi U, Rahayu RP, Ariani MD, Soesanto S. The potential of natural propolis extract combined with bovine bone graft in increasing heat shock protein 70 and osteocalcin on socket preservation. *Eur J Dent*. 2020;14(1):31–37. doi:10.1055/s-0040-1701921
14. Kresnoadi U, Yuli Prabowo TS. Expression of interleukin-1β and TGF-B due to induction with natural propolis extract and bovine bone graft combination in tooth extraction sockets leading to alveolar bone regeneration. *J Int Dent Med Res*. 2020;13(3):935–938. https://www.jidmr.com/journal/wp-content/uploads/2020/09/18-D20_1141_Jennifer_Widjaja_Utari_Kresnoadi_Indonesia.pdf. Accessed January 15, 2023.
15. Shiotani A, Takami M, Itoh K, Shibasaki Y, Sasaki T. Regulation of osteoclast differentiation and function by receptor activator of NFkB ligand and osteoprotegerin. *Anat Rec*. 2002;268(2):137–146. doi:10.1002/ar.10121
16. Muthukuru M, Darveau RP. TLR signaling that induces weak inflammatory response and SHIP1 enhances osteogenic functions. *Bone Res*. 2014;2:14031. doi:10.1038/boneres.2014.31
17. Blair HC, Larrouture QC, Li Y, et al. Osteoblast differentiation and bone matrix formation in vivo and in vitro. *Tissue Eng Part B Rev*. 2017;23(3):268–280. doi:10.1089/ten.TEB.2016.0454
18. Yuli Prabowo TS, Kresnoadi U, Hidayati HE. Effective dose of propolis extract combined with bovine bone graft on the number of osteoblasts and osteoclasts in tooth extraction socket preservation. *Dent J (Majalah Kedokteran Gigi)*. 2020;53(1):40–44. doi:10.20473/j.djmkg.v53.i1.p40-44
19. Hozzein WN, Badr G, Al Ghamdi AA, Sayed A, Al-Waili NS, Garraud O. Topical application of propolis enhances cutaneous wound healing by promoting TGF-beta/Smad-mediated collagen production in a streptozotocin-induced type I diabetic mouse model. *Cell Physiol Biochem*. 2015;37(3):940–954. doi:10.1159/000430221
20. Kresnoadi U, Widjaja J, Laksono H. Ethanol extract of propolis–bovine bone graft combination as a prospective candidate for socket preservation: Enhancing BMP7 and decreasing NFATc1. *Saudi Dent J*. 2021;33(8):1055–1062. doi:10.1016/j.sdentj.2021.05.003
21. Halim E, Hardinsyah H, Sutandyo N, Sulaeman A, Artika M, Harahap Y. Bioactive compounds and nutrients content of Indonesian and Brazilian propolis [in Indonesian]. *Jurnal Gizi dan Pangan*. 2012;7(1):1–6. https://journal.ipb.ac.id/index.php/jgizipangan/article/view/6134/4760. Accessed January 14, 2023.
22. Conti BJ, Búfalo MC, De Assis Golim M, Bankova V, Sforcin JM. Cinnamic acid is partially involved in propolis immunomodulatory action on human monocytes. *Evid Based Complement Alternat Med*. 2013;2013:109864. doi:10.1155/2013/109864
23. Almuhayawi MS. Propolis as a novel antibacterial agent. *Saudi J Biol Sci*. 2020;27(11):3079–3086. doi:10.1016/j.sjbs.2020.09.016
24. Parolia A, Thomas MS, Kundabala M, Mohan M. Propolis and its potential uses in oral health. *Int J Med Med Sci*. 2010;2(7):210–215. doi:10.5897/IJMMS.9000192
25. Khudan R, Krynytska I, Marushchak M, Korda M. Influence of chronic hyperhomocysteinemia on the features of bone metabolism in the case of lipopolysaccharide-induced periodontitis. *Dent Med Probl*. 2022;59(2):255–261. doi:10.17219/dmp/143948
26. Guney A, Karaman I, Oner M, Yerer MB. Effects of propolis on fracture healing: An experimental study. *Phytother Res*. 2011;25(11):1648–1652. doi:10.1002/ptr.3470
27. Iqbal M, Perkasa A, Yogyarti S, Harijanto E. Propolis extract gel application to the socket of *Cavia cobaya* after tooth extraction to the quantity of osteoclast [in Indonesian]. Thesis. Airlangga University, Surabaya, Indonesia. 2017.

Original paper

Do intracanal medicaments affect the bond of bioceramic sealer (BioRoot™ RCS) to radicular dentin?

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Abstract

Background. Bioceramic sealers have gained popularity due to their distinctive bioactivity and superior sealing ability in comparison with other contemporary sealers. Therefore, it is important to understand their interactions with agents used in root canal therapy, such as intracanal medicaments (ICMs), and the impact of these interactions on the bonding ability of the sealer to radicular dentin.

Objectives. The present in vitro study assessed the influence of 3 ICMs – calcium hydroxide (CH), a modified triple antibiotic paste (mTAP) and propolis (Pr)-based agents – on the bonding ability of bioceramic sealer (BioRoot™ RCS) to radicular dentin.

Material and methods. Eighteen single-rooted permanent teeth were decoronated, biomechanically prepared, and allocated into 3 groups for intracanal treatment with CH, mTAP and Pr, respectively. Sonic-activated irrigation was used for the removal of ICMs, followed by single-cone gutta-percha (GP) obturation with the BioRoot RCS sealer. Root sections were then sliced at the apical, middle and cervical levels to evaluate the bond strength at the sealer–radicular dentin interface.

Results. At the cervical root level, the CH group demonstrated significantly higher bond strength, followed by mTAP and Pr. In contrast, in the middle and apical thirds, Pr exhibited higher bond strength than both CH and mTAP.

Conclusions. All the tested ICMs affected the bonding ability of the bioceramic sealer (BioRoot RCS) to radicular dentin; however, the least adverse influence was observed with CH in the cervical third and with Pr in the middle and apical thirds.

Keywords: calcium hydroxide, propolis, bioactive, sealer, bond strength

Highlights

- Intracanal medicaments (ICMs) significantly affected the push-out bond strength (POBS) of the calcium silicate-based bioceramic sealer BioRoot RCS across different root canal levels, highlighting their clinical relevance in endodontic adhesion outcomes.
- Calcium hydroxide demonstrated the highest POBS in the cervical third, whereas propolis showed superior bond strength in the middle and apical thirds. In contrast, a modified triple antibiotic paste (mTAP) was associated with comparatively lower bond strength, potentially due to dentinal erosion and the interference with sealer adhesion caused by residual medicament traces.
- A progressive increase in the POBS of BioRoot RCS was observed from the cervical to the apical third across all experimental groups, suggesting improved sealer adaptation and retention in regions with reduced dentinal tubule density.

Introduction

Successful endodontic therapy primarily depends on adequate chemo-mechanical debridement and disinfection, followed by three-dimensional (3D) obturation to achieve a corono-apical seal. Traditionally, root canal sealers have been used in combination with semisolid/solid core materials to obtain a 3D filling with a hermetic apical seal, ensuring their infiltration into lateral and accessory canals, and thereby reducing apical and coronal microleakage.[1]

Apart from the fundamental material requisites of biocompatibility, dimensional stability and antimicrobial properties, the primary requirement of an ideal root canal sealer is the formation of an adequate adhesive bond at the radicular wall–sealer/filling material interface to ensure a superior sealing ability. Several endodontic sealers with variable sealing abilities, based on different formulations, such as zinc oxide–eugenol, glass ionomer, salicylate (calcium hydroxide-based), silicone, epoxy resin, methacrylate, and more recently bioceramic sealers (calcium silicate–based, calcium phosphate-based and mineral trioxide aggregate (MTA)-based), have been introduced into endodontics.[1]

In a systematic review conducted by Alghamdi and Abduljawad, a total of 12 in vitro studies evaluating the apical sealing potential of various root canal obturation methods with different endodontic sealers, using the non-destructive fluid filtration technique, were assessed.[2] In this review, when the apical sealing ability of a resin-based sealer (AH Plus) was compared with 2 different bioceramic sealers (iRoot SP and MTA Fillapex), one study by Zhang et al. reported no significant difference in apical sealing when iRoot SP was used with either cold lateral compaction or the single-cone gutta-percha (GP) technique.[3] In contrast, Asawaworarit et al., who evaluated MTA Fillapex using warm vertical compaction, reported relatively lower apical microleakage after 4 weeks of evaluation.[4]

A scanning electron microscopy (SEM) analysis by Haji et al. evaluated the adhesion ability of 2 calcium silicate-based bioceramic sealers (BioRoot RCS and CeraSeal RCS) in comparison with a zinc oxide–eugenol-based sealer at the sealer–radicular dentin interface in the coronal, middle and apical thirds, based on the sealing ability scores (Ray and Seltzer, 1991), which categorized adhesion as very good (no gaps), good (minor gaps) or acceptable (frequently observed gaps).[5] Both bioceramic sealers demonstrated "very good adhesion" in all thirds of the root as compared to the "acceptable adhesion" observed with the zinc oxide–eugenol sealer, thereby highlighting the superior physical properties of bioceramic sealers, such as wettability, dimensional stability, flow, and low film thickness, which promote greater surface penetrability.[5]

Another recent systematic review and meta-analysis by Rekha et al. assessed 24 in vitro studies for qualitative analysis, with sample sizes ranging from 10 to 26, of which 16 studies were included in the quantitative analysis.[6] For the evaluation of microleakage, a review of 18 studies employing different advanced methods for assessing the sealing ability concluded that, although both epoxy resin-based and bioceramic sealers exhibited comparable adhesive properties, bioceramic sealers – owing to their desirable combination of a superior sealing ability and biocompatibility – may offer improved long-term success and better prognostic outcomes for the tooth.[6]

Among the various endodontic sealers available, calcium silicate-based sealers have garnered widespread interest and gained immense popularity in recent times owing to their superior sealing ability at the radicular interfacial dentin. This phenomenon is attributed to the precipitation of hydroxyapatite and the enhanced ion diffusion resulting from the degradation of the collagenous component, caused by the alkalinity of the sealer's hydration products. Owing to their hydrophilic nature, these sealers utilize the natural moisture content within the dentinal tubules to complete the setting reaction, thereby establishing an intricate sealing bond with the radicular dentin and preventing apical leakage, which greatly contributes to the success of non-surgical endodontic therapy.[1]

For effective apical sealing, the confinement of the sealer within the canal space is crucial to prevent apical extrusion. This commonly encountered procedural mishap violates the biological objectives of obturation, as the unset sealer may act as a foreign body capable of inducing cytotoxic reactions and post-operative pain.

In a review evaluating the prevalence of sealer extrusion, a total of 439 completed non-surgical endodontic treatment procedures in permanent teeth, including both primary treatment and retreatment, were assessed.[7] The review included reports published in 62 issues of the Journal of Endodontics (2015–2020) and 9 issues of the International Endodontic Journal (2020). Based on the criterion of any radiopaque material extending beyond the radiographic apex of the root, sealer extrusion was observed in 142 cases, accounting for 32.3% of the total cases.[7]

A systematic review conducted by Aminoshariae and Kulild reported that cases with sealer extrusion exhibited a 32% greater risk of unfavorable, non-healing outcomes as compared to cases without sealer extrusion.[8] A prospective clinical trial conducted by Yu et al. reported a relatively higher incidence of sealer extrusion with the epoxy resin-based sealer AH Plus (41.5%) and the employment of the warm vertical obturation technique, compared to only 13.7% when the calcium silicate-based bioceramic sealer EndoSequence BC Sealer was used with the single-cone GP technique.[9] Similarly, a study conducted by Tan et al. demonstrated that the calcium silicate-based bioceramic sealer TotalFill BC exhibited a lower rate of apical sealer extrusion as compared to the epoxy resin-based sealer AH Plus.[10]

In a study conducted by Chybowski et al., although a high incidence of apical sealer extrusion (47.4%) was documented following single-cone obturation with the calcium silicate-based bioceramic sealer EndoSequence BC Sealer, this finding was attributed to the excellent flowability of bioceramic sealers.[11] Nevertheless, no significant adverse effects were observed, even after a follow-up period of 2.5 years. The authors suggested that, owing to the low solubility of calcium silicate components, the extruded sealer may persist within the periradicular tissues and potentially undergo bioactive interaction with tissue fluids, leading to hydroxyapatite formation within the host tissue and eventual replacement by bone.[8,11] Paradoxically, contrary to expectations, several researchers have demonstrated that even the extrusion of conventional endodontic sealers may not affect the overall outcomes of non-surgical endodontic treatment to a great extent. Goldberg et al. reported no statistically significant effect on treatment outcomes in the case of obturation with the lateral condensation technique, using epoxy resin- and zinc oxide-based sealers, with a mean follow-up period of 4.86 years.[12] Similarly, Ricucci et al. concluded that the apically extruded root canal sealers did not produce statistically significant differences in treatment outcomes, even when the extruded material was radiographically evident.[13] The authors further suggested that the presence of a pre-operative periradicular lesion was more likely to be the primary determinant of unsuccessful outcomes.[13]

The apical extrusion of the sealer and/or obturation materials is also associated with post-operative pain, one of the most common undesirable sequelae resulting from a transient inflammatory response triggered by the sensitization of the periradicular tissues. In this regard, calcium hydroxide-based sealers, such as Sealapex and Apexit Plus, are reported to be better tolerated than epoxy resin-based sealers, such as AH Plus and Resino Seal, which have been shown to release cytotoxic amines, epoxy resin components, and trace amounts of formaldehyde (3.9 ppm).[14]

A clinical study compared 2 calcium silicate-based bioceramic sealers – Bio-C Sealer and BC Sealer – with the epoxy resin-based sealer AH Plus in molar teeth diagnosed with asymptomatic irreversible pulpitis and obturated using the vertical compaction technique.[15] A relatively similar extrusion rate (1–2 mm, approx. 12%) was observed among all groups. Using a visual descriptor scale ranging from 0 to 10, the 330 patients reported no significant differences in post-obturation pain among the groups at 6, 12 and 48 h, as well as at 7 days postoperatively. The study concluded that the gradual reduction in pain symptoms over time might be attributed to the progressive decline in inflammatory activity within the tissues, along with the transition of the irritating components of the sealer into an inert state, following complete setting.[15]

Bonding of the sealer to radicular dentin is governed by the degree of adhesion, which in turn largely depends on 2 principal parameters – the intrinsic properties of the sealer material and the surface characteristics of intracanal dentin, following debridement and disinfection during chemo-mechanical instrumentation. The properties of root canal sealers that influence adhesion include surface tension, wettability, hydrophilicity, and the setting behavior (expansion or contraction) of the material when used in conjunction with obturating core materials. On the other hand, the dentinal wall factors affecting adhesion are not only related to dentin topography and intermolecular surface energy, but also to the degree of surface cleanliness with respect to the smear layer generated during instrumentation, residual traces of irrigants and intracanal medicaments (ICMs) used for disinfection, and the surface modifications induced by laser-assisted photoactivated disinfection (PAD).[16]

The hydrophobic behavior is primarily attributed to the resin component of epoxy resin-based sealers such as AH Plus, which also tend to undergo polymerization shrinkage during setting. Both factors contribute to reduced adaptation and potential detachment from the radicular dentin walls. In contrast, hydrophilic non-resin sealers based on zinc oxide–eugenol, calcium hydroxide, calcium silicate, and their combinations – such as GuttaFlow

Bioseal –exhibit superior flowability, as well as a slight expansion upon setting.[17] A study by Uzunoglu-Özyürek demonstrated greater dentinal tubule penetration with the calcium silicate–based bioceramic sealer BioRoot RCS as compared to the epoxy resin-based sealer AH Plus.[18]

Bioceramic sealers are composed of fine fillers that enhance their flowability, while their hydrophilic nature enables the utilization of the inherent moisture within the dentinal tubules. They also exhibit slight expansion during setting without polymerization shrinkage, thereby contributing to the formation of a relatively gap-free interface between the dentinal wall, the root canal sealer and the GP filling material.[19]

Routinely used intracanal irrigants, such as sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX), among others, alter the surface energy of dentin. Similarly, ICMs, such as calcium hydroxide and a triple antibiotic paste (TAP), when applied for extended periods, may leave residual deposits on the canal walls. These remnants can interfere with the wettability, flow and adaptation of sealers, thereby compromising the adhesion even of hydrophilic root canal sealers.[20]

Intracanal medicaments are stable, biocompatible temporary therapeutic and antimicrobial agents that provide enhanced canal disinfection through prolonged contact during the inter-appointment period in multi-visit non-surgical endodontic therapy. Following the use of various intracanal irrigants, these medicaments are employed to address multifactorial and challenging clinical scenarios, including long-standing large periapical pathologies, weeping canals, traumatic dental injuries, complex root canal anatomy, and patients with compromised immune responses.[21–24]

Several ICMs with diverse therapeutic properties and formulations, like calcium hydroxide, antibiotic-based pastes, CHX, and various natural alternatives, have been introduced into endodontic practice.

Calcium hydroxide is one of the oldest materials (Hermann, 1920) used as ICM in endodontics and is regarded as the gold standard, as it fulfils the majority of the requirements proposed by Grossman,[25] with a reported success rate of 73.8–80.8%.[26] When calcium hydroxide comes into contact with aqueous fluid, it slowly dissociates into calcium and hydroxyl ions, creating an alkaline root canal environment with highly reactive free radicals that exert a potent antimicrobial effect against most common endodontic microorganisms.[26] However, several studies have also reported its limited inhibitory effect against highly resistant microorganisms, such as *Enterococcus faecalis*, a Gram-positive facultative anaerobic bacterium known for its ability to form biofilms and its association with post-endodontic periradicular lesions, which account for 22–77% of endodontic failures.[27–29] Long-term application of calcium hydroxide has also been shown to exert a structural weakening effect on radicular dentin,[30] with a progressive decrease in microhardness – an indicator of mineral gain or loss – which may negatively affect the sealing ability. In comparison, propolis demonstrated a progressive increase in microhardness over a period of 7 days.[31]

For the utilization of relatively newer calcium silicate-based bioceramic sealers in both single- and multiple-visit endodontics, it is essential to evaluate their performance following the use of routinely employed ICMs, including calcium hydroxide-based preparations, antibiotic pastes and the emerging natural agents. However, given the limitations associated with calcium hydroxide-based ICMs, there is a need to compare alternative medicaments, such as antibiotic paste-based formulations with broad-spectrum antimicrobial activity and natural agents with improved biocompatibility, better tissue and tolerance fewer adverse effects. Accordingly, the present study aimed to evaluate the influence of various ICMs – calcium hydroxide (CH), a modified triple antibiotic paste (mTAP) and propolis (Pr) – on the push-out bond strength (POBS) of a calcium silicate-based bioceramic sealer BioRoot™ RCS to radicular dentin.

Material and methods

Eighteen extracted single-rooted human permanent teeth were selected after excluding specimens with surface defects or crack lines, developmental anomalies, calcified roots, internal or external resorption, open apices, severe root curvatures, or previous endodontic treatment.

Sample preparation

Decoronated root specimens were subjected to biomechanical preparation using ProTaper Gold files (Dentsply Maillefer, Ballaigues, Switzerland) up to F4 (size 40, 6% taper), with irrigation using 3% NaOCl and 17% EDTA solution. Following the final irrigation with distilled water, the root canal samples were blot dried using paper point cones and randomly allocated into 3 groups of 6 samples each to receive intracanal dressings delivered with a Lentulo spiral, as follows: group 1 (G1) – CH (calcium hydroxide and distilled water)[23]; group 2 (G2) – mTAP (a modified triple antibiotic paste prepared by combining approximately equal amounts of ciprofloxacin, metronidazole and cefaclor powders with distilled water in a 3:1 proportion)[32]; and group 3 (G3) – Pr (200 mg propolis powder (Hi-Tech Natural Products (India) Ltd., Delhi, India) mixed with 0.3 mL saline).[33]

The access cavities were sealed with a zinc oxide–eugenol-based filling material and stored at 37°C under 100% relative humidity for 2 weeks. The intracanal dressing was removed by sequential irrigation with 5 mL each of 3% NaOCl and 17% EDTA, followed by the final irrigation with distilled water activated by sonic agitation

using the EndoActivator® system (Dentsply Tulsa Dental Specialties, Tulsa, USA) for 30 s (18,000 cycles per minute). The root canals were obturated with a single GP cone, size F4, coated with the BioRoot RCS endodontic sealer (Septodont, Saint-Maur-des-Fossés, France), restored coronally with a zinc oxide–eugenol-based material and allowed to set for 1 week.

The roots were horizontally sectioned into slices approx. 2 mm thick, as verified using a digital caliper (Fig. 1), with a diamond disc to obtain apical, middle and cervical sections at 3, 7 and 11 mm levels, respectively (Fig. 2).

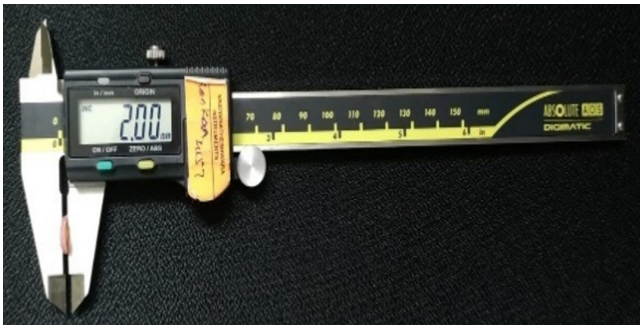

Fig. 1. Prepared root sections were measured using a digital caliper prior to push-out bond strength (POBS) testing to standardize the sample thickness, and only samples measuring 2 mm in thickness were included

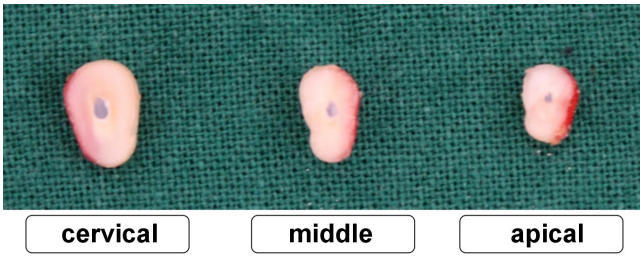

Fig. 2. Representation of cervical, middle and apical root thirds

Push-out bond strength (POBS) assessment

Root specimens mounted on a custom-made acrylic mold with a central opening to facilitate the push-out of the obturation material were subjected to compressive loading in a corono-apical direction, using a universal testing machine. Pluggers of varying tip diameters (0.3–0.8 mm) were used at a crosshead speed of 2 mm/min; they were positioned to cover most of the surface of the root-filling material without contacting the adjacent canal walls.

Failure/debonding was identified by a steep drop on the load–time graph generated by the computer software and/or by the complete dislodgement of the root-filling material. The maximum failure load was recorded in newtons and subsequently converted to megapascals. The recorded peak load was divided by the bonded surface area, estimated using a stereomicroscope (Fig. 3), to calculate bond strength using the following formula (Equation 1)[34,35]:

$$\text{bond strength} = F / A \quad [\text{mPA} / \text{mm}^2] \tag{1}$$

where:
F – maximum force [MPa];
A – bonded surface area [mm^2];
$A = \pi h \times (r_1 + r_2)$;
h – specimen thickness [mm];
r_1 – apical aspect radius;
r_2 – coronal aspect radius.

Statistical analysis

Statistical analysis was performed using the two-way analysis of variance (ANOVA), followed by Tukey's post hoc test, using the nMaster Software, v. 2 (https://nmaster.software.informer.com), and the results were considered statistically significant at $p < 0.05$.

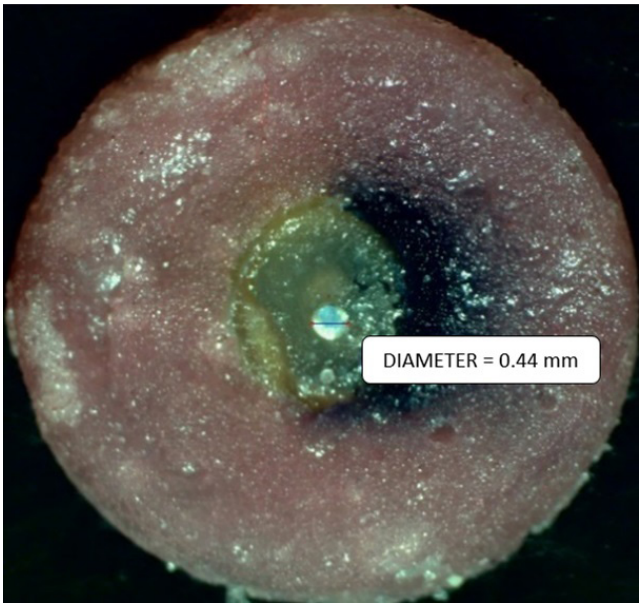

Fig. 3. Stereomicroscopic image of a root slice embedded in an acrylic mold, highlighting the root canal diameter utilized for the calculation of push-out bond strength (POBS)

Results

BioRoot RCS demonstrated higher POBS in the middle and apical sections when used following propolis, whereas calcium hydroxide showed better results in the cervical third (Table 1, Fig. 4). While the difference in the POBS values between G2 (mTAP) and G3 (Pr) was non-significant at the cervical level, G1 (CH) and G2 (mTAP) demonstrated non-significant differences at the middle and apical levels ($p > 0.05$) (Table 2).

Table 1. Comparison of bond strength between the root sections in each study group ($n = 6$)

Root section	Group	POBS [MPa]			ANOVA	
		M ±*SD*	min	max	*F*	*p*-value
C	G1	3.86 ±0.57	3.06	4.69	15.44	<0.001*
	G2	2.87 ±0.38	2.14	3.12		
	G3	2.56 ±0.26	2.24	2.95		
M	G1	5.23 ±0.91	4.19	6.45	36.33	<0.001*
	G2	5.06 ±0.86	4.05	6.52		
	G3	8.42 ±0.45	7.66	8.76		
A	G1	6.66 ±0.40	6.14	7.08	60.89	<0.001*
	G2	6.96 ±0.95	6.21	8.47		
	G3	11.71 ±1.15	10.49	12.90		

Groups: G1 – calcium hydroxide (CH); G2 – modified triple antibiotic paste (mTAP); G3 – propolis (Pr).
Root sections: C – coronal third; M – middle third; A – apical third.
POBS – push-out bond strength; *M* – mean; *SD* – standard deviation; min – minimum; max – maximum; * statistically significant.

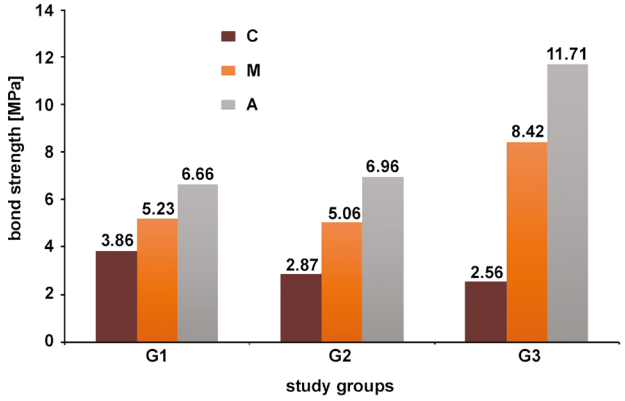

Fig. 4. Comparison of bond strength between the root sections in each study group

Groups: G1 – calcium hydroxide (CH); G2 – modified triple antibiotic paste (mTAP); G3 – propolis (Pr).
Root sections: C – coronal third; M – middle third; A – apical third.
* statistically significant.

Table 2. Pairwise comparisons of bond strength [MPa] between the study groups

Root section	Comparison		Mean difference (I–J)	*SE*	*p*-value	95% *CI*	
	group (I)	group (J)				lower bound	upper bound
C	G1	G2	0.99	0.24	0.003*	0.36	1.62
	G1	G3	1.30	0.24	<0.001*	0.67	1.93
	G2	G3	0.31	0.24	0.430	–0.32	0.94
M	G1	G2	0.17	0.44	0.920	–0.98	1.32
	G1	G3	–3.19	0.44	<0.001*	–4.33	–2.03
	G2	G3	–3.36	0.44	<0.001*	–4.50	–2.20
A	G1	G2	–0.30	0.51	0.840	–1.63	1.04
	G1	G3	–5.05	0.51	<0.001*	–6.39	–3.72
	G2	G3	–4.75	0.51	<0.001*	–6.09	–3.42

Groups: G1 – calcium hydroxide (CH); G2 – modified triple antibiotic paste (mTAP); G3 – propolis (Pr).
Root sections: C – coronal third; M – middle third; A – apical third.
SE – standard error; *CI* – confidence interval; * statistically significant.

Discussion

Obturation constitutes the final step in the triad of non-surgical endodontic treatment, following adequate access opening and biomechanical preparation of the root canal space. Traditionally, root canal obturation is most commonly accomplished using GP-based filling materials, considered the gold standard owing to their inert, thermoplastic, retreatable, and radiopaque nature, along with their wide availability. However, these materials unfortunately lack the ability to adhere to the radicular dentin walls.

Ideally, a root filling material should intimately bond to intracanal dentin and resist the dislodging forces by achieving strong adhesion. This objective is accomplished through the use of endodontic sealers at the interface, along with GP. Sealers, applied in a thin, tacky consistency, act as a luting medium for the core filling material during obturation, filling voids and entombing residual bacteria, thereby creating a fluid-tight seal. Over the years, several advancements have been made to improve the type, quality and properties of endodontic sealer materials, with the aim of establishing a continuum "the core material–the sealer–radicular dentin" as a single cohesive entity.[36]

Bioceramic materials are ceramic-based materials used in both medicine and dentistry. The newer bioceramic materials introduced into dentistry primarily consist of bioactive glass, calcium phosphate, calcium silicate, hydroxyapatite, bioinert alumina, zirconia, and other components. In dentistry, they have been applied for various purposes, including as dentin substitutes, pulp-capping agents, pulpotomy materials, apexification agents, root repair materials, retrograde filling materials, perforation sealing agents, endodontic sealers, for filling bony defects, and as adjuncts in regeneration during periradicular surgeries. These materials offer several advantages, including excellent biocompatibility, superior mechanical properties that provide a strengthening effect to the tooth structure, antimicrobial activity, intrinsic osteoinductive potential, a superior sealing ability, chemical bonding to the tooth structure, and radiopacity.[37,38]

Bioceramic sealers have been used in endodontics for the past 30 years, and are classified based on their composition into MTA-based, calcium phosphate-based and calcium silicate-based sealers. These sealers possess 2 key properties that distinguish them from other sealers – bioactivity and a superior sealing ability to radicular dentin through the formation of a distinct mineral infiltration zone (Fig. 5).[3,39–41]

BioRoot RCS, an alkaline calcium silicate-based bioceramic sealer with a pH of approx. 11–12, is a relatively new material comprising components such as tricalcium silicate and calcium chloride (Table 3). BioRoot RCS was used in the present study, as it has been reported to release nearly twice the amount of calcium ions and form a greater calcium phosphate phase as compared

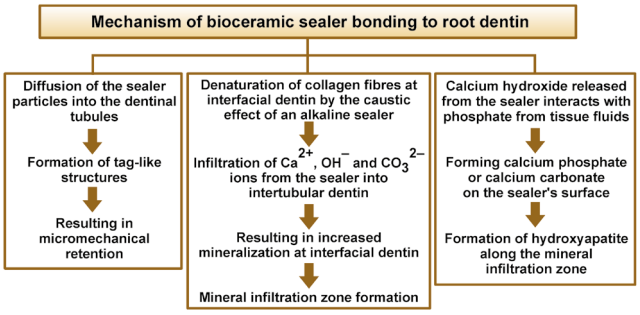

Fig. 5. Mechanism of bioceramic endodontic sealer bonding to root dentin
Ca^{2+} – calcium ions; OH^- – hydroxyl ions; CO_3^{2-} – carbonate ions.

Table 3. Composition of BioRoot RCS

Component	Composition
Powder	tricalcium silicate, povidone, zirconium oxide
Liquid	aqueous solution of calcium chloride and polycarboxylate

to EndoSequence BC Sealer.[42,43] In addition, it has been shown to stimulate human periodontal ligament cells to express angiogenic and osteogenic growth factors.[44] BioRoot RCS has also been associated with lower cytotoxicity than conventional endodontic sealers, along with antimicrobial properties and the ability to promote hard tissue deposition.[45–47] An SEM study comparing the sealing ability of Sealapex, BioRoot RCS, MTA Fillapex, and zinc oxide–eugenol-based sealers demonstrated that BioRoot RCS exhibited the best marginal adaptation, with the least number of gaps at the cervical, middle and apical root interfaces, which was attributed to its hydraulic sealing capability.[48]

As a variety of intracanal irrigants and medicaments based on chemical and natural formulations are routinely employed for root canal disinfection in contemporary endodontic practice, it is imperative to evaluate and understand their influence on the sealing ability of newer bioceramic sealers. Since ICMs remain in contact with radicular dentin for relatively prolonged durations, the present study was conducted to evaluate the influence of 3 different ICMs – calcium hydroxide (gold standard), a modified triple antibiotic paste (mTAP) and propolis (a natural bee-derived product) – on the sealing ability of the bioceramic sealer BioRoot RCS to radicular dentin at different root levels.

Calcium hydroxide, a commonly used ICM, possesses antimicrobial and anti-inflammatory properties, an alkaline pH of approx. 12.5, as well as the ability to dissolve tissue, inhibit root resorption and promote hard tissue formation with periradicular healing. However, owing to variable reports regarding its antimicrobial efficacy, alternative medicaments based on CHX, antibiotic paste combinations and natural agents such as propolis have been investigated as potential options.[22,49]

Triple antibiotic paste is a biocompatible ICM composed of the bactericidal agents ciprofloxacin and metronidazole, along with the bacteriostatic agent minocycline. TAP is effective against Gram-negative, Gram-positive and anaerobic microbiota, and helps create a favorable environment for subsequent endodontic procedures. In a microbiological study, TAP demonstrated a larger zone of inhibition against *E. faecalis* as compared to calcium hydroxide, which is considered the gold standard among ICMs.[50] However, minocycline in TAP has been associated with tooth discoloration, anti-angiogenic effects and the chelation of root dentin, leading to the demineralization and weakening of the root structure. Therefore, it has been replaced with alternatives such as cefaclor, a second-generation cephalosporin, to formulate mTAP, which was used in the present study. The mTAP formulation consisting of ciprofloxacin, metronidazole and cefaclor is effective in managing polymicrobial endodontic infections.[51]

Propolis is a well-established natural antibiotic agent derived from beeswax and consists of a complex blend of resinous substances collected by bees from plants. It contains flavonoids as its principal biologically active constituents, along with other organic compounds, such as aromatic and fatty acids, which contribute to its recognized antimicrobial efficacy as ICM against polymicrobial colonies isolated from necrotic root canals.[52,53] The chemical composition of propolis includes aromatic acids, fatty acids and their esters, flavonoids, such as naringenin, pinocembrin, phenols, and 5-methoxy-3,7-dihydroxyflavonone, as well as ketones, alcohols, terpenes, such as chrysophanol and 1-ethanone, and other trace components. In addition to its antimicrobial activity, propolis also exhibits antioxidant and anti-inflammatory properties. Besides its application as ICM, it has been used as an anticaries agent, a canal irrigant, a pulp-capping agent, a desensitizing agent, and as a transport medium for avulsed teeth.[33]

The present in vitro study evaluated the POBS of radicular dentin obturated with a calcium silicate-based sealer (BioRoot RCS), following treatment with a natural ICM (propolis), an antibiotic-based medicament (a modified triple antibiotic paste – mTAP) and a calcium hydroxide-based ICM in the cervical, middle and apical thirds of the root canal. In the cervical third, the highest bond strength was observed in G1 (CH), followed by G2 (mTAP) and G3 (Pr). In the middle third, the highest bond strength was recorded in G3 (Pr), followed by G1 (CH) and G2 (mTAP), whereas in the apical third, bond strength was highest in G3 (Pr), followed by G2 (mTAP) and G1 (CH), respectively. Statistically, no significant differences were observed between G2 and G3 in the cervical third, or between G1 and G2 in the middle and apical thirds ($p > 0.05$).

In the cervical third, G1 (CH) demonstrated higher POBS than G2 (mTAP) and G3 (Pr) (Table 1), which could be attributed to the interaction between the primary components of BioRoot RCS sealer, namely calcium silicate and calcium phosphate, and the residual calcium hydroxide.[23] In the middle and apical thirds, G3 (Pr) exhibited the highest POBS, possibly facilitated by the preferentially

strong interaction of the aromatic and fatty acids present in propolis with the hydrophilic components of the dentin surface[22] and BioRoot RCS, which warrants further investigation to better understand this chemical interaction.

Group G2 (mTAP) demonstrated only moderate POBS at all root levels, which may be attributed to the reduced dislodgement resistance resulting from the significant erosion of the dentinal tubule orifices by the residual medicament. Similar findings were observed in an SEM study by Akman et al., which evaluated mTAP and double antibiotic paste (DAP) dressings used with BioAggregate apical plugs and Biodentine.[36]

The POBS recorded in all 3 groups was highest in the apical third, followed by the middle and cervical thirds, respectively. This finding is consistent with the observations of Mannocci et al., who reported an association between higher bond strength and lower densities of the dentinal tubules.[54] In addition, Cimpean et al. suggested that the precise adaptation of the GP cone in the middle and apical sections of the prepared canals contributes to improved sealer diffusion into the dentinal tubules.[55]

While ICMs have been implicated in both negatively and positively influencing the dislodgement resistance of root-filling materials, some studies have also reported negligible effects. Nagas et al. demonstrated the detrimental impact of residual traces of ICMs on cement adhesion, with the exception of calcium hydroxide,[56] whereas Topçuoğlu et al. reported that calcium hydroxide might positively enhance bonding strength by chelating with calcium in dentin.[57] In the latter study, the residues of mTAP appeared to interfere with chemical bonding between radicular dentin and the sealer by acting as a physical barrier, whereas the removal of the sticky propolis-based ICM by the circulating irrigants was relatively limited in the apical and middle thirds as compared with the cervical third.[22,57]

As the presence of residual medicaments plays a significant role in sealer adhesion, their effective removal must be considered during POBS evaluation. Although ultrasonic-activated irrigation has shown insufficiency in removing calcium hydroxide remnants from root canals,[18] it has been found to be effective when used in combination with NaOCl for the removal of TAP from artificial grooves.[58] Several researchers have reported EDTA as the most effective agent for eliminating calcium hydroxide from the canal space[55]; hence, a combination of NaOCl and EDTA with ultrasonic irrigation was employed as the final debridement and cleansing protocol in this study. The NaOCl–EDTA irrigation protocol prior to the use of a bioceramic sealer (TotalFill BC) has been shown to yield higher POBS values than etidronic acid and NaOCl alone, as EDTA roughens the dentin surface, thereby enhancing the micromechanical interlocking of the sealer with the dentinal tubules.[59] However, the delivery and activation methods used in the irrigation protocol may also influence POBS outcomes.

A study by Bogari et al. evaluated the bonding ability and adaptation of a bioceramic sealer (TotalFill BC), using a POBS test and SEM following irrigation with 3 protocols – conventional syringe-needle irrigation, ultrasonic activation, and neodymium-doped yttrium aluminum garnet (Nd:YAG) laser activation at a wavelength of 975 nm in the pulsed mode.[60] The highest POBS values were observed in the Nd:YAG laser group (6.46 ±0.5 MPa), which was statistically significant in comparison with the ultrasonic (4.21 ±2.2 MPa) and conventional syringe-needle irrigation (3.33 ±1.8 MPa) groups. The SEM analysis of the radicular wall–GP/sealer interface demonstrated the largest gaps in the conventional syringe-needle group (~61 μm), followed by the ultrasonic group (~58 μm), while the least gaps were observed in the Nd:YAG laser group (~25 μm), which also produced a smoother, cleaner dentin surface conducive to sealer penetration.[60] These findings indicate that the irrigation protocols combined with different activation methods not only enhance canal debridement and smear layer removal, but also influence the bonding performance of bioceramic sealers to the canal walls. Similarly, a pilot study by Esteves-Oliveira et al. investigating the effects of lasers on the permeability and morphology of radicular dentin reported that the Nd:YAG laser significantly reduced dentin permeability as compared to the erbium-doped yttrium aluminum garnet (Er:YAG) and diode lasers.[61] Hence, it can be concluded that although different lasers exhibit varying properties, they show promising potential as adjuncts for disinfection and debridement in endodontic treatment.[60–64] Accordingly, the future scope of the present study includes evaluating different ICMs in combination with various irrigation activation methods, including laser-assisted protocols, to further assess their impact on the POBS of bioceramic sealers to radicular dentin.

The POBS assessment tests are widely employed to evaluate the effectiveness of bonding between dental materials and radicular dentin,[24] as dislodgement resistance during restorative procedures, post-space preparation and tooth flexure not only helps prevent corono-apical leakage, but also enhances resistance to root fracture.[65] A material exhibiting high POBS contributes to improved longevity and a better prognosis of endodontically treated teeth.[24,65]

Two-millimeter-thick root sections were used for bond strength assessment in this study to minimize early debonding and sealer detachment during slicing. The plugger-to-specimen diameter ratio is an important parameter, as ratios below 55% may reduce bond strength values, whereas ratios between 70% and 90% do not significantly influence the results.[66] Since the plugger diameter is required to be approx. 0.85 times smaller than the diameter of the obturation filling while still being broad enough to avoid scoring the material surface,[67] pluggers of varying diameters (0.3–0.8 mm) were used in this study.

As obturation with lateral condensation has inherent disadvantages, including the lack of GP homogeneity, poor adaptation to the canal walls, a higher proportion of the sealer in the apical region, and an increased risk of vertical root fracture,[68] and as warm vertical compaction is unsuitable for bioceramic sealers due to the potential heat-induced alteration of their adhesive properties, single-cone GP obturation was employed in this study.[69] Although the use of a core material with a sealer as a root canal filling may present limitations, such as voids at the sealer–core interface and the lack of homogeneity, it remains the accepted standard in contemporary endodontics. Accordingly, a single GP cone (size F4) was used as the core material in combination with the calcium silicate–based sealer. In addition, previous studies have reported that endodontic sealers used in conjunction with a core material exhibit lower POBS as compared to the sealer-only obturation techniques,[56] while other investigations have suggested that dislodgement resistance testing may yield inaccurate values due to the deformation of GP.[70] Hence, further studies are warranted to evaluate the influence of prior application of ICMs on the POBS of calcium silicate-based materials to radicular dentin when bulk sealer obturation is used.

Limitations

As an intracanal medicament, propolis shows promising potential; however, further in vivo and long-term clinical studies are required to evaluate its sustained clinical performance. Another limitation of this in vitro study is the lack of assessment of residual medicament traces on the radicular dentin surface following the completion of the irrigation protocol. The presence of residual ICMs within the root canal should be considered when interpreting the final results.

Conclusions

Within the limitations of this in vitro study, all tested ICMs affected the bonding ability of the calcium silicate-based bioceramic sealer (BioRoot RCS) to radicular dentin; however, the least adverse effect was observed with calcium hydroxide in the cervical third and with propolis in the middle and apical thirds.

Calcium hydroxide demonstrated the highest POBS of BioRoot RCS to root canal dentin, followed by mTAP and propolis, with no statistically significant difference between mTAP and propolis in the cervical third. In contrast, propolis exhibited the highest bond strength, followed by calcium hydroxide and mTAP in the middle and apical thirds, with no statistically significant differences between calcium hydroxide and mTAP in these regions. Overall, the POBS of BioRoot RCS to radicular dentin increased from the cervical to the apical third across all 3 study groups.

Future perspectives

Further research may be conducted to evaluate the following parameters: the detailed interaction between propolis ICM residues and calcium silicate-based bioceramic sealers; the effect of prior application of various ICMs, such as calcium hydroxide, mTAP and propolis, on the POBS of bulk-fill bioceramic sealers without GP cones to root canal dentin; and the influence of propolis residues on the POBS of bioceramic sealers, with particular emphasis on comparing the efficacy of different irrigation protocols for their removal.

In addition, studies may assess the effect of these ICMs on the POBS of other commercially available bioceramic-based sealers apart from BioRoot RCS. Scanning electron microscopy analysis may be employed to evaluate adhesion at the sealer–dentin interface, along with the characterization of the type of bond failure between bioceramic sealers and radicular dentin, using advanced analytical techniques. These investigations may provide valuable insights that could enhance material performance in clinical settings.

Ethics approval and consent to participate

The Institutional Ethics Committee at the AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), NITTE (Deemed to be University), Mangaluru, India, approved and granted clearance to this study (ABSM/EC/102/2021). Written informed consent was obtained from all participants.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Komabayashi T, Colmenar D, Cvach N, Bhat A, Primus C, Imai Y. Comprehensive review of current endodontic sealers. *Dent Mater J*. 2020;39(5):703–720. doi:10.4012/dmj.2019-288

2. Alghamdi F, Abduljawad F. Comparative evaluation of the apical sealing ability among different root canal obturation techniques and endodontic sealers by using fluid filtration method: A systematic review. *Int Dent J Stud Res*. 2019;7(1):1–8. doi:10.18231/j.idjsr.2019.001
3. Zhang W, Li Z, Peng B. Assessment of a new root canal sealer's apical sealing ability. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107(6):e79–e82. doi:10.1016/j.tripleo.2009.02.024
4. Asawaworarit W, Yachor P, Kijsamanmith K, Vongsavan N. Comparison of the apical sealing ability of calcium silicate-based sealer and resin-based sealer using the fluid-filtration technique. *Med Princ Pract*. 2016;25(6):561–565. doi:10.1159/000450577
5. Haji TH, Selivany BJ, Suliman AA. Sealing ability in vitro study and biocompatibility in vivo animal study of different bioceramic based sealers. *Clin Exp Dent Res*. 2022;8(6):1582–1590. doi:10.1002/cre2.652
6. Rekha R, Kavitha R, Venkitachalam R, Prabath SV, Deepthy S, Krishnan V. Comparison of the sealing ability of bioceramic sealer against epoxy resin based sealer: A systematic review and meta-analysis. *J Oral Biol Craniofac Res*. 2023;13(1):28–35. doi:10.1016/j.jobcr.2022.10.006
7. Abulhamael A, Lim DY, Chiang K, Alghamdi F, Roges R. The prevalence of cases with apical sealer extrusion published in recent articles of the endodontic literature. *Ann Dent Spec*. 2022;10(1):62–64. doi:10.51847/2IbOT5bD4r
8. Aminoshariae A, Kulild JC. The impact of sealer extrusion on endodontic outcome: A systematic review with meta-analysis. *Aust Endod J*. 2020;46(1):123–129. doi:10.1111/aej.12370
9. Yu YH, Kushnir L, Kohli M, Karabucak B. Comparing the incidence of postoperative pain after root canal filling with warm vertical obturation with resin-based sealer and sealer-based obturation with calcium silicate-based sealer: A prospective clinical trial. *Clin Oral Investig*. 2021;25(8):5033–5042. doi:10.1007/s00784-021-03814-x
10. Tan HSG, Lim KC, Lui JN, Lai WMC, Yu VSH. Postobturation pain associated with tricalcium silicate and resinbased sealer techniques: A randomized clinical trial. *J Endod*. 2021;47(2):169–177. doi:10.1016/j.joen.2020.10.013
11. Chybowski EA, Glickman GN, Patel Y, Fleury A, Solomon E, He J. Clinical outcome of non-surgical root canal treatment using a single-cone technique with EndoSequence bioceramic sealer: A retrospective analysis. *J Endod*. 2018;44(6):941–945. doi:10.1016/j.joen.2018.02.019
12. Goldberg F, Cantarini C, Alfie D, Macchi RL, Arias A. Relationship between unintentional canal overfilling and the long-term outcome of primary root canal treatments and nonsurgical retreatments: A retrospective radiographic assessment. *Int Endod J*. 2020;53(1):19–26. doi:10.1111/iej.13209
13. Ricucci D, Rôças IN, Alves FR, Loghin S, Siqueira Jr. JF. Apically extruded sealers: Fate and influence on treatment outcome. *J Endod*. 2016;42(2):243–249. doi:10.1016/j.joen.2015.11.020
14. Shashirekha G, Jena A, Pattanaik S, Rath J. Assessment of pain and dissolution of apically extruded sealers and their effect on the periradicular tissues. *J Conserv Dent*. 2018;21(5):546–550. doi:10.4103/JCD.JCD_224_18
15. Drumond JP, Maeda W, Nascimento WM, et al. Comparison of postobturation pain experience after apical extrusion of calcium silicate- and resin-based root canal sealers. *J Endod*. 2021;47(8):1278–1284. doi:10.1016/j.joen.2021.05.008
16. Huang Y, Orhan K, Celikten B, Orhan AI, Tufenkci P, Sevimay S. Evaluation of the sealing ability of different root canal sealers: A combined SEM and micro-CT study. *J Appl Oral Sci*. 2018;26:e20160584. doi:10.1590/1678-7757-2016-0584
17. Ferreira MM, Martinho JP, Duarte I, et al. Evaluation of the sealing ability and bond strength of two endodontic root canal sealers: An in vitro study. *Dent J* (Basel). 2022;10(11):201. doi:10.3390/dj10110201
18. Uzunoglu-Özyürek E, Erdoğan Ö, Türker SA. Effect of calcium hydroxide dressing on the dentinal tubule penetration of 2 different root canal sealers: A confocal laser scanning microscopic study. *J Endod*. 2018;44(6):1018–1023. doi:10.1016/j.joen.2018.02.016
19. Hess D, Solomon E, Spears R, He J. Retreatability of a bioceramic root canal sealing material. *J Endod*. 2011;37(11):1547–1549. doi:10.1016/j.joen.2011.08.016
20. Iman M. Al-Zaka IM, Atta-Allah A, Al-Gharrawi HA, Mehdi JA. The effect of different root canal irrigants on the sealing ability of Bioceramic sealer. *Mustansiria Dental Journal (MDJ)*. 2018;10(1):1–7. doi:10.32828/mdj.v10i1.177
21. Akcay M, Arslan H, Topcuoglu HS, Tuncay O. Effect of calcium hydroxide and double and triple antibiotic pastes on the bond strength of epoxy resin-based sealer to root canal dentin. *J Endod*. 2014;40(10):1663–1667. doi:10.1016/j.joen.2014.05.006
22. Üstün Y, Arslan S, Aslan T. Effects of calcium hydroxide and propolis intracanal medicaments on bond strength of resin-based endodontic sealer as assessed by push-out test. *Dent Mater J*. 2013;32(6):913–919. doi:10.4012/dmj.2013-094
23. Gokturk H, Bayram E, Bayram HM, Aslan T, Ustun Y. Effect of double antibiotic and calcium hydroxide pastes on dislodgement resistance of an epoxy resin-based and two calcium silicate-based root canal sealers. *Clin Oral Investig*. 2017;21(4):1277–1282. doi:10.1007/s00784-016-1877-1
24. Huffman BP, Mai S, Pinna L, et al. Dislocation resistance of ProRoot Endo Sealer, a calcium silicate-based root canal sealer, from radicular dentine. *Int Endod J*. 2009;42(1):34–46. doi:10.1111/j.1365-2591.2008.01490.x
25. Gopikrishna V. *Grossman's Endodontic Practice*. 14th ed. Gurugram, India: Wolters Kluwer; 2020:309.
26. Alghamdi F, Alkhattab O. Effectiveness of intracanal calcium hydroxide medicament in treating periapical lesions: A systematic review. *J Stoma*. 2022;75(1):44–54. doi:10.5114/jos.2022.114502
27. Badr AE, Omar N, Badria FA. A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. *Int Endod J*. 2011;44(1):51–58. doi:10.1111/j.1365-2591.2010.01794.x
28. Hegde S, Lala PK, Dinesh RB, Shubha AB. An in vitro evaluation of antimicrobial efficacy of primary root canal filling materials. *J Clin Pediatr Dent*. 2012;37(1):59–64. doi:10.17796/jcpd.37.1.v6305588xw525505
29. Mattigatti S, Ratnakar P, Moturi S, Varma S, Rairam S. Antimicrobial effect of conventional root canal medicaments vs propolis against *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*. *J Contemp Dent Pract*. 2012;13(3):305–309. doi:10.5005/jp-journals-10024-1142
30. Rosenberg B, Murray PE, Namerow K. The effect of calcium hydroxide root filling on dentin fracture strength. *Dent Traumatol*. 2007;23(1):26–29. doi:10.1111/j.1600-9657.2006.00453.x
31. Naeem MM, Sarwar H, Nisar A, et al. Effect of propolis on root dentine microhardness when used as an intracanal medicament: An in vitro study. *J Funct Biomater*. 2023;14(3):144. doi:10.3390/jfb14030144
32. Alsubait S, Alsaad N, Alahmari S, Alfaraj F, Alfawaz H, Alqedairi A. The effect of intracanal medicaments used in endodontics on the dislocation resistance of two calcium silicate-based filling materials. *BMC Oral Health*. 2020;20(1):57. doi:10.1186/s12903-020-1044-6
33. Shabbir J, Qazi F, Farooqui W, Ahmed S, Zehra T, Khurshid Z. Effect of Chinese propolis as an intracanal medicament on post-operative endodontic pain: A double-blind randomized controlled trial. *Int J Environ Res Public Health*. 2020;17(2):445. doi:10.3390/ijerph17020445
34. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. *Int Endod J*. 2011;44(6):491–498. doi:10.1111/j.1365-2591.2010.01848.x
35. Sagsen B, Ustün Y, Demirbuga S, Pala K. Push-out bond strength of two new calcium silicate-based endodontic sealers to root canal dentine. *Int Endod J*. 2011;44(12):1088–1091. doi:10.1111/j.1365-2591.2011.01925.x
36. Akman M, Akbulut MB, Güneşer MB, Eldeniz AÜ. Effect of intracanal medicaments on the push-out bond strength of Biodentine in comparison with Bioaggregate apical plugs. *J Adhes Sci Technol*. 2016;30(5):459–467. doi:10.1080/01694243.2015.1106425
37. Khalil WA, Alghamdi F, Aljahdali E. Strengthening effect of bioceramic cement when used to repair simulated internal resorption cavities in endodontically treated teeth. *Dent Med Probl*. 2020;57(2):165–169. doi:10.17219/dmp/116743
38. Raghavendra SS, Jadhav GR, Gathani KM, Kotadia P. Bioceramics in endodontics – a review. *J Istanb Uni Fac Dent*. 2017;51(3 Suppl 1):S128–S137. doi:10.17096/jiufd.63659

39. Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine. *Int Endod J*. 2011;44(12):1081–1087. doi:10.1111/j.1365-2591.2011.01924.x
40. Atmeh AR, Chong EZ, Richard G, Festy F, Watson TF. Dentin-cement interfacial interaction: Calcium silicates and polyalkenoates. *J Dent Res*. 2012;91(5):454–459. doi:10.1177/0022034512443068
41. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. *J Endod*. 2009;35(7):1051–1055. doi:10.1016/j.joen.2009.04.022
42. Viapiana R, Moinzadeh AT, Camilleri L, Wesselink PR, Filho MT, Camilleri J. Porosity and sealing ability of root fillings with gutta-percha and BioRoot RCS or AH Plus sealers. Evaluation by three ex vivo methods. *Int Endod J*. 2016;49(8):774–782. doi:10.1111/iej.12513
43. Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi MG. Properties of BioRoot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. *Int Endod J*. 2017;50(Suppl 2):e120–e136. doi:10.1111/iej.12856
44. Camps J, Jeanneau C, El Ayachi I, Laurent P, About I. Bioactivity of a calcium silicate-based endodontic cement (BioRoot RCS): Interactions with human periodontal ligament cells in vitro. *J Endod*. 2015;41(9):1469–1473. doi:10.1016/j.joen.2015.04.011
45. Dimitrova-Nakov S, Uzunoglu E, Ardila-Osorio H, et al. In vitro bioactivity of BioRoot™ RCS, via A4 mouse pulpal stem cells. *Dent Mater*. 2015;31(11):1290–1297. doi:10.1016/j.dental.2015.08.163
46. Prüllage RK, Urban K, Schäfer E, Dammaschke T. Material properties of a tricalcium silicate-containing, a mineral trioxide aggregate-containing, and an epoxy resin-based root canal sealer. *J Endod*. 2016;42(12):1784–1788. doi:10.1016/j.joen.2016.09.018
47. Arias-Moliz MT, Camilleri J. The effect of the final irrigant on the antimicrobial activity of root canal sealers. *J Dent*. 2016;52:30–36. doi:10.1016/j.jdent.2016.06.008
48. Shandilya A, Azhar A, Maheswari D, Ratre S, Agrawal N, Siddiqui T. Evaluation of the sealing ability of gutta percha with BioRoot RCS, MTA Fillapex and Sealapex – an SEM study. *J Pharm Bioallied Sci*. 2022;14(Suppl 1):S893–S896. doi:10.4103/jpbs.jpbs_91_22
49. Kvist T, Molander A, Dahlén G, Reit C. Microbiological evaluation of one- and two-visit endodontic treatment of teeth with apical periodontitis: A randomized, clinical trial. *J Endod*. 2004;30(8):572–576. doi:10.1097/01.don.0000121607.87969.6e
50. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: A literature review – Part I. In vitro studies. *Restor Dent Endod*. 2014;39(4):241–252. doi:10.5395/rde.2014.39.4.241
51. Mohammadi Z, Abbott PV. On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. *Int Endod J*. 2009;42(7):555–567. doi:10.1111/j.1365-2591.2009.01564.x
52. Koo H, Gomes BP, Rosalen PL, Ambrosano GM, Park YK, Cury JA. In vitro antimicrobial activity of propolis and *Arnica montana* against oral pathogens. *Arch Oral Biol*. 2000;45(2):141–148. doi:10.1016/s0003-9969(99)00117-x
53. Ramos IFdAS, Biz MT, Paulino N, et al. Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy: An in vivo study. *J Appl Oral Sci*. 2012;20(1):50–56. doi:10.1590/s1678-77572012000100010
54. Mannocci F, Pilecki P, Bertelli E, Watson TF. Density of dentinal tubules affects the tensile strength of root dentin. *Dent Mater*. 2004;20(3):293–296. doi:10.1016/S0109-5641(03)00106-4
55. Cimpean SI, Colceriu Burtea AL, Chiorean RS, et al. Evaluation of bond strength of four different root canal sealers. *Materials (Basel)*. 2022;15(14):4966. doi:10.3390/ma15144966
56. Nagas E, Cehreli Z, Uyanik MO, Durmaz V. Bond strength of a calcium silicate-based sealer tested in bulk or with different main core materials. *Braz Oral Res*. 2014;28(1):S1806-832420140001002561-7. doi:10.1590/1807-3107bor-2014.vol28.0046
57. Topçuoğlu HS, Arslan H, Akçay M, Saygili G, Çakici F, Topçuoğlu G. The effect of medicaments used in endodontic regeneration technique on the dislocation resistance of mineral trioxide aggregate to root canal dentin. *J Endod*. 2014;40(12):2041–2044. doi:10.1016/j.joen.2014.08.018
58. Arslan H, Capar ID, Saygili G, et al. Efficacy of various irrigation protocols on the removal of triple antibiotic paste. *Int Endod J*. 2014;47(6):594–599. doi:10.1111/iej.12194
59. Sfeir G, Bukiet F, Hage W, El Hachem R, Zogheib C. Impact of final irrigation protocol on the push-out bond strength of two types of endodontic sealers. *Materials (Basel)*. 2023;16(5):1761. doi:10.3390/ma16051761
60. Bogari DF, Alessa M, Aljaber M, et al. The biological and mechanical effect of using different irrigation methods on the bond strength of Bioceramic sealer to root dentin walls. *Cureus*. 2022;14(4):e24022. doi:10.7759/cureus.24022
61. Esteves-Oliveira M, De Guglielmi CA, Ramalho KM, Arana-Chavez VE, De Eduardo CP. Comparison of dentin root canal permeability and morphology after irradiation with Nd:YAG, Er:YAG, and diode lasers. *Lasers Med Sci*. 2010;25(5):755–760. doi:10.1007/s10103-010-0775-z
62. Cetinkaya Ý, Bodrumlu E, Koçak MM, Koçak S, Sağlam BC, Türker SA. Effect of modified NaOCl irrigation solution on bond strength of bioceramic-based root canal sealer to dentin. *Indian J Dent Res*. 2020;31(4):574–578. doi:10.4103/ijdr.IJDR_747_18
63. Gyulbenkiyan E, Gusiyska A, Vassileva R, Dyulgerova E. Scanning electron microscopic evaluation of the sealer/dentine interface of two sealers using two protocols of irrigation. *J IMAB*. 2020;26(1):2887–2891. doi:10.5272/jimab.2020261.2887
64. Shokouhinejad N, Hoseini A, Gorjestani H, Shamshiri AR. The effect of different irrigation protocols for smear layer removal on bond strength of a new bioceramic sealer. *Iran Endod J*. 2013;8(1):10–13. doi:10.22037/iej.v8i1.3968
65. Atasoy Ulusoy OI, Genç O, Arslan S, Alaçam T, Görgül G. Fracture resistance of roots obturated with three different materials. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;104(5):705–708. doi:10.1016/j.tripleo.2007.07.013
66. Pane ES, Palamara JE, Messer HH. Critical evaluation of the push-out test for root canal filling materials. *J Endod*. 2013;39(5):669–673. doi:10.1016/j.joen.2012.12.032
67. Chen WP, Chen YY, Huang SH, Lin CP. Limitations of push-out test in bond strength measurement. *J Endod*. 2013;39(2):283–287. doi:10.1016/j.joen.2012.11.002
68. Taşdemir T, Er K, Yildirim T, et al. Comparison of the sealing ability of three filling techniques in canals shaped with two different rotary systems: A bacterial leakage study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108(3):e129–e134. doi:10.1016/j.tripleo.2009.05.007
69. Donnermeyer D, Urban K, Bürklein S, Schäfer E. Physico-chemical investigation of endodontic sealers exposed to simulated intracanal heat application: Epoxy resins and zinc oxide-eugenols. *Int Endod J*. 2020;53(5):690–697. doi:10.1111/iej.13267
70. Williams C, Loushine RJ, Weller RN, Pashley DH, Tay FR. A comparison of cohesive strength and stiffness of Resilon and gutta-percha. *J Endod*. 2006;32(6):553–555. doi:10.1016/j.joen.2005.08.002

Original paper

In vitro evaluation of discoloration and surface alteration in self-adhesive resin cements using CIELAB and 3D profilometry

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Abstract

Background. Self-adhesive resin cements (SARCs) are widely used in the field of restorative dentistry due to their simplified application and adequate bonding properties. However, their long-term color and surface stability, particularly under the influence of pigment-rich dietary substances, remain a critical concern in aesthetic restorations.

Objectives. The aim of the study was to evaluate the color stability and surface roughness of 4 contemporary SARCs after immersion in various staining media using digital photography.

Material and methods. A total of 160 disc-shaped specimens ($n = 40$ per group) were fabricated from 4 SARCs: Maxcem Elite™ (MXC); G-CEM ONE™ (GCO); SpeedCEM® Plus (SPC); and RelyX™ Universal (RLX). The specimens were immersed in artificial saliva, coffee, red wine, or matcha tea at 37°C for 0 (T0), 7 (T1), 14 (T2), 21 (T3), and 28 (T4) days. Color changes (ΔE_{00}) were measured, and surface roughness parameters were evaluated using 3D laser scanning microscopy. The statistical analysis was performed using linear mixed models (LMMs) and Tukey's honestly significant difference (HSD) post hoc test ($\alpha = 0.05$).

Results. All materials showed visually perceptible color changes ($\Delta E_{00} > 1.1$). The highest discoloration and roughness values were observed in MXC, especially after exposure to red wine. RelyX™ Universal exhibited the greatest resistance to staining and surface degradation. 2-Hydroxyethyl methacrylate (HEMA)-based SARCs (MXC, GCO) were more susceptible to pigment infiltration and roughness increase than urethane dimethacrylate (UDMA)-based cements (RLX, SPC).

Conclusions. The aesthetic stability of SARCs is influenced by both material composition and exposure to staining agents. Red wine caused the most severe discoloration and surface changes, followed by coffee, matcha tea and artificial saliva. Higher water sorption and surface roughness were associated with increased staining. Preserving surface integrity is essential for maintaining the long-term aesthetic performance of SARCs.

Keywords: surface roughness, color stability, CIELAB, self-adhesive resin cement, staining media

Cite as
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Highlights

- Color stability and surface roughness of 4 self-adhesive resin cements (SARCs) were evaluated after exposure to common staining agents.
- Red wine caused the most intense discoloration and surface changes, followed by coffee and matcha tea.
- Increases in surface roughness were correlated with higher levels of discoloration.
- Urethane dimethacrylate (UDMA)-based SARCs (RelyX™ Universal, SpeedCEM® Plus) demonstrated greater resistance to staining and surface roughening, supporting their suitability for aesthetically demanding restorations.

Introduction

In recent years, there has been a noticeable shift in the aesthetic expectations of dental patients. The demand for natural-looking, tooth-colored restorations, particularly in the visible anterior region, has increased.[1] In addition to personal beauty ideals, digital media and social networks play a decisive role in shaping patients' perceptions of dental aesthetics.[2] As a result, the long-term color stability of restorative materials has become a central concern in clinical practice and dental research. Both the choice of ceramic and luting cement significantly influence the natural appearance of restorations.[3] Even minimal discoloration of the cement can markedly affect the overall aesthetic outcome when using highly translucent ceramics.[4]

Self-adhesive resin cements (SARCs) have become established materials for bonding indirect restorations due to their ease of use, clinical efficiency and reliable adhesion.[5] Chemically, they are based on a combination of functional monomers, such as bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA), along with filler particles that improve their mechanical performance.[6] In contrast to conventional resin cements, SARCs contain acidic monomers (e.g., phosphoric acid esters or carboxylate groups) that induce mild demineralization and enable chemical self-adhesion to the tooth substrate.[5] This is particularly beneficial in deep, pulp-near preparations, as it offers protection against hypersensitivities caused by excessive etching. While this modified composition enhances adhesive properties, it may compromise the long-term stability of the bond due to reduced surface pretreatment.[6]

In this context, the resistance to hydrolytic degradation has become a key factor in determining the clinical durability of SARCs.[7] Owing to their hydrophilic monomer content, these materials are more susceptible to water sorption, which weakens the polymer matrix, destabilizes cross-linking and alters the surface morphology.[7] These changes not only increase the risk of marginal breakdown but also promote the adsorption of exogenous pigments.[8,9] Increased surface roughness further facilitates the accumulation of staining substances and hampers effective surface cleaning. As a consequence, colorants from the oral environment can more easily penetrate the material, progressively compromising long-term color stability.[8] In addition to intrinsic material factors, extrinsic elements play a crucial role in the color stability of SARCs.[10] Staining solutions such as red wine, coffee and matcha tea contain high concentrations of anthocyanins, tannins and oxidized polyphenols, which, due to their small molecular size and high lipophilicity, can deeply infiltrate the polymer matrix.[10] Once inside, these molecules interact with the composite's organic components, especially unpolymerized monomers, ester groups or free radicals, leading to chemical reactions such as oxidation, hydrolysis or chain scission, which may cause irreversible discoloration.[11] The extent of these effects depends largely on the pH of the staining medium and on the hydrophilicity and water sorption behavior of the respective SARC.[12] Strongly acidic or basic solutions tend to destabilize polymer networks and facilitate pigment diffusion, while more hydrophilic SARCs are prone to deeper penetration and internal staining due to higher water uptake.[13,14]

To objectively quantify color changes, digital imaging technologies such as the eLAB system (Emulation S.Hein, Freiburg im Breisgau, Germany) are increasingly utilized. Unlike traditional spectrophotometry, the eLAB system allows for standardized calibration of light sources, camera sensors and white balance,[15] ensuring reproducible and visually accurate color analysis under clinically realistic conditions. This advanced technique is particularly relevant for the evaluation of aesthetic restorative materials, as it provides a more precise and clinically meaningful assessment of color dynamics. Although numerous studies have investigated the color and surface stability of SARCs, there remains a paucity of research on the behavior of newly formulated SARCs when exposed to various staining agents.[5,16] Given the ongoing development of dental materials, a differentiated analysis is needed to assess their long-term aesthetic stability under simulated aging conditions. This study systematically investigates how pigment-rich substances, such as coffee, red wine and matcha tea, affect the color stability (ΔE_{00})

and surface roughness (arithmetic mean height (Sa) and maximum peak-to-valley height (Sz)) of several modern SARCs. The goal is to identify material-dependent differences in discoloration and surface degradation and to evaluate the suitability of these cements for aesthetically demanding indirect restorations. The findings are intended to provide an evidence-based foundation for material selection in restorative dentistry and offer clinically relevant recommendations for minimizing discoloration.

The following null hypotheses were tested:

1. Storage in different staining solutions (artificial saliva, coffee, red wine, and matcha tea) does not result in significant differences in the color change (ΔE_{00}) of the tested SARCs;
2. The surface roughness values (Sa, Sz) of the SARCs do not change significantly over the course of the investigation.

Material and methods

Preparation of study samples

The study flowchart is presented in Fig. 1. A total of 160 disc-shaped samples (n = 40/SARC; diameter: 10 mm, thickness: 2 mm) were fabricated from 4 different SARCs: Maxcem Elite™ (Kerr Corporation, Orange, USA) (MXC); G-CEM ONE™ (GC Corporation, Tokyo, Japan) (GCO); SpeedCEM® Plus (Ivoclar Vivadent, Schaan, Liechtenstein) (SPC); and RelyX™ Universal (3M Deutschland GmbH, Neuss, Germany) (RLX). The specific compositions and batch numbers of the tested materials are detailed in Table 1. Each composite was placed into a standardized Teflon mold and polymerized in two 1-mm layers, starting with the occlusal side, for 20 s using a light-emitting diode (LED) light-curing unit (Bluephase Style, 1,200 mW/cm^2; Ivoclar Vivadent). After removing the mold, an additional 20-second post-curing was performed to ensure complete polymerization. A randomly selected surface of each sample was polished under wet conditions using silicon carbide abrasive papers (P320 to P1200) on a benchtop grinding and polishing machine (Ecomet® 6; Buehler, Lake Bluff, USA). Following polishing, the samples were ultrasonically cleaned in distilled water for 8 min,[17] thoroughly rinsed, and stored in distilled water at 37°C for 24 h.

Exposure to staining media and color measurements

Each sample was randomly assigned to one of 4 experimental staining media: artificial saliva; coffee (Capsa Prodomo; Alois Dallmayr Kaffee oHG, Munich, Germany); red wine (Doppio Passo, 13% alcohol content; Casa Vinicola Botter, Fossalta di Piave, Italy); and matcha tea (Bio Matcha; Health Bar GmbH, Berlin, Germany) without pH measurement. Artificial saliva was prepared by the institutional hospital pharmacy in accordance with the formulation described by Manfro et al.[18] The solution contained 22.1 mmol/L of bicarbonate, 16.1 mmol/L of potassium, 14.1 mmol/L of sodium hydrogen, 2.6 mmol/L of phosphate, 0.8 mmol/L of boric acid, 0.7 mmol/L of calcium, 0.4 mmol/L of thiocyanate, and 0.2 mmol/L of magnesium. The samples were stored in an incubator (schülke Cultura; Schülke & Mayr GmbH, Norderstedt, Germany) protected from light at a constant temperature of 37°C to simulate physiological oral conditions. After staining, all specimens were rinsed with distilled water for 10 s to remove loosely adherent surface residues and air-dried for 2 min using lint-free absorbent tissue. Color measurements were conducted at 5 defined time points: T0 (baseline); T1 (7 days); T2 (14 days); T3 (21 days); and T4 (28 days). For standardized image acquisition, a digital single-lens reflex camera (EOS 6D Mark II; Canon Inc., Tokyo, Japan) equipped with a 100-mm f/2.8 macro lens and a Macro Twin Lite MT-24EX flash system (Canon Inc.) was utilized. A cross-polarization filter (polar_eyes; Emulation, Freiburg, Germany) was employed to eliminate distracting reflections and ensure reproducible color capture. The camera settings were aligned in accordance with the methodology provided by Hein et al.: aperture of f/22; shutter speed of 1/125 s; and ISO 100.[15] During image acquisition, the samples were placed into a precision-engineered acrylic plate (Palavit® L; Kulzer, Hanau, Germany)

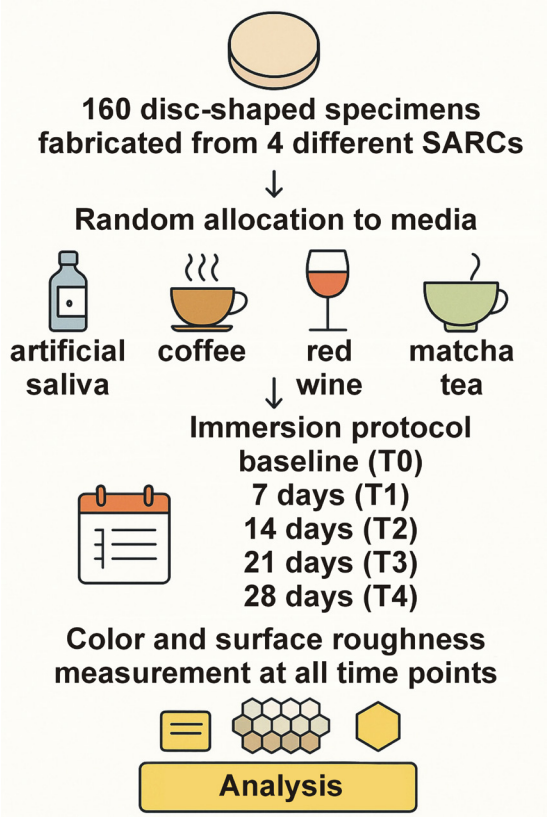

Fig. 1. Flowchart of the study

SARCs – self-adhesive resin cements.

Table 1. Characteristics of the materials used in the study

Brand/manufacturer	Abbreviation	Batch number	Chemical composition
G-CEM ONE™; GC Corporation, Tokyo, Japan	GCO	2312051	7,7,9-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bis(methacrylate) 2-hydroxy-1,3-dimethacryloxypropane 10-methacryloyloxydecyl dihydrogen phosphate α,α-dimethylbenzyl hydroperoxide 6-tert-butyl-2,4-xylenol
Maxcem Elite™; Kerr Corporation, Orange, USA	MXC	10463885	2-hydroxyethyl methacrylate 4-methoxyphenol cumene hydroperoxide α,α-dimethylbenzyl hydroperoxide methacrylate ester monomer titanium dioxide pigment
SpeedCEM® Plus; Ivoclar Vivadent, Schaan, Liechtenstein	SPC	Z06T2Z	urethane dimethacryclate triethylene glycol dimethacrylate ytterbium(III) fluoride methacrylate phosphoric acid ester dibenzoyl peroxide 1,10-decanediol dimethacrylate polyethylene glycol dimethacrylate
RelyX™ Universal; 3M Deutschland GmbH, Neuss, Germany	RLX	10896493	urethane dimethacrylate ytterbium(III) fluoride glass powder (65997-17-3) surface treated with 2-propenoic acid, 2-methyl-,3-(trimethoxysilyl) propyl ester (2530-85-0) and trimethoxyphenylsilane (2996-92-1) bulk material triethylene glycol dimethacrylate L-ascorbic acid 6-hexadecanoate hydrate (1:2) triethoxy(octyl)silane hydrolysis products with silica 2-hydroxyethyl methacrylate titanium dioxide triphenyl phosphite

and fixed in a phantom head (frasaco GmbH, Tettnang, Germany) to ensure consistent positioning. For the calibration of the eLAB system, a white balance card (Emulation S.Hein) was positioned on the acrylic plate. The captured CIE L*a*b* color values were converted into the LCh color system (L*, C*, h°) to facilitate a more nuanced analysis of color changes. Color deviations were calculated using the CIEDE2000 equation (ΔE_{00}),[4] which, as an advanced color difference formula, provides a more realistic weighting of differences in lightness, saturation and hue. The scaling factors, namely S_L, S_C and S_H account for varying perceptual sensitivities to lightness, chroma and hue. The k_L, k_C and k_H parameters are used to adjust for specific viewing conditions. Additionally, the rotation term (RT) corrects perceptual distortions, particularly within the blue color spectrum. To evaluate color changes, clinically established thresholds, as defined by Paravina et al., were applied.[19] The perceptibility threshold (PT) was set at ΔE_{00} = 1.1, and the clinically acceptable tolerance threshold was established at ΔE_{00} = 2.7. These values are derived from studies conducted under standardized lighting conditions, a neutral gray background, and a viewing distance of approx. 30–40 cm.[20]

Surface roughness analysis

A quantitative analysis of surface roughness was performed using a 3D laser scanning microscope (VK-X3050; Keyence Corporation, Osaka, Japan) in the vertical scanning interferometry (VSI) mode, in accordance with ISO 25178-2:2021.[21] For each sample, 3 randomly selected measurement areas were captured, and their mean values were used for statistical evaluation. To assess potential microstructural changes after exposure to the staining media, the roughness parameters, namely arithmetic mean height (Sa) and maximum peak-to-valley height (Sz), were analyzed.

Statistical analysis

The sample size was calculated using G*Power software (Heinrich Heine University, Düsseldorf, Germany), based on an effect size of f = 0.4, a significance level of α = 0.05, and a test power of 80%, as established in the study by Chen et al.[21] The statistical analysis was conducted using RStudio (R v. 4.4.3; Posit PBC, Boston, USA). A linear mixed model (LMM) was employed to investigate

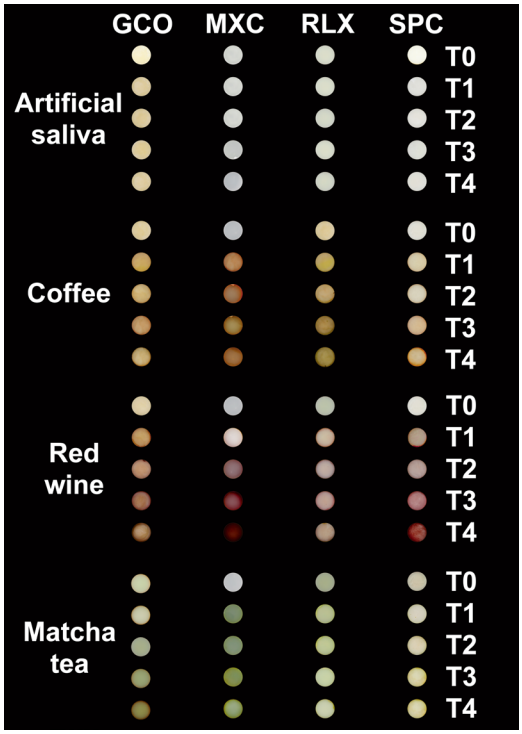

Fig. 2. Representative images of self-adhesive resin cement (SARC) specimens immersed in different staining solutions at baseline (T0) and after 7 (T1), 14 (T2), 21 (T3), and 28 (T4) days

GCO – G-CEM ONE™; MXC – Maxcem Elite™; RLX – RelyX™ Universal; SPC – SpeedCEM® Plus.

the effects of different composite types, staining media and time points on color differences (ΔE_{00}) and surface roughness (Sa, Sz). Post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test, with the significance level set at $\alpha = 0.05$.

Results

Color change

Figure 2 presents representative samples following immersion in the respective staining media across 5 defined time points (T0–T4). The LMM analysis revealed significant effects for the SARC type ($F(3, 121.4)$, $\eta^2 = 0.716$, $p < 0.001$), staining medium ($F(3, 1273.2)$, $\eta^2 = 0.964$, $p < 0.001$) and measurement time point ($F(3, 1864.0)$, $\eta^2 = 0.928$, $p < 0.001$). Statistically significant interactions between these factors were also observed ($F(27, 45.3)$, $\eta^2 = 0.739$, $p < 0.001$).

The ΔE_{00} values increased progressively over time (Fig. 3), with MXC consistently demonstrating the most pronounced color change across all staining media. In contrast, RLX and SPC exhibited the highest overall color stability. Among the staining agents, red wine caused the most pronounced discoloration, particularly

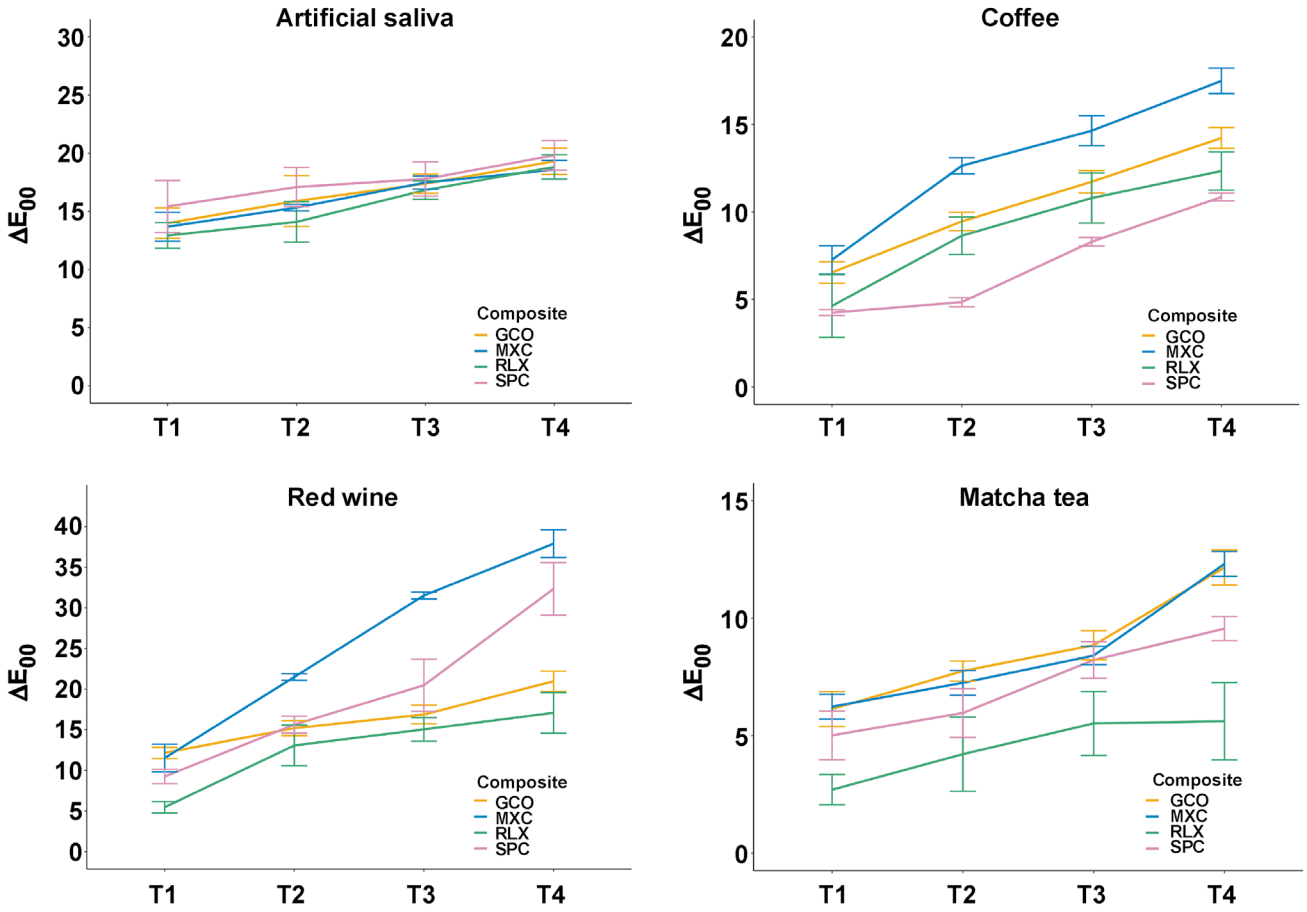

Fig. 3. Mean color change (ΔE_{00}) of the tested self-adhesive resin cements (SARCs) across immersion intervals following exposure to different staining solutions

for MXC (37.91 ±2.37), while RLX showed the lowest discoloration potential in this medium (17.11 ±3.50). Compared to red wine, coffee induced less discoloration, but resulted in a significantly higher color change than matcha tea. Within the coffee group, SPC demonstrated the highest color stability, presenting the lowest ΔE_{00} values. Across all staining agents, RLX showed the most stable performance, with the lowest degree of discoloration observed in matcha tea (5.61 ±2.30).

Pairwise comparisons confirmed significant differences across time points and staining media. No significant differences were found between GCO and SPC in artificial saliva and matcha tea up to 21 days; however, substantial differences between these materials were observed in coffee at all time points, in matcha tea at 28 days, and in red wine at 7, 21 and 28 days. In contrast, significant distinctions were consistently observed in comparisons involving MXC and RLX. The detailed significance levels relative to established perceptibility and acceptability thresholds are summarized in Table 2. The ΔE_{00} values for all materials consistently exceeded the perceptibility threshold of $\Delta E_{00} = 1.1$, indicating that the color changes were visually detectable. For all tested SARCs, the ΔE_{00} values in all staining media additionally surpassed the clinically acceptable threshold of $\Delta E_{00} = 2.7$.

Surface roughness

Surface roughness parameters (Sa and Sz) varied significantly depending on the SARC type, staining medium and measurement time point. The LMM analysis revealed significant effects for the SARC type (F(3, 1549.0), $\eta^2 = 0.866$, $p < 0.001$), staining medium (F(3, 2578.9), $\eta^2 = 0.915$, $p < 0.001$) and time point (F(3, 2343.3), $\eta^2 = 0.929$, $p < 0.001$), as well as significant interaction effects (F(36, 10.3), $\eta^2 = 0.340$, $p < 0.001$).

Table 2. Mean color change (ΔE_{00}) of the tested materials across immersion intervals following exposure to staining solutions

Time point	Medium	Material				No significance
		GCO	MXC	RLX	SPC	
T1	artificial saliva	1.42 ±0.18	1.37 ±0.17	1.29 ±0.15	1.54 ±0.31	all
	matcha tea	6.13 ±1.04	6.23 ±0.74	2.72 ±0.90	5.01 ±1.45	GCO–SPC MXC–GCO MXC–SPC
	coffee	6.54 ±0.86	7.27 ±1.13	4.63 ±2.50	4.25 ±0.24	MXC–GCO GCO–RLX RLX–SPC
	red wine	12.21 ±0.96	11.54 ±2.37	5.47 ±1.00	9.24 ±1.22	MXC–GCO MXC–SPC
	p-value			<0.001		
T2	artificial saliva	1.59 ±0.31	1.57 ±0.04	1.41 ±0.24	1.71 ±0.23	all
	matcha tea	7.75 ±0.61	7.25 ±0.74	4.21 ±2.20	5.97 ±1.45	GCO–SPC SPC–RLX GCO–MXC MXC–SPC
	coffee	9.46 ±0.73	12.62 ±0.64	8.65 ±1.50	4.85 ±0.36	GCO–RLX
	red wine	15.21 ±1.31	21.53 ±0.58	13.12 ±3.50	15.62 ±1.45	GCO–RLX SPC–GCO SPC–RLX
	p-value			<0.001		
T3	artificial saliva	1.74 ±0.12	1.75 ±0.08	1.68 ±0.11	1.78 ±0.21	all
	matcha tea	8.85 ±0.86	8.41 ±0.55	5.52 ±1.90	8.22 ±1.08	MXC–SPC GCO–MXC GCO–SPC
	coffee	11.71 ±0.91	14.62 ±1.19	10.83 ±2.00	8.34 ±0.34	GCO–RLX
	red wine	16.90 ±1.59	31.51 ±0.61	15.02 ±2.00	20.51 ±4.50	SPC–GCO GCO–RLX
	p-value			<0.001		
T4	artificial saliva	1.93 ±0.16	1.86 ±0.11	1.88 ±0.15	1.98 ±0.18	all
	matcha tea	12.61 ±1.64	12.34 ±0.74	5.61 ±2.30	9.55 ±0.72	GCO–MXC
	coffee	14.22 ±0.83	17.51 ±1.02	12.33 ±1.53	10.92 ±0.31	–
	red wine	21.02 ±1.72	37.91 ±2.37	17.11 ±3.50	32.43 ±4.50	–
	p-value			<0.001		

Data presented as mean ± standard deviation (*M ±SD*); T1 – after 7 days; T2 – after 14 days; T3 – after 21 days; T4 – after 28 days.

Among the staining agents, red wine induced the highest surface roughness values, particularly for GCO (Sa: 258.95 ±3·63 nm) and MXC (Sa: 284.02 ±4.22 nm) (Fig. 4). Coffee also led to a considerable increase in surface roughness, especially in MXC (Sa: 259.01 ±5.78 nm) and GCO (Sa: 253.01 ±14.7 nm). In comparison, matcha tea exhibited a lower roughening potential. The lowest Sa and Sz values were observed in artificial saliva (Table 3).

Post hoc testing confirmed statistically significant pairwise differences for most comparisons ($p < 0.001$). Detailed significance levels are listed in Table 3.

Discussion

The findings of this investigation led to the rejection of both null hypotheses, as color change (ΔE_{00}) and surface roughness parameters (Sa, Sz) of the tested SARCs exhibited statistically significant differences over time and across different staining media. Among the evaluated materials, RLX demonstrated the highest color stability and the least increase in surface roughness, whereas MXC exhibited the most pronounced alterations across both parameters. Among the staining solutions, red wine induced

Table 3. Mean surface roughness values of the tested materials across immersion intervals following exposure to staining solutions

Parameter	Medium	Material	T0	T1	T2	T3	T4
Sa [nm]	artificial saliva	GCO	149.03 ±7.08	154.96 ±3.24	163.02 ±2.80	173.07 ±5.38	177.94 ±3.36
		MXC	145.11 ±3.68	153.04 ±3.63	156.98 ±2.38	164.06 ±4.77*	178.02 ±2.30*
		RLX	129.97 ±4.02*[a]	139.05 ±6.08*[a]	146.01 ±4.84*[a]	150.96 ±4.27*[a]	164.09 ±4.77*
		SPC	143.06 ±8.20[b]	149.02 ±7.08	156.08 ±6.27	154.95 ±3.24*[a]	173.11 ±9.24
	matcha tea	GCO	160.04 ±3.71	166.12 ±14.50	197.96 ±7.17	199.08 ±3.26	218.93 ±4.65
		MXC	171.02 ±4.86*	180.91 ±12.60	183.05 ±6.52*	198.97 ±10.10	204.11 ±6.38*
		RLX	132.94 ±6.52*[a]	131.08 ±13.40*[a]	144.03 ±7.46*[a]	164.09 ±7.34*[a]	200.95 ±4.67*
		SPC	141.07 ±7.14*[a]	133.96 ±10.30*[a]	149.01 ±6.84*[a]	162.92 ±7.62*[a]	181.06 ±8.51*[ab]
	coffee	GCO	163.05 ±9.40	199.02 ±2.94	201.07 ±8.74	222.96 ±10.10	253.01 ±14.70
		MXC	173.04 ±6.67	194.06 ±9.22	210.09 ±6.53	232.03 ±10.80	259.01 ±5.78
		RLX	130.02 ±8.73*[a]	146.08 ±11.40*[a]	158.95 ±6.66*[a]	199.06 ±7.18*[a]	210.04 ±5.06*[a]
		SPC	139.01 ±4.34*[a]	150.05 ±9.06*[a]	178.07 ±4.86*[ab]	195.09 ±9.98*[a]	205.02 ±4.78*[a]
	red wine	GCO	175.06 ±3.16	214.97 ±3.96	227.03 ±3.31	238.09 ±3.77	258.95 ±3.63
		MXC	193.04 ±7.06*	221.08 ±8.89	238.01 ±4.99*	266.06 ±4.48*	284.02 ±4.22*
		RLX	148.03 ±3.62*[a]	163.05 ±5.10*[a]	178.07 ±3.63*[a]	220.01 ±5.19*[a]	238.04 ±4.20*[a]
		SPC	147.98 ±2.16*[a]	156.06 ±3.33*[a]	209.03 ±4.40*[ab]	222.08 ±5.27*[a]	232.96 ±4.97*[a]
p-value	<0.001	<0.001			<0.001		
Sz [µm]	artificial saliva	GCO	1.45 ±0.12	1.48 ±0.10	1.59 ±0.07	1.73 ±0.07	1.83 ±0.06
		MXC	1.42 ±0.05	1.49 ±0.03	1.57 ±0.04	1.75 ±0.04	1.86 ±0.06
		RLX	1.35 ±0.04*[a]	1.49 ±0.10*[a]	1.52 ±0.09*[a]	1.68 ±0.12*[a]	1.74 ±0.25*[a]
		SPC	1.30 ±0.06	1.42 ±0.05*[ab]	1.54 ±0.04*[a]	1.67 ±0.06*[a]	1.75 ±0.07*[a]
	matcha tea	GCO	2.18 ±0.12	2.39 ±0.14	2.56 ±0.11	2.73 ±0.09	2.88 ±0.08
		MXC	2.25 ±0.10	2.47 ±0.13	2.61 ±0.15	2.79 ±0.12	2.94 ±0.10
		RLX	2.03 ±0.09*[a]	2.22 ±0.10*[a]	2.41 ±0.13*[a]	2.53 ±0.12*[a]	2.64 ±0.11*
		SPC	2.07 ±0.11*[a]	2.21 ±0.17*[a]	2.43 ±0.17*[a]	2.51 ±0.11*[a]	2.62 ±0.07*[a]
	coffee	GCO	2.28 ±0.08	2.42 ±0.06	2.61 ±0.08	2.84 ±0.05	3.02 ±0.04
		MXC	2.21 ±0.05*	2.36 ±0.07	2.59 ±0.06	2.71 ±0.10*	2.95 ±0.23
		RLX	1.92 ±0.14*[a]	2.04 ±0.72*[a]	2.15 ±0.12*[a]	2.29 ±0.67*[a]	2.37 ±0.05*
		SPC	1.85 ±0.06*[a]	1.94 ±0.05*[ab]	2.07 ±0.09*[a]	2.21 ±0.04*[a]	2.33 ±0.06*[a]
	red wine	GCO	3.12 ±0.09	3.41 ±0.06	3.74 ±0.08	3.93 ±0.05	4.19 ±0.07
		MXC	3.04 ±0.07	3.28 ±0.05*	3.59 ±0.06*	3.87 ±0.06	4.08 ±1.20*
		RLX	2.66 ±0.05*[a]	2.87 ±0.77*[a]	3.13 ±0.07*[a]	3.35 ±0.06*[a]	3.56 ±0.07*[a]
		SPC	2.59 ±0.06*[a]	2.81 ±0.07*[a]	3.05 ±0.08*[ab]	3.29 ±0.09*[a]	3.44 ±0.04*[ab]
p-value	<0.001	<0.001			<0.001		

Data presented as *M* ±*SD*; Sa – arithmetic mean height; Sz – maximum peak-to-valley height; T0 – baseline. Superscript symbols (*, [a], [b]) indicate statistically significant differences ($p < 0.05$) between materials at the same time point, as follows: * significantly different from GCO; [a] significantly different from MXC; [b] significantly different from RLX.

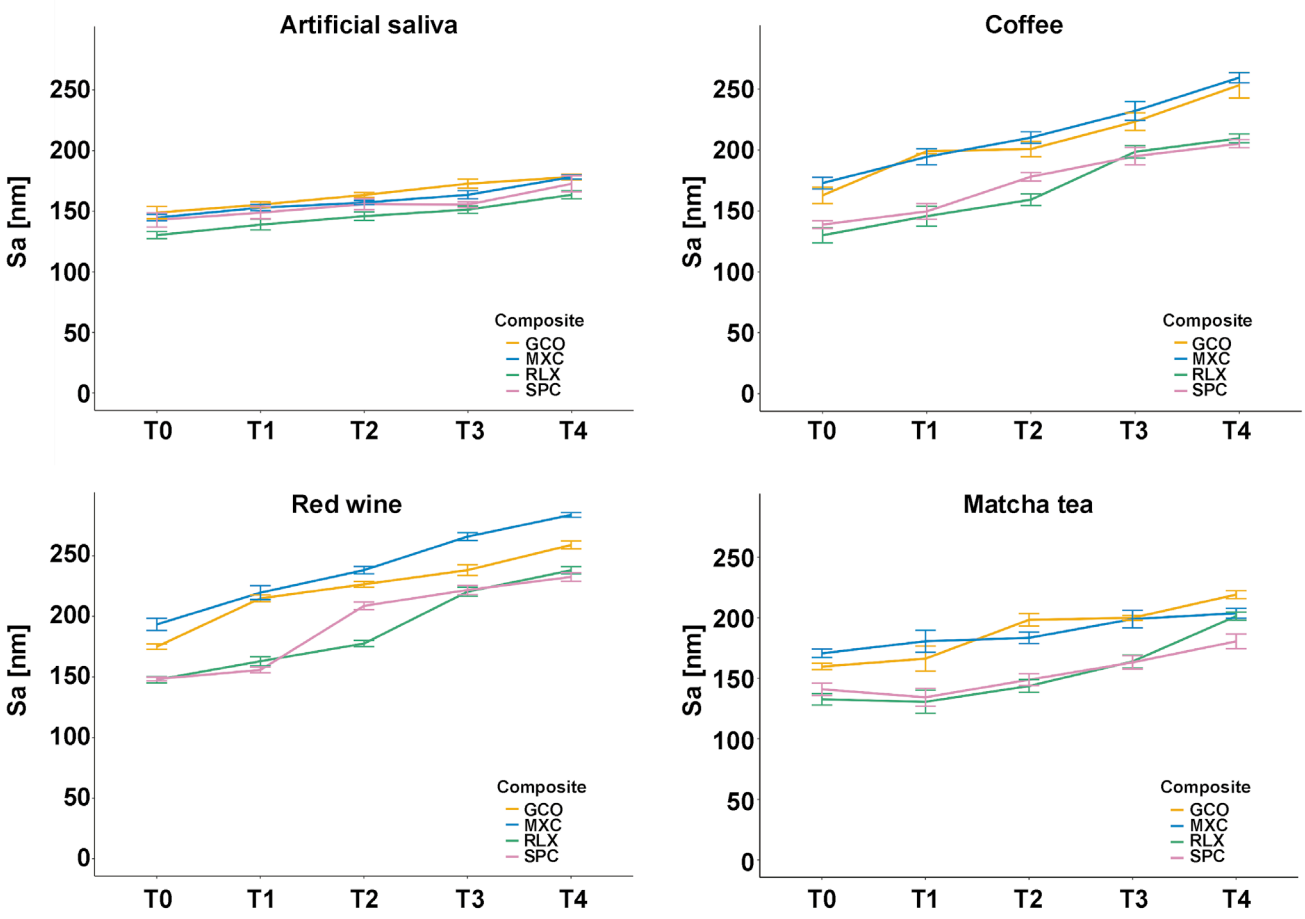

Fig. 4. Changes in surface roughness (Sa) of the tested self-adhesive resin cements (SARCs) across immersion intervals following exposure to different staining solutions

the most substantial color and surface changes, followed by coffee and matcha tea. These findings emphasize the clinical relevance of material selection for highly aesthetic anterior restorations and underscore the importance of considering material-inherent properties and external influences when selecting SARCs for aesthetic cases.

Influence of staining media and their chemical composition

Central to the present study was the investigation of how various staining agents affect the color stability of different SARCs. A differentiated analysis of the chemical properties of each staining medium helped elucidate the mechanisms underlying the observed differences. The extent of discoloration varied significantly depending on the medium. The most intense staining was observed in response to red wine, followed by coffee, matcha tea and artificial saliva. This can be attributed to the high concentration of chromophoric molecules, such as anthocyanins, tannins and oxidized polyphenols, which exhibit a strong affinity for polymer matrices due to their chemical structure.[22] Furthermore, the acidic pH of red wine (pH: 3.2–3.8) may promote hydrolytic degradation on the surface of the material,[22] which, when combined with increased surface roughness, likely enhances pigment infiltration. Acidic environments may also trigger the breakdown of peroxide-based initiator systems, structurally weakening the polymer matrix and exposing filler particles, both of which contribute to an increase in roughness and susceptibility to staining.

Coffee also caused significant discoloration, with several materials surpassing the clinically acceptable ΔE_{00} threshold. Melanoidins and oxidized tannins, which are formed during the roasting process, are considered primary contributors due to their intense chromophoric activity.[21] These hydrophilic pigments preferentially interact with matrix systems characterized by low cross-link density, an issue which is particularly relevant in 2-hydroxyethyl methacrylate (HEMA)-based SARCs, such as MXC and GCO. The material-dependent behavior observed in this study aligns with previous research that identified coffee as a highly staining medium.[23] In contrast to prior studies, which frequently employed immersion temperatures of 65–80°C, this investigation maintained a constant temperature of 37°C to better replicate intraoral conditions. Despite the moderate temperature, substantial discoloration occurred, underscoring the strong affinity of coffee-derived pigments for hydrophilic polymer components.[14] The immersion protocol used here is also considered

clinically relevant. Guler et al. demonstrated that a 24-hour immersion in coffee corresponds to approx. 1 month of real-life consumption.[24] Thus, the 28-day protocol used in this study simulates the effect of nearly 2 years and 8 months of habitual coffee intake.

Immersion in matcha tea led to moderate but measurable discoloration. Although matcha has a nearly neutral pH (6.0–7.0), it is rich in polyphenolic catechins, particularly epigallocatechin gallate (EGCG), that interact with the organic matrix via polar hydroxyl groups.[25,26] Several SARCs exhibited ΔE_{00} values comparable to those seen in the coffee group, indicating the clinical relevance of this increasingly popular beverage. Like coffee, matcha tea is prepared with hot water, meaning that both thermal and chemical mechanisms may contribute to the discoloration observed.[25] Additionally, its intense green coloration—due to chlorophyll pigments—and fine particle size may promote greater surface adsorption, particularly in hydrophilic materials.[25] Artificial saliva produced the least discoloration. However, HEMA-based SARCs consistently exceeded the perceptibility threshold of ΔE_{00} = 1.1, suggesting the occurrence of hygroscopic alterations in the polymer matrix. These findings confirm that only artificial saliva resulted in discoloration below the clinical acceptability threshold, while all pigment-rich solutions led to perceptible and potentially unacceptable changes. Notably, HEMA-based SARCs (MXC, GCO) were significantly more susceptible to staining than their UDMA-based counterparts (RLX, SPC), favoring the latter in aesthetic clinical scenarios.

Color change, surface roughness and the role of material composition

The observed variations in both color stability and surface roughness can be largely attributed to differences in material composition. In particular, the interaction between the polymer matrix and the filler system appears pivotal in mediating resistance to external influences.[27,28] One possible mechanism underlying these changes is the degradation of peroxide-based initiators (e.g., cumene hydroperoxide, α,α-dimethylbenzyl hydroperoxide) under acidic conditions.[29] This degradation compromises the polymer structure and exposes filler particles, thereby increasing surface roughness and pigment adsorption. A positive correlation between surface roughness and color change was observed both in the present study and in previous literature.[30]

In the context of surface roughness, Sa increased significantly over time for the HEMA-based composites (MXC and GCO), especially after 21 and 28 days of immersion in red wine and coffee. Conversely, RLX and SPC, both of which are UDMA-based, exhibited only minimal changes in Sa and Sz, indicating superior surface stability under the tested conditions. This trend was particularly evident for SPC, which maintained the lowest Sz values (peak-to-valley height) across all staining cycles. These findings support the hypothesis that hydrophobic resin matrices and well-integrated filler systems contribute to structural integrity, whereas hydrophilic monomers promote matrix degradation and surface deterioration. Clinically, increased surface roughness may accelerate plaque accumulation and discoloration, thus compromising both aesthetics and material longevity. Material-dependent behavior can be explained by the chemical nature of SARCs. Maxcem Elite™ contains a highly hydrophilic monomer (HEMA), which facilitates water uptake and enhances pigment infiltration. Conversely, SPC and RLX are UDMA-based materials, incorporating a less hydrophilic monomer that promotes denser matrix structures and offers superior resistance to discoloration and surface degradation. While the organic matrix composition is widely recognized as a key factor in color stability, its role in surface roughness evolution has received comparatively less attention. Direct comparative studies between HEMA- and UDMA-based materials remain limited.[31,32] Filler content may also play a role in the superior performance of RLX. The combination of ytterbium(III) fluoride and silanized glass fillers may contribute to increased matrix density and reduced water sorption. The extent to which smaller filler particles enhance color stability and surface resilience remains unclear, as a particle-size analysis was not included in the scope of this study.

Recent studies have emphasized the importance of both polishing technique and material composition in determining the surface resilience of resin-based restoratives under acidic conditions. In particular, a recent investigation by Szalewski et al. revealed that nanohybrid composites showed no significant increase in surface roughness after immersion in highly acidic environments (pH = 2) over 21 days.[33] This finding suggests that newer materials may exhibit enhanced resistance to acid-induced degradation due to optimized filler–matrix coupling, improved polymer networks, and the elimination of the oxygen inhibition layer.

While the present study focused on SARCs rather than nanohybrid composites, a similar trend was observed: RLX, a UDMA-based material with high filler content, exhibited minimal surface degradation across all media, including red wine. This finding aligns with the hypothesis that dense, hydrophobic matrices and well-integrated filler systems contribute to enhanced structural integrity in challenging environments. In contrast, HEMA-based materials, such as MXC and GCO, were more susceptible to pigment adsorption and roughness increase, likely due to their hydrophilic nature and increased water sorption.

Although polishing protocols were not part of the present experimental design, it is worth noting that surface finishing significantly influences roughness parameters, especially under acidic stress. Future investigations should integrate standardized polishing procedures and long-term acidic exposures to better simulate clinical realities,

particularly in patients with high dietary acid intake or systemic conditions associated with reduced oral pH.

These results are in line with findings from a recent systematic review, which emphasized that the combination of mechanical and chemical surface treatments significantly enhances the bonding performance and surface integrity of dental ceramics and resin-based materials.[34] This further supports the importance of optimized filler–matrix interactions and hydrophobic resin networks in resisting both discoloration and surface degradation, as observed in the present study for UDMA-based composites.

To address the inherent susceptibility of HEMA-based systems to degradation, recent experimental approaches have focused on modifying their composition using phosphate copolymers. These modifications aim to improve mechanical properties of the material and reduce water sorption, thereby enhancing both the structural stability and resistance to discoloration.[35]

Clinical interpretation based on the established thresholds

The clinical relevance of the observed color changes was evaluated using perceptibility and acceptability thresholds for ΔE_{00}.[36] In all staining conditions, the ΔE_{00} values for all tested SARCs exceeded the clinically acceptable threshold of $\Delta E_{00} = 2.7$, indicating a heightened risk of aesthetically relevant discoloration. This was particularly evident in the red wine group, where all samples surpassed the acceptability threshold. None of the tested materials remained below the perceptibility threshold of $\Delta E_{00} = 1.1$, thus confirming that all changes were visually detectable. Color changes exceeding the threshold of acceptability are generally considered disruptive to aesthetics and may compromise the long-term appearance of indirect restorations. However, clinical interpretation remains context-dependent: in the anterior region, especially in high-aesthetic cases such as veneers, even minor deviations may be unacceptable, whereas in posterior zones or at conversational viewing distances (e.g., 50–60 cm), values up to 3.7 for ΔE_{00} may still be considered tolerable.[20,36] These findings stress the importance of selecting highly color-stable SARCs such as RLX in anterior restorations, while slightly more stain-prone materials may be acceptable in less visible regions.

Limitations

Although conducted under rigorously standardized in vitro conditions, the findings of this study are limited in their generalizability to clinical practice. Intraoral factors such as salivary flow, biofilm formation, masticatory forces, temperature variations, and self-cleansing mechanisms could not be fully replicated. Moreover, common oral hygiene practices, such as tooth brushing or the use of dental cleansers, were not included in the study, despite their potential to substantially influence surface topography and stain accumulation. The choice of staining agents, while clinically relevant, does not encompass the full range of dietary habits. Furthermore, the interactions between dietary chromogens, salivary enzymes and oral microbiota were not addressed. To minimize the risk of overestimating discoloration due to superficial residue, all specimens were rinsed with distilled water and gently dried before undergoing color analysis. However, this standardized cleaning method cannot fully replicate the dynamic effects of intraoral self-cleansing mechanisms. Future investigations should include clinical studies that validate the in vitro results under realistic oral conditions, including the presence of biofilm, mechanical abrasion and the effects of oral hygiene interventions.

Consistent with the findings of Malysa et al., our results suggest that SARC materials may be particularly susceptible to hydrolytic degradation, especially under artificial aging conditions.[37] Future studies should, therefore, integrate both thermal and mechanical aging protocols in order to better simulate intraoral challenges.

Conclusions

The color stability and surface integrity of contemporary SARCs are strongly influenced by their chemical composition and exposure to staining agents. Hydrophilic, HEMA-containing SARCs (MXC, GCO) exhibited the most pronounced changes in color and surface roughness, likely due to increased water uptake and pigment permeability. Red wine exhibited the strongest staining potential, which can be attributed to its high levels of anthocyanins and tannins. Coffee, matcha tea and artificial saliva showed progressively less staining potential. Increased surface roughness was associated with greater discoloration, emphasizing the importance of surface texture in pigment adhesion. This effect was most notable in MXC, which showed the greatest increase in roughness. In clinical setting, more hydrophobic, UDMA-based SARCs (RLX, SPC) are recommended, as they exhibit denser matrix structures, reduced water sorption and greater resistance to staining.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Klages U, Bruckner A, Zentner A. Dental aesthetics, self-awareness, and oral health-related quality of life in young adults. *Eur J Orthod*. 2004;26(5):507–514. doi:10.1093/ejo/26.5.507
2. Vanga A, Gomasani S. Impact of social media on dental treatment choices among medical and nonmedical graduates in Nellore city: A cross-sectional survey. *J Oral Health Comm Dent*. 2024;18(1):9–12. doi:10.5005/jp-journals-10062-0181
3. de Oliveira NG, Espíndola-Castro LF, Rocha JC, et al. Influence of the self-adhering strategy on microhardness, sorption, solubility, color stability, and cytotoxicity compared to bulk-fill and conventional resin composites. *Clin Oral Investig*. 2022;26(11):6663–6670. doi:10.1007/s00784-022-04624-5
4. Janson M, Liebermann A, Schoppmeier CM. Color stability and surface roughness of novel single-shade universal composite resins exposed to staining solutions: An in vitro study. *Saudi Dent J*. 2025;37(4–6):28. doi:10.1007/s44445-025-00035-w
5. Alvarenga M, Machado L, Prado A, Veloso S, Monteiro G. Self-adhesive resin cement versus conventional cements on the failure rate of indirect single-tooth restorations: A systematic review and meta-analysis of randomized clinical trials. *J Prosthet Dent*. 2024;132(5):880.e881–880.e888. doi:10.1016/j.prosdent.2024.04.027
6. Heboyan A, Vardanyan A, Karobari MI, et al. Dental luting cements: An updated comprehensive review. *Molecules*. 2023;28(4):1619. doi:10.3390/molecules28041619
7. Petropoulou A, Vrochari AD, Hellwig E, Stampf S, Polydorou O. Water sorption and water solubility of self-etching and self-adhesive resin cements. *J Prosthet Dent*. 2015;114(5):674–679. doi:10.1016/j.prosdent.2015.06.002
8. Durand LB, Ruiz-López J, Perez BG, et al. Color, lightness, chroma, hue, and translucency adjustment potential of resin composites using CIEDE2000 color difference formula. *J Esthet Restor Dent*. 2021;33(6):836–843. doi:10.1111/jerd.12689
9. Villarroel M, Fahl N, De Sousa AM, De Oliveira OB. Direct esthetic restorations based on translucency and opacity of composite resins. *J Esthet Restor Dent*. 2011;23(2):73–87. doi:10.1111/j.1708-8240.2010.00392.x
10. Liebermann A, Roos M, Stawarczyk B. The effect of different storage media on color stability of self-adhesive composite resin cements for up to one year. *Materials (Basel)*. 2017;10(3):300. doi:10.3390/ma10030300
11. Elmalawany LM, El-Refai DA, Alian GA. Change in surface properties of two different dental resin composites after using various beverages and brushing. *BMC Oral Health*. 2023;23(1):966. doi:10.1186/s12903-023-03710-8
12. Roedel L, Bednarzig V, Belli R, Petschelt A, Lohbauer U, Zorzin J. Self-adhesive resin cements: pH-neutralization, hydrophilicity, and hygroscopic expansion stress. *Clin Oral Investig*. 2017;21(5):1735–1741. doi:10.1007/s00784-016-1947-4
13. Uctasli MB, Garoushi S, Uctasli M, Vallittu PK, Lassila L. A comparative assessment of color stability among various commercial resin composites. *BMC Oral Health*. 2023;23(1):789. doi:10.1186/s12903-023-03515-9
14. Ertaş E, Güler AU, Yücel AC, Köprülü H, Güler E. Color stability of resin composites after immersion in different drinks. *Dent Mater J*. 2006;25(2):371–376. PMID:16916243.
15. Hein S, Modrić D, Westland S, Tomeček M. Objective shade matching, communication, and reproduction by combining dental photography and numeric shade quantification. *J Esthet Restor Dent*. 2021;33(1):107–117. doi:10.1111/jerd.12641
16. Borouziniat A, Majidinia S, Shirazi AS, Kahnemuee F. Comparison of bond strength of self-adhesive and self-etch or total-etch resin cement to zirconia: A systematic review and meta-analysis. *J Conserv Dent Endod*. 2024;27(2):113–125. doi:10.4103/jcde.Jcde_225_23
17. Liebermann A, Detzer J, Stawarczyk B. Impact of recently developed universal adhesives on tensile bond strength to computer-aided design/manufacturing ceramics. *Oper Dent*. 2019;44(4):386–395. doi:10.2341/18-017-L
18. Manfro ARG, Reis A, Loguercio AD, Imparato JCP, Raggio DP. Effect of different concentrations of chlorhexidine on bond strength of primary dentin. *Pediatr Dent*. 2012;34(2):e11–e15. PMID:22583871.
19. Paravina RD, Ghinea R, Herrera LJ, et al. Color difference thresholds in dentistry. *J Esthet Restor Dent*. 2015;27 Suppl 1:S1–S9. doi:10.1111/jerd.12149
20. Khashayar G, Bain PA, Salari S, Dozic A, Kleverlaan CJ, Feilzer AJ. Perceptibility and acceptability thresholds for colour differences in dentistry. *J Dent*. 2014;42(6):637–644. doi:10.1016/j.jdent.2013.11.017
21. Chen S, Zhu J, Yu M, Jin C, Huang C. Effect of aging and bleaching on the color stability and surface roughness of a recently introduced single-shade composite resin. *J Dent*. 2024;143:104917. doi:10.1016/j.jdent.2024.104917
22. Catelan A, Briso ALF, Sundfeld RH, Goiato MC, dos Santos PH. Color stability of sealed composite resin restorative materials after ultraviolet artificial aging and immersion in staining solutions. *J Prosthet Dent*. 2011;105(4):236–241. doi:10.1016/s0022-3913(11)60038-3
23. Tavangar M, Bagheri R, Kwon TY, Mese A, Manton DJ. Influence of beverages and surface roughness on the color change of resin composites. *J Investig Clin Dent*. 2018;9(3):e12333. doi:10.1111/jicd.12333
24. Guler AU, Yilmaz F, Kulunk T, Guler E, Kurt S. Effects of different drinks on stainability of resin composite provisional restorative materials. *J Prosthet Dent*. 2005;94(2):118–124. doi:10.1016/j.prosdent.2005.05.004
25. Jakubczyk K, Kochman J, Kwiatkowska A, et al. Antioxidant properties and nutritional composition of matcha green tea. *Foods*. 2020;9(4):483. doi:10.3390/foods9040483
26. Najman K, Sadowska A, Wolińska M, Starczewska K, Buczak K. The content of bioactive compounds and technological properties of matcha green tea and its application in the design of functional beverages. *Molecules*. 2023;28(20):7018. doi:10.3390/molecules28207018
27. Fazlioğlu L, Oğlakçi B, Özduman ZC, Eligüzeloğlu Dalkiliç E. The color change of a novel single-shade composite immersed in different beverages. *Bezmialem Science*. 2022;10(6):716–721. doi:10.14235/bas.galenos.2022.35220
28. Gönülol N, Yilmaz F. The effects of finishing and polishing techniques on surface roughness and color stability of nanocomposites. *J Dent*. 2012;40 Suppl 2:e64–e70. doi:10.1016/j.jdent.2012.07.005
29. Lamparth I, Angermann J, Fässler P, et al. Influence of the hydroperoxide structure on the reactivity and mechanical properties of self-cure dental composites. *Dent Mater*. 2024;40(8):1191–1198. doi:10.1016/j.dental.2024.06.008
30. Guler S, Unal M. The evaluation of color and surface roughness changes in resin based restorative materials with different contents after waiting in various liquids: An SEM and AFM study. *Microsc Res Tech*. 2018;81(12):1422–1433. doi:10.1002/jemt.23104
31. Wu D, Yao Y, Cifuentes-Jimenez CC, et al. Long-term dentin bonding performance of universal adhesives: The effect of HEMA content and bioactive resin composite. *J Funct Biomater*. 2024;15(12):379. doi:10.3390/jfb15120379
32. Fonseca ASQS, Labruna Moreira AD, de Albuquerque PP, de Menezes LR, Pfeifer CS, Schneider LF. Effect of monomer type on the CC degree of conversion, water sorption and solubility, and color stability of model dental composites. *Dent Mater*. 2017;33(4):394–401. doi:10.1016/j.dental.2017.01.010
33. Szalewski L, Wójcik D, Sowa M, Vivcharenko V, Pałka K. Influence of low pH on the microhardness and roughness surface of dental composite – a preliminary study. *Materials (Basel)*. 2024;17(14):3443. doi:10.3390/ma17143443
34. Malysa A, Wezgowiec J, Orzeszek S, Florjanski W, Zietek M, Wieckiewicz M. Effect of different surface treatment methods on bond strength of dental ceramics to dental hard tissues: A systematic review. *Molecules*. 2021;26(5):1223. doi:10.3390/molecules26051223

35. Golbari N, Valian A, Najafi F. Effect of coating fillers with HEMA-phosphate copolymer on the mechanical properties of an experimental composite resin. *Dent Med Probl*. 2025;62(1):115–124. doi:10.17219/dmp/134147
36. Paravina RD, Ghinea R, Herrera LJ, et al. Color difference thresholds in dentistry. *J Esthet Restor Dent*. 2015;27 Suppl 1:S1–S9. doi:10.1111/jerd.12149
37. Malysa A, Wezgowiec J, Grzebieluch W, Danel DP, Wieckiewicz M. Effect of thermocycling on the bond strength of self-adhesive resin cements used for luting CAD/CAM ceramics to human dentin. *Int J Mol Sci*. 2022;23(2):745. doi:10.3390/ijms23020745

Original paper

Amplification and overexpression of a novel m6A reader FXR1 are associated with poor prognosis in head and neck squamous cell carcinoma

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Abstract

Background. Fragile X-related protein 1 (FXR1), an RNA-binding protein (RBP) implicated in various cancers, has emerged as a potential contributor to the progression of head and neck squamous cell carcinoma (HNSCC).

Objectives. The aim of the study was to comprehensively analyze FXR1 genetic alterations, mRNA expression, protein levels, and immune infiltration in HNSCC, in order to elucidate its molecular role and therapeutic potential.

Material and methods. Data from The Cancer Genome Atlas (TCGA)–HNSCC cohort was analyzed. Copy number variation (CNV) was assessed using cBioPortal, FXR1 expression profiling was performed using the University of ALabama at Birmingham CANcer data analysis Portal (UALCAN), and immunohistochemistry (IHC) examination was conducted using the Human Protein Atlas (HPA). The TIMER 2.0 and TISIDB databases were employed for immune infiltration analysis.

Results. Genetic alterations in *FXR1*, including DNA amplification, were observed in 22% of HNSCC patients and were significantly correlated with elevated mRNA expression. FXR1 mRNA and protein levels were upregulated in HNSCC tumors, and this upregulation was associated with poor prognosis. The expression of *FXR1* was also correlated with clinical features, including disease stage, grade, metastasis, and human papillomavirus (HPV) status. Immune infiltration analysis revealed a negative correlation between *FXR1* mRNA expression and $CD8^+$ T cells, suggesting a potential role in immune modulation.

Conclusions. FXR1 may serve as a potential biomarker and immune regulator in HNSCC, with genetic alterations influencing its mRNA expression. This study sheds light on the multifaceted role of FXR1 in the progression of HNSCC, and suggests potential avenues for targeted therapeutic strategies. Further research, including functional experiments and large-scale cohort analyses, is warranted to validate and translate these findings into clinical applications.

Keywords: TCGA, prognosis, copy number variation, head and neck carcinoma, FXR1

Cite as
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Highlights

- FXR1 may serve as a potential biomarker and immune regulator in head and neck squamous cell carcinoma (HNSCC), with genetic alterations influencing its mRNA expression.
- FXR1 appears to play a multifaceted role in HNSCC progression, including potential involvement in tumor–immune interactions, and may represent a target for future therapeutic strategies.
- Further research, including functional studies and large-scale cohort analyses, is required to validate these findings and support their clinical translation.

Introduction

Head and neck squamous cell carcinoma (HNSCC) constitutes a diverse group of tumors, predominantly squamous cell carcinomas (SCCs), accounting for approx. 90% of diagnoses and ranking as the 6th most prevalent cancer worldwide.[1,2] Oropharyngeal cancers are primarily driven by human papillomavirus (HPV), while alcohol consumption and tobacco use are risk factors for cancers of the oral cavity, hypopharynx and larynx. Notably, HPV-positive HNSCC generally exhibits a more favorable prognosis.[3] The development of HNSCC involves intricate genetic and epigenetic alterations that contribute to hallmark features of cancer, including uncontrolled proliferation and metastasis.[4–8]

The discovery of human fragile X-related protein 1 (FXR1) (3q28) resulted from fragile X messenger ribonucleoprotein 1 (FMR1) sequencing, revealing an autosomal gene with 60% amino acid sequence identity to other family members.[9] FXR1 shares a common structure but differs in its C-termini, implying distinct roles. The protein contains several domains, including a Tandem agenet-like domain, a non-classical nuclear localization signal (NLS), 3 K homology (KH) domains, a nuclear export signal (NES), and an arginine–glycine–glycine (RGG) box, classifying FXR1 as an RNA-binding protein (RBP). Exportin-1 (XPO-1) shuttles FXR1 to the cytoplasm, where RGG motifs facilitate binding to DNA or RNA structures. FXR1 is ubiquitously expressed and undergoes various post-translational modifications, including SUMOylation, ubiquitylation, acetylation, methylation, and phosphorylation at S420.[10,11]

FXR1 regulates microRNA (miRNA) processing, stabilizes specific miRNAs, and influences their subcellular localization.[12] It interacts with nucleoporins during mitosis, affecting protein export. Additionally, FXR1 stabilizes mRNA through adenylate–uridylate (AU)-rich elements (AREs) in the 3'-untranslated region (3'-UTR), thereby influencing mRNA translation and stability. It also modulates mRNA translation through interactions with miRNAs, argonaute-2 (AGO2) and the RNA interference (RNAi) pathway. Furthermore, FXR1 has an influence on the cell cycle, synaptic protein function and muscle development. It plays a crucial role in tumor necrosis factor alpha (TNF-α)–ARE-mediated translational activation, impacting cell growth. Overall, these multifaceted functions highlight the significance of FXR1 in post-transcriptional gene regulation and underscore its potential diagnostic and therapeutic implications.[10,13]

Recent studies have highlighted the involvement of FXR1 in cancer.[14,15] Elevated expression of FXR1 has been observed in various malignancies, including bladder cancer, colorectal cancer, non-small cell lung cancer, and hepatocellular carcinoma (HCC).[14–18] In these contexts, FXR1 acts as an oncogene, promoting tumor growth and invasion through mechanisms such as mRNA stabilization, enhanced protein translation and inactivation of tumor suppressor genes.[19–21]

Despite its pro-tumorigenic attributes, the RNA-binding mechanisms of FXR1 offer promising therapeutic avenues. Targeting these interactions using small molecules or RNAi-based strategies may disrupt its oncogenic effects. In HNSCC, genetic alterations, particularly copy number variation (CNV), play a crucial role in activating oncogenes or inactivating tumor suppressor genes.[22–24] Recent studies have also demonstrated the overexpression of FXR1 in HNSCC tissues, correlating it with tumor growth and invasion.[25] This study, utilizing data from The Cancer Genome Atlas (TCGA)–HNSCC cohort, aimed to analyze *FXR1* genetic variations, including the expression of DNA, mRNA, protein, as well as to unravel its molecular role and therapeutic potential in HNSCC. FXR1 is significantly associated with HNSCC, thus providing a link between the prognosis and tumor infiltration in HNSCC.

Material and methods

Copy number variation analysis

The cBioPortal (https://www.cbioportal.org), a user-friendly and interactive online resource, facilitates the visualization, analysis and download of extensive cancer genomics datasets.[26] In this investigation, the cBioPortal was used to examine alterations in the FXR1 gene among

HNSCC patients. Comprehensive clinical data for 528 HNSCC patients is presented in Table 1. The prognostic significance of *FXR1* genetic alterations in patients with HNSCC was assessed using the Kaplan–Meier plotter analysis.

FXR1 expression analysis

The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN) (http://ualcan.path.uab.edu)[27] was used to analyze expression profiles in HNSCC. The UALCAN integrates datasets from TCGA and the Clinical Proteomic Tumor Analysis Consortium (CPTAC).

Table 1. Demographic and clinical characteristics of The Cancer Genome Atlas (TCGA)–head and neck squamous cell carcinoma (HNSCC) cohort used for FXR1 analysis (*N* = 528)

Variable		Patients, *n* (%)
Sex	male	383 (72.5)
	female	136 (25.8)
	not available	9 (1.7)
Age [years]	21–40	20 (3.8)
	41–60	236 (44.7)
	61–80	237 (44.9)
	81–100	24 (4.5)
	not available	11 (2.1)
Patient race	Caucasian	444 (84.1)
	African American	47 (8.9)
	Asian	11 (2.1)
	not available	26 (4.9)
Tumor grade	well differentiated (grade 1)	62 (11.7)
	moderately differentiated (grade 2)	303 (57.4)
	poorly differentiated (grade 3)	125 (23.7)
	undifferentiated (grade 4)	7 (1.3)
	not available	31 (5.9)
Cancer stage	1	27 (5.1)
	2	71 (13.4)
	3	81 (15.3)
	4	264 (50.0)
	not available	85 (16.1)
Lymph node metastasis status	no regional lymph node metastasis (N0)	176 (33.3)
	metastases in 1–3 axillary lymph nodes (N1)	67 (12.7)
	metastases in 4–9 axillary lymph nodes (N2)	12 (2.3)
	metastases in ≥10 axillary lymph nodes (N3)	8 (1.5)
	not available	265 (50.2)
HPV status	positive	80 (15.2)
	negative	434 (82.2)
	not available	14 (2.7)
TP53 mutation status	TP53 mutant	327 (61.9)
	TP53 non-mutant	175 (33.1)
	not available	26 (4.9)

HPV – human papillomavirus.

The present study also involved a comprehensive analysis of the relationship between *FXR1* expression and clinicopathological features of HNSCC, such as disease stage, histological grade, lymph node involvement, and HPV status.

Immunohistochemistry analysis

The Human Protein Atlas (HPA) (http://www.proteinatlas.org)[28] is a database that provides information on human protein-coding genes, including RNA and protein expression levels as well as location. Immunohistochemistry (IHC) images illustrating the expression of FXR1 protein in clinical specimens from HNSCC patients and normal tissues were obtained from the HPA database. To analyze the expression levels of FXR1, staining intensity was classified as low, moderate or high. According to HPA data, Anti-FXR1 rabbit monoclonal antibodies (HPA018246 and HPA055475; Sigma-Aldrich, St. Louis, USA) were utilized for IHC, with dilution ratios of 1:150 and 1:50, respectively.

Immune infiltration analysis

Given the pivotal role of immune genes in the pathogenesis of HNSCC, we analyzed the relationship between our target gene, *FXR1*, and key immune genes involved in HNSCC pathogenesis. Tumor infiltration was assessed using TIMER 2.0 (http://TIMER.cistrome.org)[29] and the TISIDB (http://cis.hku.hk/TISIDB)[30] database. The TIMER server was employed to systematically evaluate immune infiltration across various malignancies, employing the TIMER method to quantify $CD4^+$ T cells, $CD8^+$ T cells, B cells, neutrophils, macrophages, and dendritic cells. The gene module was applied to examine *FXR1* expression in different cancers and the extent of immune cell infiltration in HNSCC. Additional analyses included the exploration of immunomodulators, chemokines and their receptors. Specifically, the top 3 negatively correlated immunomodulatory genes, encompassing major histocompatibility complex (MHC) molecules, immunostimulators and immune inhibitors, were identified in relation to *FXR1* mRNA expression in HNSCC patients.

Statistical analysis

Data was analyzed using various statistical methods depending on the specific research question. To compare FXR1 expression between the groups, Student's *t*-tests were performed to calculate *p*-values and determine fold changes, along with gene rank information. The Kaplan–Meier analysis was used to assess the relationship between variables and survival outcomes, with the log-rank test applied for comparisons between survival curves. To comprehensively evaluate the impact of FXR1 expression and other clinicopathological factors on survival, a multivariate Cox proportional hazards model was employed.

Correlations between gene expression levels were assessed using Spearman's correlation analysis in the TIMER 2.0 databases. The TISIDB database provided both Spearman's and Pearson's correlation analyses to evaluate the strength of association between variables. A *p*-value <0.05 was considered statistically significant unless stated otherwise.

Results

Association between genetic alterations in *FXR1* and increased mRNA expression in HNSCC

Genetic alterations in *FXR1* were observed in 22% of HNSCC patients among the 528 individuals analyzed. These alterations were primarily characterized by *FXR1* gene amplification, while some cases exhibited missense mutations (Fig. 1A). Notably, *FXR1* amplification was significantly correlated with elevated *FXR1* mRNA expression in HNSCC patients (Fig. 1B) ($p < 0.05$).

Upregulation of FXR1 mRNA and protein levels in HNSCC

The mRNA expression level of *FXR1* was significantly upregulated in patients with primary HNSCC tumors ($n = 520$) compared to normal tissues ($n = 44$) in the TCGA–HNSCC dataset (Fig. 2A) ($p < 0.001$). Similarly, FXR1 protein levels were significantly increased in primary HNSCC tumor tissues ($n = 108$) compared to normal tissues ($n = 71$) in the CPTAC–HNSCC dataset (Fig. 2B) ($p < 0.001$). Immunohistochemical analysis further confirmed that FXR1 protein was markedly higher in HNSCC tumor tissues than in normal tissues (Fig. 2C). Moreover, the Kaplan–Meier curve displayed the relationship between *FXR1* genetic alterations and HNSCC patient survival. In particular, patients with altered *FXR1* exhibited poorer overall survival, suggesting its potential prognostic significance (Fig. 2D) ($p < 0.05$).

Association of dysregulated *FXR1* mRNA with stage, grade, metastasis status, and HPV status in HNSCC

FXR1 mRNA expression was significantly altered across different tumor stages, grades and metastasis statuses compared to normal tissues (Fig. 3A–C) ($p < 0.05$). In terms of tumor stage, significant differences were noted between normal tissues and stages 2, 3 and 4, as well as between stages 3 and 4 (Fig. 3A) ($p < 0.05$). Regarding HNSCC grade, substantial alterations were observed between normal tissues and grades 2, 3 and 4, as well as between grade 1 and grades 2 and 3 (Fig. 3B) ($p < 0.05$). In terms of metastasis status, *FXR1* expression in normal tissues differed significantly from N0–N3 groups.

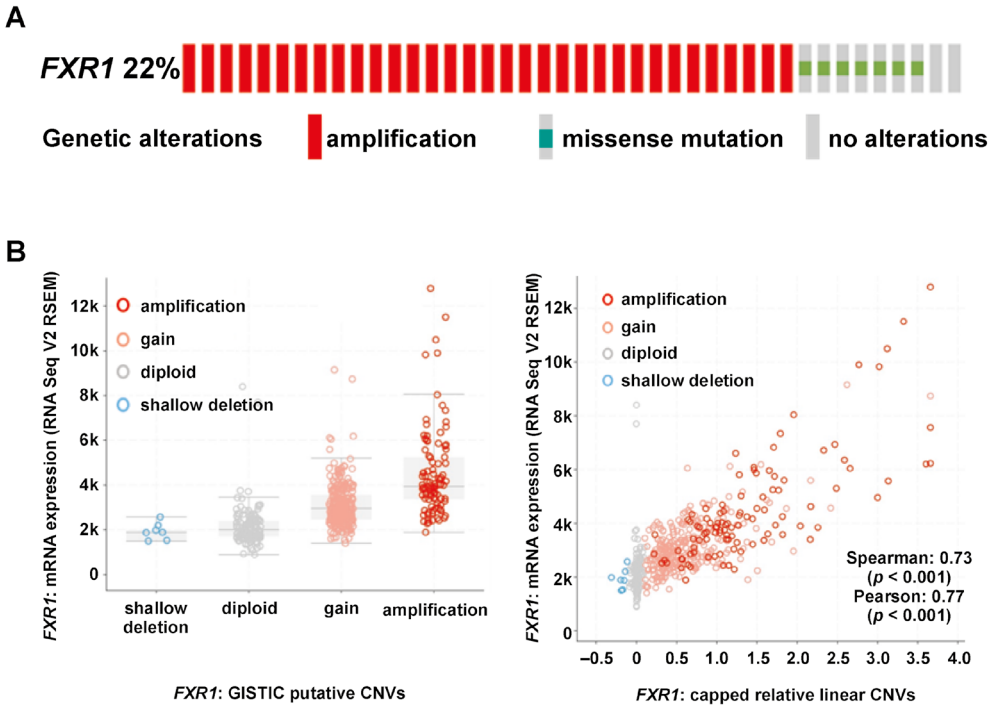

Fig. 1. *FXR1* gene alterations in head and neck squamous cell carcinoma (HNSCC) (source: cBioPortal)

A. OncoPrint illustrating genomic alterations of *FXR1* in HNSCC patients; B. Correlation between *FXR1* mRNA expression and copy number variations (CNVs) in HNSCC tissues, assessed using Spearman's and Pearson's correlation analyses.
RSEM – RNA-Seq by Expectation Maximization.

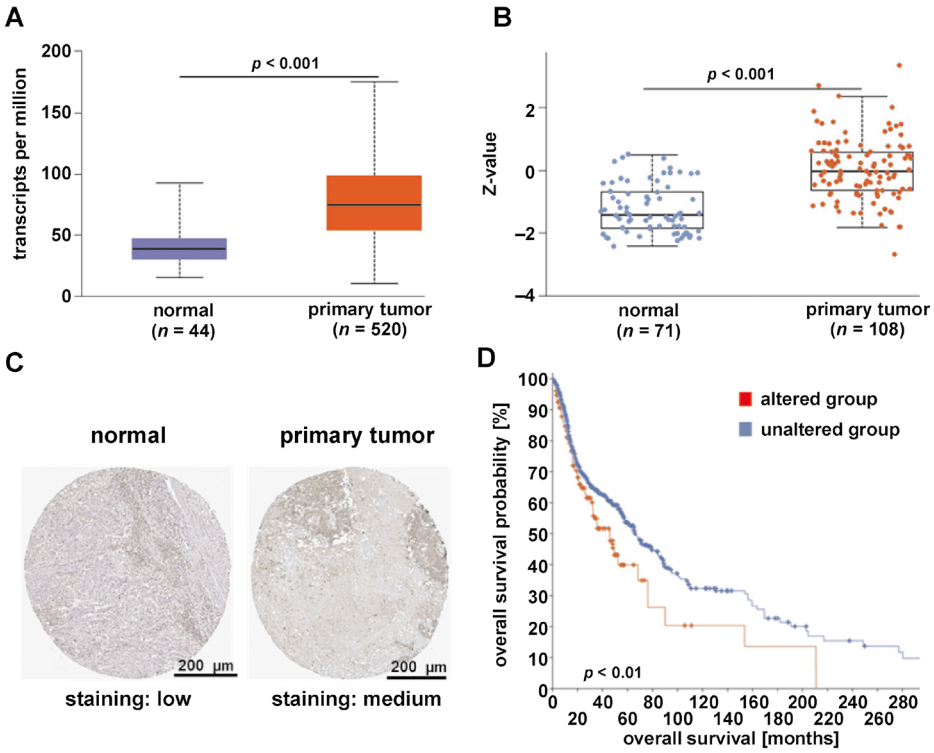

Fig. 2. FXR1 expression and prognosis in head and neck squamous cell carcinoma (HNSCC) (sources: University of ALabama at Birmingham CANcer data analysis Portal (UALCAN), Human Protein Atlas (HPA) and cBioPortal)

A. *FXR1* mRNA expression in HNSCC tumor tissues compared to normal tissues (The Cancer Genome Atlas (TCGA)–HNSCC cohort); B. FXR1 protein expression in HNSCC tumor tissues compared to normal tissues (Clinical Proteomic Tumor Analysis Consortium (CPTAC)–HNSCC cohort); C. Immunohistochemical staining confirming elevated FXR1 protein expression in HNSCC tumor tissues compared to normal tissues (TCGA–HNSCC cohort); D. Overall survival of patients with *FXR1* genetic alterations compared to those without alterations (TCGA–HNSCC cohort).

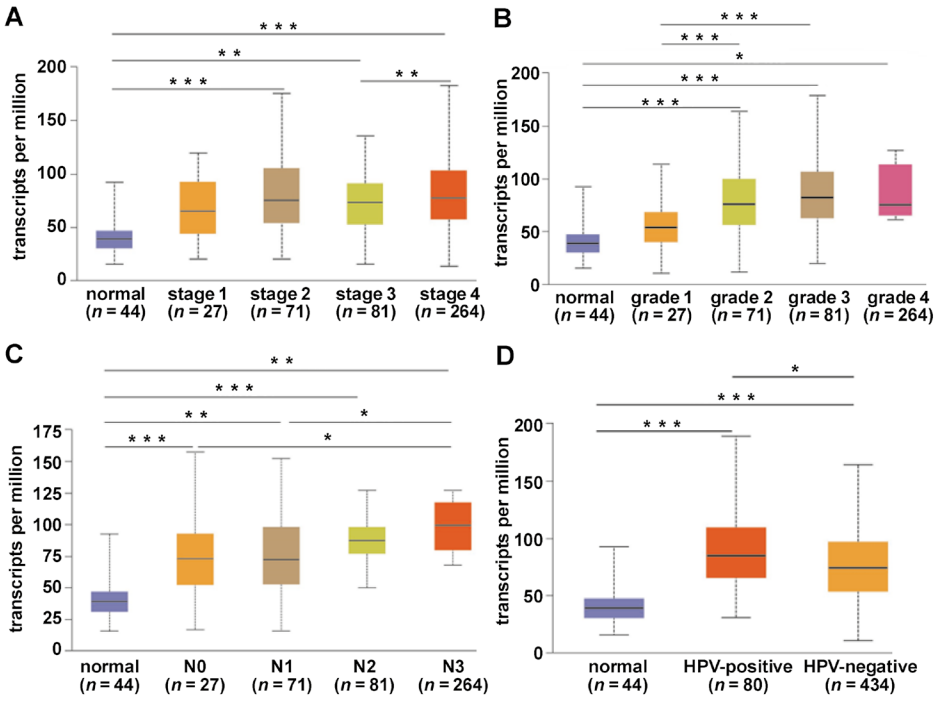

Fig. 3. *FXR1* expression and clinicopathological features in head and neck squamous cell carcinoma (HNSCC) (source: University of ALabama at Birmingham CANcer data analysis Portal (UALCAN))

A. *FXR1* mRNA expression according to cancer stage; B. *FXR1* mRNA expression according to tumor grade; C. *FXR1* mRNA expression according to lymph node metastasis status; D. *FXR1* mRNA expression in human papillomavirus (HPV)-positive and HPV-negative HNSCC patients.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Additionally, significant differences were observed between N0 and N3, and between N1 and N3 (Fig. 3C) ($p < 0.05$). Furthermore, *FXR1* expression was significantly higher in HPV-positive HNSCC patients compared to HPV-negative patients and normal tissues (Fig. 3D) ($p < 0.05$).

Impact of *FXR1* mRNA expression on tumor immune infiltration in HNSCC

The mRNA expression of *FXR1* exhibited a significant negative correlation with $CD8^+$ T cells (Fig. 4A). High *FXR1* expression was associated with poorer prognosis in HNSCC patients, including those with HPV-negative tumors (Fig. 4A,B). The mRNA expression of *FXR1* and genes involved in MHC molecules, immunostimulators and immunoinhibitors, were analyzed in HNSCC patients, as depicted in Supplementary Figure 1 (available on request from the corresponding author). These groups function as immunomodulators in humans. The top 3 negatively correlated genes in each category were identified, with MHC molecules (*HLA-DMA*, *HLA-DPB1*, *HLA-DRB1*), immunostimulators (*KLRK1*, *CD27*, *TNFSF13B*) and immune inhibitors (*CD244*, *CTLA4*, *CD96*) showing significant negative correlations with *FXR1* expression in HNSCC patients (Fig. 5A–C) ($p < 0.001$).

Discussion

Among RNA-binding proteins, FXR1 is a well-characterized oncogene involved in multiple cancers. The overexpression of FXR1 has been reported in several malignancies, including glioma, lung carcinoma, HCC, breast cancer, head and neck carcinoma, ovarian cancer, and colorectal carcinoma.[14,15,17,18,20,25]

Copy number variation has been associated with changes in gene expression; yet, the specific cellular associations between CNV and gene expression alterations remain unknown.[31] The present study investigated genetic alterations and their functional implications, as well as the association between *FXR1* mRNA expression and HNSCC. Genetic alterations in *FXR1* were observed in 22% of HNSCC patients and were primarily characterized by gene amplification, with a smaller proportion of cases exhibiting missense mutations. Notably, *FXR1* amplification was significantly correlated with elevated *FXR1* mRNA expression, indicating a potential regulatory mechanism. This observation confirms previous findings demonstrating the influence of gene copy number on expression levels. Additionally, earlier studies have reported enrichment of both amplified and downregulated genes within the ubiquitin–proteasome system, suggesting potential consequences for protein degradation pathways in HNSCC.[31] Further research by Baltaci et al.[32] and Carron et al.[33] demonstrated that CNVs, particularly gene amplification, contribute to HNSCC carcinogenesis through the cooperation of oncogenes and inherited CNV, influencing the occurrence and prognosis of oropharyngeal cancer, respectively. Beyond oncology, genetic alterations in *FXR1* have also been implicated in congenital multiminicore myopathy and Fragile X syndrome.[34,35]

FXR1 emerges as a potential biomarker and immune regulator in HNSCC. Both mRNA and protein levels were elevated in tumor tissues compared to healthy tissues, suggesting a role in disease progression. This upregulation is associated with patient survival and correlates with diverse clinical features, including HPV status. Previous studies have examined the role of FXR1 in cancer. Xiao et al. demonstrated the importance of *FXR1* across 25 cancer types, including breast and cervical cancers, showcasing its diagnostic potential and association with prognosis.[36] The enrichment analysis revealed the influence of FXR1 on tumor development and progression through multiple pathways such as cell cycle control, mitochondrial metabolism, immune response, and skin barrier formation.[36] Notably, RBPs have emerged as key regulators of these processes, influencing RNA splicing, transport, translation, and localization.[37] Further studies have demonstrated the diverse roles of FXR1, ranging from influencing RNA metabolism in glioblastoma to impacting muscle development and adenovirus interactions.[20,38,39] These findings reveal the broad involvement of FXR1 in various cancer types and disease-related processes.

While existing research explores the role of FXR1 in cancer, several key mechanisms remain shrouded. In ovarian cancer, FXR1 has been shown to regulate c-MYC translation and stabilization, promoting its oncogenic activity.[40] Moreover, cancer cells lacking both p53 and FXR2 ("double knockout") exhibit increased sensitivity to FXR1 inhibition. This vulnerability depends on the FXR1 tandem Tudor domain and its interaction with STAT1/3 proteins. FXR1 binds to gene promoters, including *ARID1A* and *CASC4*, and recruits STAT1/3 to promote transcription. The inhibition of FXR1 or JAK (activating STAT1/3) suppresses FXR1 target genes and reduces cancer cell growth.[41] These findings reveal a novel synergy between FXR1 and STAT1/3 in regulating cell proliferation, opening therapeutic avenues for double-knockout cancers. Furthermore, in HCC, FXR1 has been implicated in the intricate transforming growth factor beta (TGF-β)/SMAD signaling pathway. Elevated FXR1 expression correlates with dismal prognosis and promotes cancer cell motility, invasion and proliferation through the modulation of the SMAD2/3 axis.[18] Moreover, Majumder et al. highlighted the ability of FXR1 to bypass cellular senescence in oral cancer, revealing its contribution to tumor cell survival and growth.[42] These outcomes suggest that FXR1 presents additional therapeutic opportunities across diverse cancer landscapes.

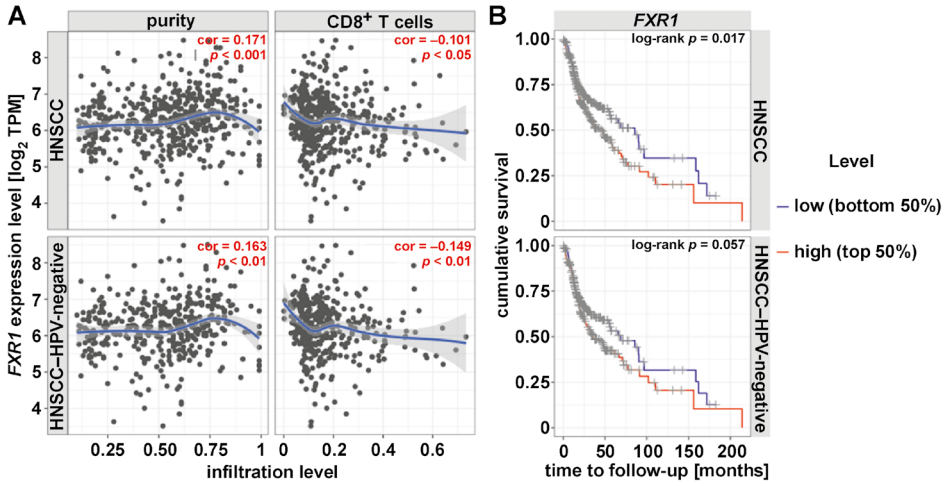

Fig. 4. *FXR1* expression and immune infiltration in head and neck squamous cell carcinoma (HNSCC) (source: TIMER 2.0)

A. Correlation between *FXR1* expression and CD8+ T cell infiltration in HNSCC patients; B. Association between *FXR1* expression and survival outcomes in HNSCC patients.
TPM – transcripts per million.

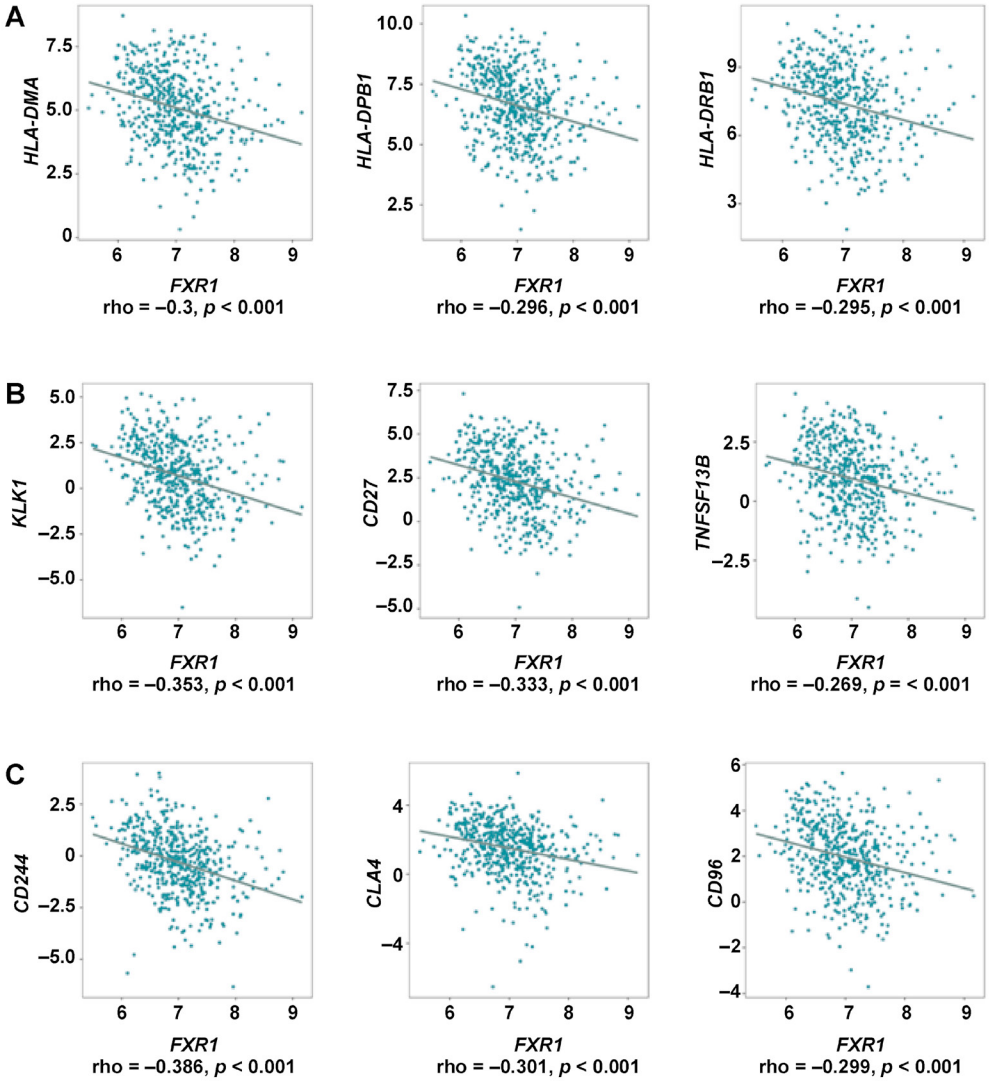

Fig. 5. Expression of immune regulatory genes and FXR1 in head and neck squamous cell carcinoma (HNSCC) (source: TISIDB; Spearman's correlation analysis, $n = 522$)

A. Major histocompatibility complex (MHC) molecule genes; B. Immunostimulatory genes; C. Immune inhibitory genes.

A growing number of studies have shown that the immune microenvironment plays a vital role in the development and progression of HNSCC.[43] Previous studies have shown that *FXR1* expression is significantly correlated with the expression of immunomodulators across various cancers.[36] In this study, *FXR1* expression was negatively correlated with $CD8^+$ T cells, implying a role in the modulation of the immune microenvironment. Further analyses indicated that *FXR1* interacts with key immune-related genes, supporting its potential as an immunotherapeutic target. These findings warrant further investigation into the multifaceted role of FXR1 in HNSCC progression and immune evasion.

The current study, based on the TCGA–HNSCC dataset and various bioinformatics tools, provides valuable insights into the potential of FXR1 as a biomarker and prognostic indicator in HNSCC. However, further research is required to fully grasp its potential. Large-scale cohort analysis of genetic variants and expression patterns across diverse experimental methods could significantly enhance the accuracy and robustness of these findings. Functional experiments and in vivo studies are crucial for validating the therapeutic potential of FXR1 in HNSCC. Our initial data has shed light on how genetic variants focusing on CNV influence the pathogenesis of HNSCC by altering FXR1 expression and regulation. Building upon this foundation, further research can translate these findings into tangible therapeutic strategies.

Conclusions

This study demonstrates a significant association between *FXR1* genetic alterations and aberrant expression in HNSCC. *FXR1* gene amplification contributes to elevated FXR1 mRNA and protein levels in HNSCC patients. This upregulation is associated with poor prognosis and correlates with various clinical features, including disease stage, grade, lymph node metastasis status, and HPV status. Additionally, our findings suggest that FXR1 may play a role in modulating the immune microenvironment, emphasizing its multifaceted involvement in HNSCC progression and immune evasion. Taken together, FXR1 emerges as a promising biomarker and therapeutic target, warranting further exploration of its molecular mechanisms and therapeutic potential in HNSCC.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

ChatGPT (OpenAI, San Francisco, USA) was used to improve readability. The content was then subjected to a thorough review and editing process to ensure the highest standards of quality. The authors accept full responsibility for the publication.

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References

1. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. *Nat Rev Dis Primers*. 2020;6(1):92. doi:10.1038/s41572-020-00224-3
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
3. Barsouk A, Aluru JS, Rawla P, Saginala K, Barsouk A. Epidemiology, risk factors, and prevention of head and neck squamous cell carcinoma. *Med Sci (Basel)*. 2023;11(2):42. doi:10.3390/medsci11020042
4. Kannan B, Pandi C, Pandi A, Jayaseelan VP, Murugan MS, Arumugam P. Altered expression of GLS2 indicates a poor prognosis and correlates with clinicopathological features of oral squamous cell carcinoma. *Int J Oral Maxillofac Surg*. 2024;53(8):635–643. doi:10.1016/j.ijom.2024.01.011
5. Avs KR, Pandi C, Kannan B, Pandi A, Jayaseelan VP, Arumugam P. RFC3 serves as a novel prognostic biomarker and target for head and neck squamous cell carcinoma. *Clin Oral Investig*. 2023;27(11):6961–6969. doi:10.1007/s00784-023-05316-4
6. Balachander K, Paramasivam A. Ferroptosis: An emerging therapeutic target for oral cancer. *Oral Oncol*. 2022;131:105970. doi:10.1016/j.oraloncology.2022.105970
7. Romanowska K, Sobecka A, Rawłuszko-Wieczorek AA, Suchorska WM, Golusiński W. Head and neck squamous cell carcinoma: Epigenetic landscape. *Diagnostics (Basel)*. 2020;11(1):34. doi:10.3390/diagnostics11010034
8. Ju Y, Wu X, Wang H, et al. Genomic landscape of head and neck squamous cell carcinoma across different anatomic sites in Chinese population. *Front Genet*. 2021;12:680699. doi:10.3389/fgene.2021.680699
9. Siomi MC, Siomi H, Sauer WH, Srinivasan S, Nussbaum RL, Dreyfuss G. FXR1, an autosomal homolog of the fragile X mental retardation gene. *EMBO J*. 1995;14(11):2401–2408. doi:10.1002/j.1460-2075.1995.tb07237.x
10. Khan FA, Fang N, Zhang W, Ji S. The multifaceted role of Fragile X-Related Protein 1 (FXR1) in cellular processes: An updated review on cancer and clinical applications. *Cell Death Dis*. 2024;15(1):72. doi:10.1038/s41419-023-06413-8
11. Majumder M, Johnson RH, Palanisamy V. Fragile X-related protein family: A double-edged sword in neurodevelopmental disorders and cancer. *Crit Rev Biochem Mol Biol*. 2020;55(5):409–424. doi:10.1080/10409238.2020.1810621
12. van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. *Nat Rev Cancer*. 2011;11(9):644–656. doi:10.1038/nrc3107
13. St Paul A, Corbett C, Peluzzo A, et al. FXR1 regulates vascular smooth muscle cell cytoskeleton, VSMC contractility, and blood pressure by multiple mechanisms. *Cell Rep*. 2023;42(4):112381. doi:10.1016/j.celrep.2023.112381

14. Qian J, Hassanein M, Hoeksema MD, et al. The RNA binding protein FXR1 is a new driver in the 3q26-29 amplicon and predicts poor prognosis in human cancers. *Proc Natl Acad Sci U S A*. 2015;112(11):3469–3474. doi:10.1073/pnas.1421975112
15. Comtesse N, Keller A, Diesinger I, et al. Frequent overexpression of the genes *FXR1*, *CLAPM1* and *EIF4G* located on amplicon 3q26-27 in squamous cell carcinoma of the lung. *Int J Cancer*. 2007;120(12):2538–2544. doi:10.1002/ijc.22585
16. Deng M, Wang N, Li Z, et al. FXR1 can bind with the CFIm25/CFIm68 complex and promote the progression of urothelial carcinoma of the bladder by stabilizing TRAF1 mRNA. *Cell Death Dis*. 2022;13(2):170. doi:10.1038/s41419-022-04614-1
17. Jin X, Zhai B, Fang T, Guo X, Xu L. FXR1 is elevated in colorectal cancer and acts as an oncogene. *Tumour Biol*. 2016;37(2):2683–2690. doi:10.1007/s13277-015-4068-9
18. Zhao K, Gao J, Shi J, et al. FXR1 promotes proliferation, invasion and migration of hepatocellular carcinoma in vitro and in vivo. *Oncol Lett*. 2023;25(1):22. doi:10.3892/ol.2022.13608
19. Cao H, Gao R, Yu C, Chen L, Feng Y. The RNA-binding protein FXR1 modulates prostate cancer progression by regulating FBXO4. *Funct Integr Genomics*. 2019;19(3):487–496. doi:10.1007/s10142-019-00661-8
20. Cao S, Zheng J, Liu X, et al. FXR1 promotes the malignant biological behavior of glioma cells via stabilizing MIR17HG. *J Exp Clin Cancer Res*. 2019;38(1):37. doi:10.1186/s13046-018-0991-0
21. Datta C, Truesdell SS, Wu KQ, et al. Ribosome changes reprogram translation for chemosurvival in G0 leukemic cells. *Sci Adv*. 2022;8(43):eabo1304. doi:10.1126/sciadv.abo1304
22. Yang J, Chen Y, Luo H, Cai H. The landscape of somatic copy number alterations in head and neck squamous cell carcinoma. *Front Oncol*. 2020;10:321. doi:10.3389/fonc.2020.00321
23. Chen Y, Chen C. DNA copy number variation and loss of heterozygosity in relation to recurrence of and survival from head and neck squamous cell carcinoma: A review. *Head Neck*. 2008;30(10):1361–1383. doi:10.1002/hed.20861
24. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. *Arch Oral Biol*. 2021;122:105030. doi:10.1016/j.archoralbio.2020.105030
25. Qie S, Majumder M, Mackiewicz K, et al. Fbxo4-mediated degradation of Fxr1 suppresses tumorigenesis in head and neck squamous cell carcinoma. *Nat Commun*. 2017;8(1):1534. doi:10.1038/s41467-017-01199-8
26. de Bruijn I, Kundra R, Mastrogiacomo B, et al. Analysis and visualization of longitudinal genomic and clinical data from the AACR Project GENIE Biopharma collaborative in cBioPortal. *Cancer Res*. 2023;83(23):3861–3867. doi:10.1158/0008-5472.CAN-23-0816
27. Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: An update to the integrated cancer data analysis platform. *Neoplasia*. 2022;25:18–27. doi:10.1016/j.neo.2022.01.001
28. Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. *Science*. 2017;357(6352):eaan2507. doi:10.1126/science.aan2507
29. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res*. 2020;48(W1):W509–W514. doi:10.1093/nar/gkaa407
30. Ru B, Wong CN, Tong Y, et al. TISIDB: An integrated repository portal for tumor-immune system interactions. *Bioinformatics*. 2019;35(20):4200–4202. doi:10.1093/bioinformatics/btz210
31. Shao X, Lv N, Liao J, et al. Copy number variation is highly correlated with differential gene expression: A pan-cancer study. *BMC Med Genet*. 2019;20(1):175. doi:10.1186/s12881-019-0909-5
32. Baltaci E, Karaman E, Dalay N, Buyru N. Analysıs of gene copy number changes in head and neck cancer. *Clin Otolaryngol*. 2018;43(4):1004–1009. doi:10.1111/coa.12686
33. Carron J, Torricelli C, Silva JK, et al. Association of inherited copy number variation in *ADAM3A* and *ADAM5* pseudogenes with oropharynx cancer risk and outcome. *Genes (Basel)*. 2022;13(12):2408. doi:10.3390/genes13122408
34. Estañ MC, Fernández-Núñez E, Zaki MS, et al. Recessive mutations in muscle-specific isoforms of FXR1 cause congenital multi-minicore myopathy. *Nat Commun*. 2019;10(1):797. doi:10.1038/s41467-019-08548-9
35. Zang JB, Nosyreva ED, Spencer CM, et al. A mouse model of the human Fragile X syndrome I304N mutation. *PLoS Genet*. 2009;5(12):e1000758. doi:10.1371/journal.pgen.1000758
36. Xiao K, Ullah I, Yang F, et al. Comprehensive bioinformatics analysis of FXR1 across pan-cancer: Unraveling its diagnostic, prognostic, and immunological significance. *Medicine (Baltimore)*. 2023;102(48):e36456. doi:10.1097/MD.0000000000036456
37. Mangus DA, Evans MC, Jacobson A. Poly(A)-binding proteins: Multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol*. 2003;4(7):223. doi:10.1186/gb-2003-4-7-223
38. Smith JA, Curry EG, Blue RE, et al. FXR1 splicing is important for muscle development and biomolecular condensates in muscle cells. *J Cell Biol*. 2020;219(4):e201911129. doi:10.1083/jcb.201911129
39. Hajikhezri Z, Kaira Y, Schubert E, et al. Fragile X-related protein FXR1 controls human adenovirus capsid mRNA metabolism. *J Virol*. 2023;97(2):e0153922. doi:10.1128/jvi.01539-22
40. George J, Li Y, Kadamberi IP, et al. RNA-binding protein FXR1 drives cMYC translation by recruiting eIF4F complex to the translation start site. *Cell Rep*. 2021;37(5):109934. doi:10.1016/j.celrep.2021.109934
41. Fan Y, Yue J, Xiao M, et al. FXR1 regulates transcription and is required for growth of human cancer cells with *TP53/FXR2* homozygous deletion. *Elife*. 2017;6:e26129. doi:10.7554/eLife.26129
42. Majumder M, House R, Palanisamy N, et al. Correction: RNA-binding protein FXR1 regulates p21 and TERC RNA to bypass p53-mediated cellular senescence in OSCC. *PLoS Genet*. 2016;12(10):e1006411. doi:10.1371/journal.pgen.1006411
43. Chen SMY, Krinsky AL, Woolaver RA, Wang X, Chen Z, Wang JH. Tumor immune microenvironment in head and neck cancers. *Mol Carcinog*. 2020;59(7):766–774. doi:10.1002/mc.23162

Original paper

Antimicrobial and cytotoxic evaluation of biosurfactant-coated copper nanoparticles: A study on selected endodontic pathogens

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Abstract

Background. Endodontic infections caused by pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus oralis*, and *Enterococcus* spp. pose significant challenges due to biofilm formation and resistance to conventional treatments. Copper nanoparticles (CuNPs) exhibit promising antimicrobial properties, and coating them with biosurfactants may enhance stability while reducing cytotoxicity.

Objectives. The aim of the study was to synthesize and characterize CuNPs stabilized with biosurfactants derived from *Achromobacter xylosoxidans* and *Pseudomonas aeruginosa*, and to evaluate their antimicrobial efficacy and cytotoxicity.

Material and methods. Copper nanoparticles were synthesized using a chemical reduction method and coated with biosurfactants to prevent oxidation and aggregation. The nanoparticles were characterized using Fourier transform infrared spectroscopy (FTIR), ultraviolet–visible (UV–Vis) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX). Antimicrobial activity against *E. coli*, *S. aureus*, *S. oralis*, and *Enterococcus* spp. was evaluated using the well diffusion method and the minimum inhibitory concentration (MIC). Cytotoxicity was assessed using an MTT assay on L929 fibroblast cell lines.

Results. Characterization confirmed the formation of spherical CuNPs with sizes ranging from 90 nm to 100 nm. Both CuO+P5C and CuO+P11 nanoparticles demonstrated potent antimicrobial activity against all tested pathogens, with a MIC value of 125 µg/mL. Cytotoxicity results showed minimal toxicity, with nearly 78–80% cell viability maintained at concentrations of up to 100 µg/mL, indicating excellent biocompatibility.

Conclusions. Biosurfactant-coated CuNPs represent a stable, eco-friendly and effective antimicrobial alternative for addressing persistent endodontic infections. Further in vivo studies and extended cytotoxicity evaluations are needed to confirm their clinical application.

Keywords: copper nanoparticles, biosurfactants, *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*, endodontic pathogens, antimicrobial activity

Highlights

- Stable biosurfactant-coated copper nanoparticles (CuNPs) with a size range of 90–100 nm were successfully synthesized.
- The CuNPs demonstrated antimicrobial activity against key endodontic pathogens, with a minimum inhibitory concentration (MIC) of 125 µg/mL.
- Favorable cytocompatibility was observed, with approximately 78% cell viability, supporting their potential suitability for endodontic applications.

Introduction

Endodontic infections are frequently caused by microbial species such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus oralis*, and *Enterococcus* spp., which produce virulence factors like biofilms and toxins, complicating the outcomes of root canal treatment.[1–3] These pathogens thrive in the nutrient-rich, anaerobic environment of infected root canals, contributing to tissue destruction and the formation of periapical lesions.[4,5] A comprehensive understanding of the resilience and virulence of these microorganisms is critical for the development of targeted strategies aimed at promoting healing, preventing reinfection and improving patient outcomes.[6]

Nanotechnology offers an innovative platform to combat resistant microorganisms by employing nanoscale materials (1–100 nm) with high surface areas and enhanced reactivity.[7] Among various nanoparticles, copper nanoparticles (CuNPs) are particularly notable for their ability to release reactive ions that disrupt microbial membranes.[8,9] These properties position CuNPs as promising alternatives to conventional antibiotics, particularly in challenging biomedical applications such as endodontic therapy. Their small size and high surface-to-volume ratio facilitate effective penetration into biofilms and microbial membranes, making them highly effective antimicrobial agents.[10,11]

Historically, copper compounds have been widely used in agriculture for their bactericidal properties. However, recent advancements have focused on the application of CuNPs in the biomedical field. Coating CuNPs with biosurfactants enhances their stability and reduces cytotoxicity, broadening their potential applications.[12] Biosurfactants are amphiphilic compounds produced by microorganisms such as bacteria, fungi and yeasts, and are known for their eco-friendly, biodegradable nature and surface-active properties. These compounds reduce interfacial tension, act as natural capping agents that stabilize nanoparticles, and prevent aggregation and oxidation.[13–15] The functional groups present in biosurfactants enable controlled nanoparticle formation and facilitate effective interactions with microbial cell walls, thereby enhancing antimicrobial efficacy.[16,17]

Biosurfactants, including glycolipids such as rhamnolipids derived from *Achromobacter xylosoxidans* and *Pseudomonas aeruginosa*, exhibit excellent emulsifying properties. These characteristics allow them to form coatings on CuNPs, enhancing dispersibility, biocompatibility and antimicrobial activity while reducing cytotoxicity.[14] Given the microbial challenges associated with root canal infections, biosurfactant-coated CuNPs represent a novel, eco-friendly approach to improving treatment outcomes.

Objectives

The objective of this study was to compare the antimicrobial activity of CuNPs coated with biosurfactants derived from gram-negative endodontic bacteria. By leveraging recent advancements in biosurfactant-coated nanoparticles, this research aimed to explore their potential as effective alternatives to traditional antimicrobial agents for managing endodontic infections.

Hypotheses

The following hypothesis was formulated: Biosurfactant-coated CuNPs exhibit enhanced antimicrobial activity and reduced cytotoxicity compared to uncoated CuNPs when tested against endodontic pathogens. The null hypothesis states that biosurfactant-coated CuNPs do not significantly differ from uncoated CuNPs in terms of antimicrobial efficacy or cytotoxicity.

Material and methods

Chemicals and reagents

Copper sulfate pentahydrate and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, USA). Deionized water was used to prepare the copper sulfate solution and plant extract. Sodium hydroxide, nitric acid and sodium bicarbonate, used in the synthesis process, were procured from Qualigens Pharma Pvt. Ltd. (Khalapur, India).

Synthesis of CuNPs

Copper nanoparticles were synthesized using a chemical reduction method as described by Ahamed et al.[18] and Chowdhury et al.[19] A 0.1 M solution of copper sulfate

pentahydrate was prepared and stirred for 30 min. Then, 50 mL of 0.1 M ascorbic acid was added as a reducing agent, followed by 30 mL of 1 M sodium hydroxide for pH adjustment. The reaction was carried out at 80°C for 2 h, during which the color of the solution changed from yellow to reddish-brown, indicating the formation of CuNPs. The resulting product was left to settle for 24 h, washed with deionized water and ethanol, and stored at 4°C for further analysis.

Production of biosurfactants from microorganisms

Mineral salt medium (MSM) containing 2% glucose and adjusted to pH 7 was prepared and sterilized in a 250-mL Erlenmeyer flask. Subsequently, 5 mL of culture broth from *A. xylosoxidans* (P5C) or *P. aeruginosa* (P11), each at a cell concentration of 1×10^9 cells/mL, was inoculated into the medium, along with 2 mL of petrol. The flask was incubated on an orbital shaker at 120 rpm and 35°C for 3 days. The culture broth was then centrifuged at 15,000 rpm and 4°C for 10 min to obtain a cell-free supernatant, which was filtered through a 0.2-µm Whatman filter paper. The supernatant was acidified to pH 2 using 6 N HCl, extracted with an organic solvent, and evaporated at 45°C (Table 1). The remaining solutes were dissolved in a solvent, and the biosurfactant stock was maintained at 1 mg/mL. Fourier transform infrared spectroscopy (FTIR) and liquid chromatography–mass spectrometry (LC–MS) analyses confirmed the biosurfactant composition.[20]

Coating of copper nanoparticles with biosurfactants

The pre-synthesized CuNPs were dissolved in 10 mL of deionized water and stirred with 200 ppm biosurfactant solution. Then, 2 M nitric acid was added dropwise to adjust the pH. A 1:1 ratio of the CuNP solution to biosurfactant (P5C or P11) dispersed in 50 mL of sodium bicarbonate was prepared. The reaction mixture was incubated at 37°C for 48 h, centrifuged at 2,500 rpm for 15 min, and the resulting biosurfactant-coated CuNPs were washed with deionized water and ethanol before being stored at 4°C.[21]

Characterization of copper nanoparticles

Ultraviolet–visible spectroscopy analysis

The optical properties of the nanoparticles were analyzed using an ultraviolet–visible (UV–Vis) spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). The absorbance peaks were measured to confirm nanoparticle formation. The suspension was prepared in a suitable solvent, after which the absorbance was evaluated.[22]

Table 1. Biosurfactant extraction procedure

Solvent	Procedure
Methanol:chloroform (2:1 ratio)	An equal volume of solvent was added to the culture supernatant.
	The mixture was acidified to pH 2 using 6 N HCl, followed by extraction with an equal volume of methanol:chloroform.

Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy analysis (Nicolet iS10; Thermo Fisher Scientific, Waltham, USA) was used to identify functional groups associated with the nanoparticles and the biosurfactant coating. The minimum required quantity of samples was loaded onto the sampler.[22]

Zeta potential analysis

Zeta potential was measured using a Zetasizer Nano ZS (Malvern Panalytical, Malvern, UK) to evaluate nanoparticle stability. Deionized water was used to prepare the sample suspension, and the sample was injected into the sample holder to prevent air bubbles. Subsequently, the sample holder was retained inside the apparatus, and the zeta potential of the particles was analyzed using the electrophoretic light scattering method.[22]

Scanning electron microscopy and transmission electron microscopy analyses

Morphological characteristics were examined using scanning electron microscopy (SEM) (JSM-6390LV; JEOL Ltd., Tokyo, Japan) and transmission electron microscopy (TEM) (Tecnai G2 Spirit BioTWIN; FEI, Hillsboro, USA). Copper nanoparticles were dissolved in an appropriate solvent to prepare the sample suspension, which was then sonicated for 1 min. A sample drop was placed on the conductive substrate. Once the material had dried, the samples were analyzed using a TEM instrument set to 80–200 keV and an SEM device set to 10–20 keV.[22]

Energy-dispersive X-ray spectroscopy analysis

The elemental composition was determined using energy-dispersive X-ray spectroscopy (EDX) by depositing CuNPs on a carbon film and analyzing them under a microscope (JSM-IT300; JEOL Ltd.).[23]

Antimicrobial activity

Gram-positive pathogens, including *S. aureus*, *S. oralis* and *Enterococcus* spp., as well as gram-negative pathogens, including *E. coli*, were procured from the CSIR–National

Collection of Industrial Microorganisms (NCIM) (Pune, India) and inoculated onto nutrient agar plates at 37°C. The antimicrobial activity of CuNPs was determined against 4 different endodontic pathogens, namely *E. coli* (EC009), *S. aureus* (RC008), *S. oralis* (XaBDOO5), and *Enterococcus* spp. (XCOO4) using a 96-well plate method.[24–27] These gram-negative, rod-shaped bacteria cause pathogenic diseases in many plants. Depending on the host plant and specific strain, they can lead to leaf spots, cankers, wilting, stunting, and fruit blemishes. In the present study, the method used was the resazurin assay.[28,29]

Well diffusion method

Nutrient agar (15 mL) was poured into sterilized Petri dishes, inoculated with 100 µL of bacterial broth, and spread evenly. Wells with a diameter of 6 mm were created in the agar using a sterile borer, and 100 µL of the CuNP solution (100 µg/mL) was added. The plates were incubated at 37°C for 24 h, and the diameter of the zone of inhibition (ZOI) was measured. Streptomycin (1 mg/mL) and dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively.

Minimum inhibitory concentration analysis

The minimum inhibitory concentration (MIC) was determined using the resazurin assay in a 96-well plate. Serial dilutions of CuNPs were prepared, and 100 µL of each concentration was added to the wells, followed by 10 µL of bacterial suspension and 50 µL of nutrient broth. After incubation at 37°C for 18–24 h, MIC values were assessed by observing the color change of resazurin from blue to pink.[24,25] The percentage of inhibition was calculated using the following formula (Equation 1):

$$\text{inhibition [\%]} = [(\text{control} - \text{test})/\text{control}] \times 100 \quad (1)$$

Cytotoxicity analysis over normal human fibroblast cell lines

Normal fibroblast cells (L929) were procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco™, Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich) under standard conditions (37°C, 5% CO_2). The cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and incubated for 24 h. They were then treated with CuNPs (20, 40, 60, 80, 100 µg/mL) for 24 h. Subsequently, 10 µL of the MTT solution (5 mg/mL in phosphate-buffered saline (PBS)) was added and incubated for 3–4 h at 37°C. After removing the medium, 100 µL of DMSO was added to dissolve the formazan crystals, and the absorbance was measured at 570 nm.[30] Cell viability was calculated as follows (Equation 2):

$$\text{cell viability [\%]} = [(OD_{control} - OD_{test})/OD_{control}] \times 100 \quad (2)$$

where:
OD – optical density.

Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation ($M \pm SD$). The statistical analysis was conducted using GraphPad Prism 9.0 software (GraphPad Software, Inc., San Diego, USA). Differences between experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. A p-value <0.05 was considered statistically significant.

Results

Characterization of biosurfactants

The FTIR analysis of the biosurfactants showed characteristic peaks confirming their composition. Peaks corresponding to proteins were observed at 1,656.24 cm^{-1}, carbohydrates at 1,110.42 cm^{-1} and 1,018.53 cm^{-1}, and fatty acids at 2,832.58 cm^{-1} and 2,945.00 cm^{-1}. A broad O–H stretching peak was identified at 3,334.35 cm^{-1}, indicating hydroxyl groups. These spectra confirmed the biosurfactants produced by *A. xylosoxidans* and *P. aeruginosa*, as shown in Fig. 1 and Fig. 2.

Characterization of CuNPs (UV–Vis spectroscopy)

UV–Vis spectroscopy confirmed the formation of CuNPs, with maximum absorbance peaks observed at 222 cm^{-1} and 225 cm^{-1}. These peaks are characteristic of biosynthesized CuNPs, as presented in Fig. 3.

FTIR analysis for CuNPs

The FTIR spectra of CuNP samples revealed key functional groups associated with the nanoparticles. Peaks at 701 cm^{-1} corresponded to Cu–O stretching, confirming the presence of copper oxide. O–H stretching was observed at 3,363.40 cm^{-1}, and C≡C stretching was identified at 2,137.72 cm^{-1}. A peak at 1,643.14 cm^{-1} indicated C=O stretching vibrations, while 1,422.58 cm^{-1} corresponded to C–H bending. These findings confirm the successful synthesis and capping of CuNPs, as shown in Fig. 4.

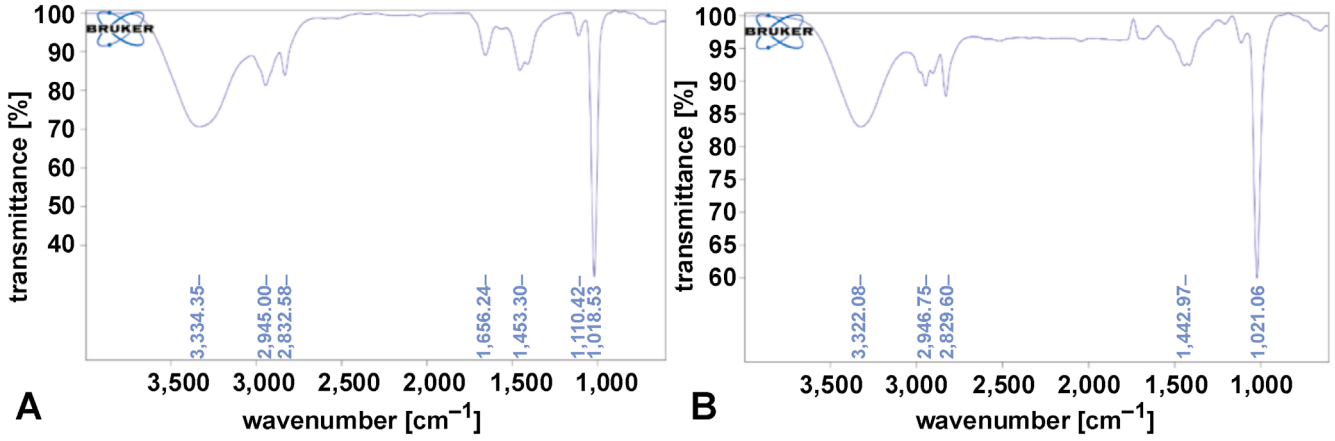

Fig. 1. Fourier transform infrared spectroscopy (FTIR) spectra of biosurfactants derived from *A. xylosoxidans* (P5C) (A) and *P. aeruginosa* (P11) (B)

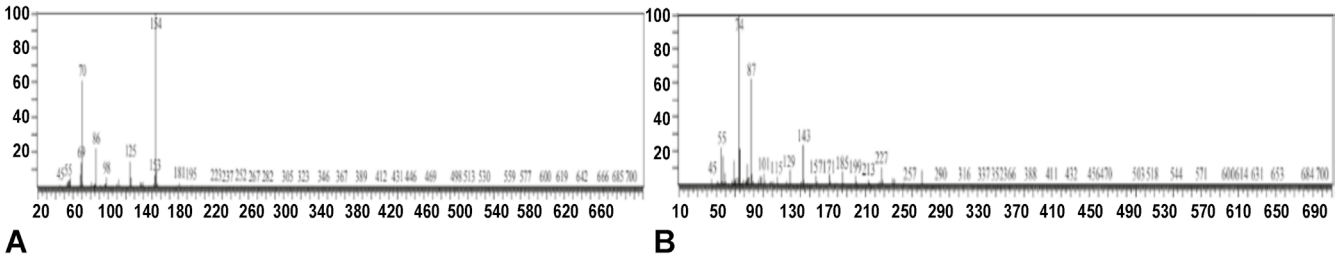

Fig. 2. Liquid chromatography–mass spectrometry (LC–MS) spectra of biosurfactants derived from *A. xylosoxidans* (P5C) (A) and *P. aeruginosa* (P11) (B)

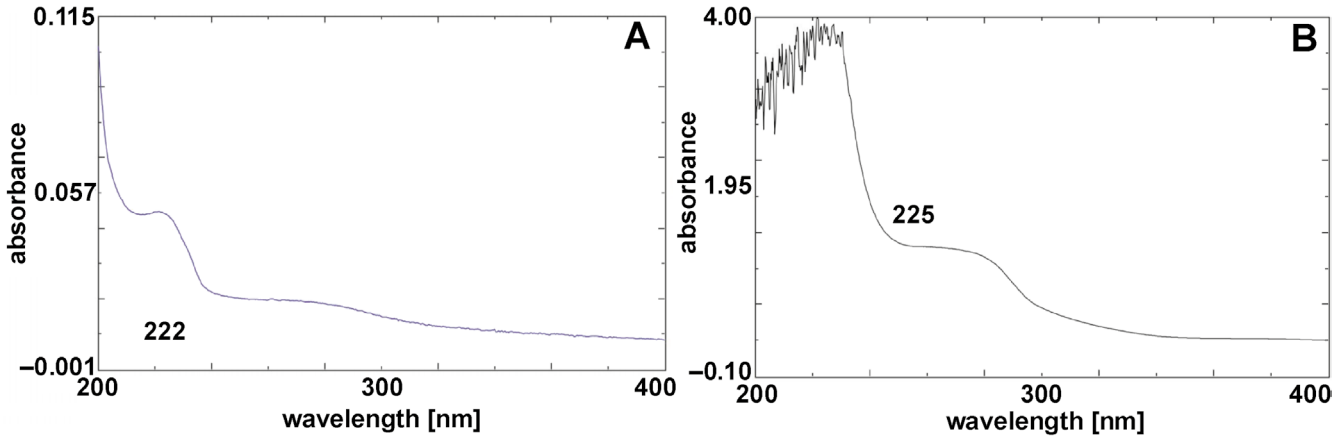

Fig. 3. Ultraviolet visible (UV-Vis) spectra of CuO+P5C (A) and CuO+P11 (B) nanoparticles (NPs)

Zeta potential analysis

Zeta potential measurements were conducted to evaluate the stability of CuNPs. The CuO+P5C nanoparticles exhibited a zeta potential of –24.1 mV, while the CuO+P11 nanoparticles showed a zeta potential of –12.1 mV, indicating moderate stability. These moderately negative charges suggest reduced toxicity and enhanced biological compatibility. Figure 5 displays zeta potential distributions for both nanoparticle types.

SEM analysis

Scanning electron microscopy revealed distinct morphological characteristics of CuNPs. The CuO+P5C nanoparticles exhibited irregular morphology with sizes ranging from 90 nm to 100 nm, whereas the CuO+P11 nanoparticles displayed more granular and spherical morphology with sizes between 85 nm and 95 nm. Full-field SEM images are presented in Fig. 6.

Morphological analysis by TEM

Transmission electron microscopy images confirmed the spherical shape and size distribution of CuNPs. The nanoparticles ranged in diameter from 90 nm to 100 nm. Figures 7 and 8 illustrate the spherical morphology and size distribution of CuNP samples.

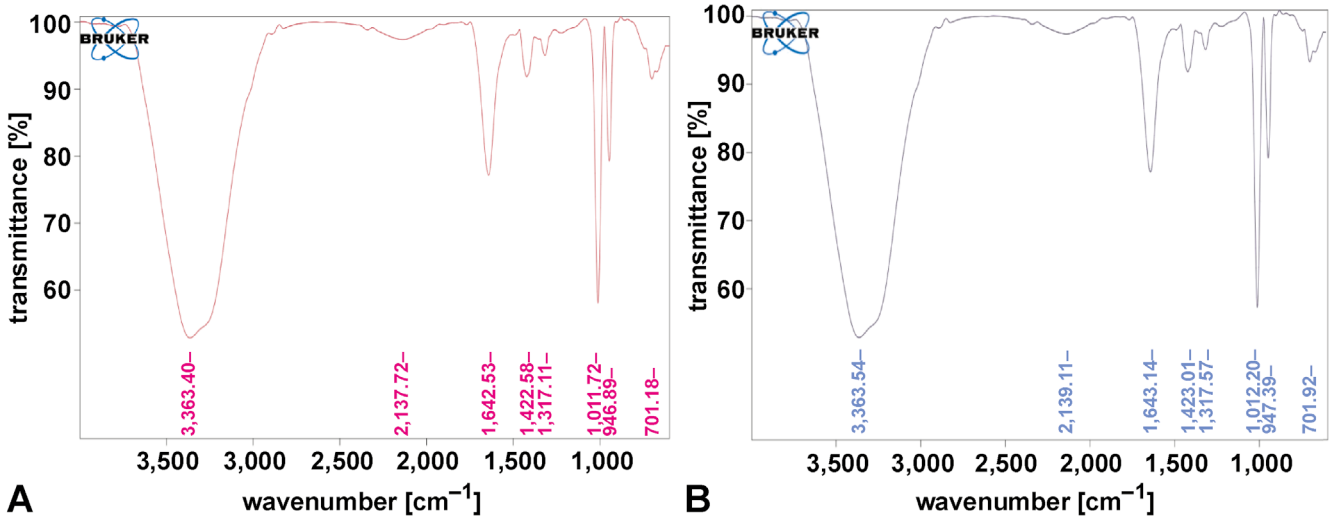

Fig. 4. Fourier transform infrared spectroscopy (FTIR) spectra of CuO+P5C (A) and CuO+P11 (B) nanoparticles (NPs)

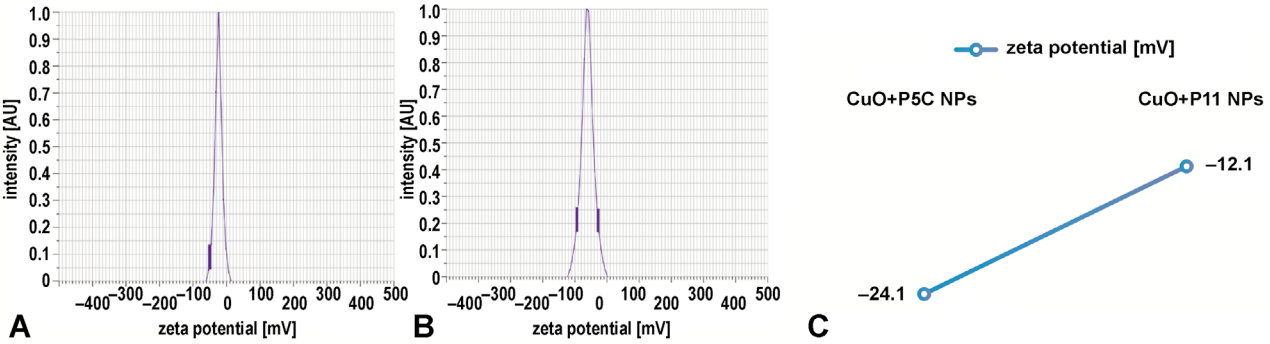

Fig. 5. Zeta potential analysis of CuO+P5C nanoparticles (NPs) (A), CuO+P11 NPs (B), and the comparative distribution (C)

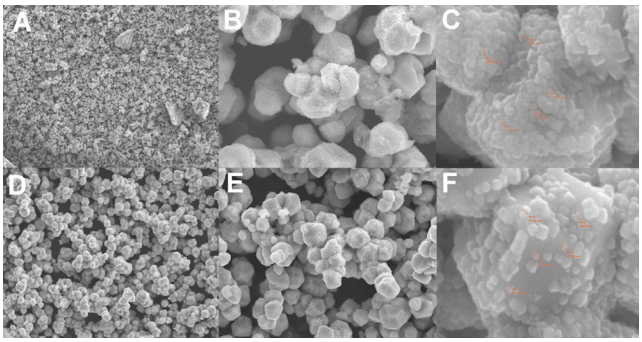

Fig. 6. Scanning electron microscopy (SEM) images of CuO+P5C and CuO+P11 nanoparticles (NPs)

A–C. Spherical CuO+P5C NPs; D–F. Spherical and granular CuO+P11 NPs.

Composition analysis by EDX

Energy-dispersive X-ray spectroscopy confirmed the elemental composition of the nanoparticles. Copper accounted for 95.51 ±3.33% of the total weight in the CuO+P5C samples and 95.44 ±3.20% in the CuO+P11 samples. Prominent peaks at 8.04 keV further validated the high copper content in the nanoparticles (Fig. 9).

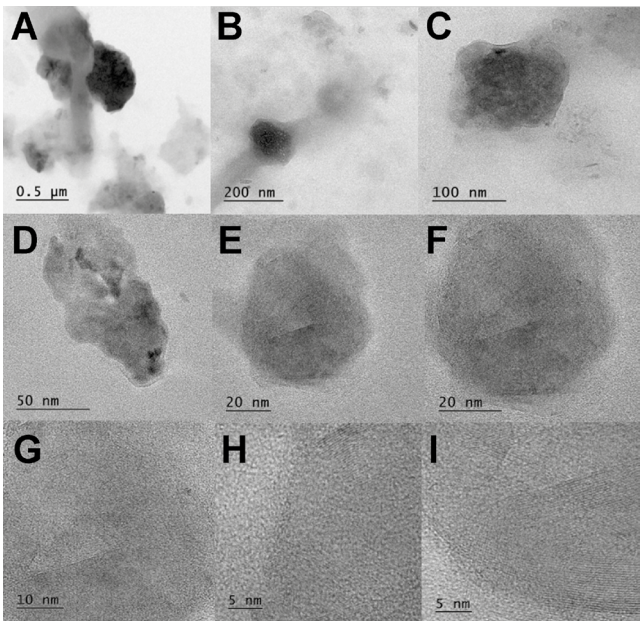

Fig. 7. Transmission electron microscopy (TEM) images of CuO+P11 nanoparticles (NPs) at different magnifications showing spherical morphology (A–F) and the particle core (G–I)

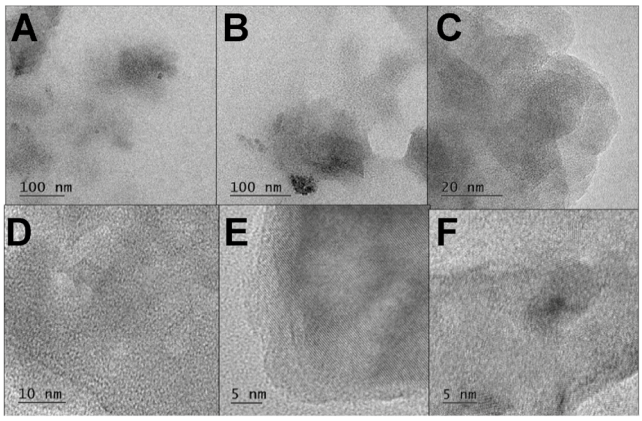

Fig. 8. Transmission electron microscopy (TEM) images of CuO+P5C nanoparticles (NPs) at different magnifications, showing spherical morphology (A–D) and particle structure (E,F)

Antimicrobial activity of CuNPs

The antimicrobial efficacy of CuNPs was assessed against *E. coli*, *S. aureus*, *S. oralis*, and *Enterococcus* spp. The ZOI results, summarized in Table 2 and Fig. 10, indicated significant activity in both the CuO+P5C and CuO+P11 samples. *Escherichia coli* exhibited the highest sensitivity to the nanoparticles. Streptomycin was used as a positive control. Biosurfactant-coated CuNPs demonstrated potent antimicrobial activity against all tested strains.

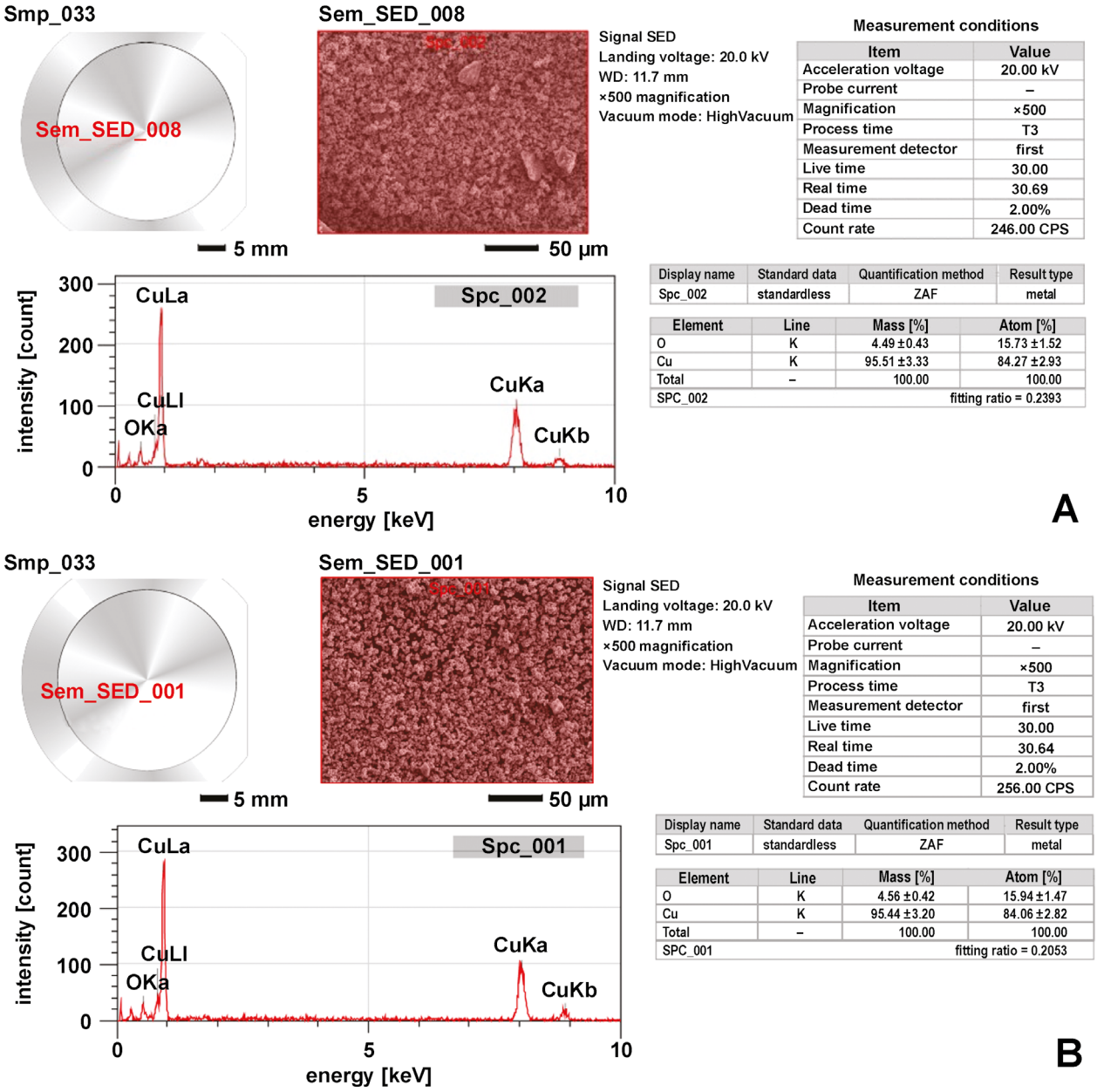

Fig. 9. Energy-dispersive X-ray spectroscopy (EDX) analysis of CuO+P5C (A) and CuO+P11 (B) nanoparticles (NPs)
SED – secondary electron detector; WD – working distance.

Table 2. Antimicrobial activity of biosurfactant-coated copper nanoparticles (CuNPs) against selected endodontic pathogens

Sample	Concentration [µg/mL]	ZOI against *E. coli*	ZOI against *S. aureus*	ZOI against *S. oralis*	ZOI against *Enterococcus* spp.
Positive control	1,000	29	29	29	29
CuO+P5C	250	21	18	16	16
	500	22	21	21	19
	1,000	24	23	23	21
CuO+P11	250	17	14	19	12
	500	23	16	22	18
	1,000	25	22	24	21

ZOI – zone of inhibition.

Minimum inhibitory concentration analysis

The MIC of both CuO+P5C and CuO+P11 nanoparticles was found to be 125 µg/mL for all tested microorganisms, as measured by the resazurin assay. The results, summarized in Table 3, confirm the strong antimicrobial potential of biosurfactant-coated CuNPs.

Cytotoxicity analysis

The cytotoxicity of CuNPs was assessed using the MTT assay on L929 fibroblast cell lines. The CuO+P5C and CuO+P11 nanoparticles exhibited good cytoprotective activity, maintaining nearly 78–80% of cell viability at concentrations up to 100 µg/mL. These results, compared to 70% ethanol as a positive control, indicate the superior biocompatibility of the nanoparticles (Fig. 11).

Table 3. Minimum inhibitory concentration (MIC) of biosurfactant-coated copper nanoparticles (CuNPs) against selected endodontic pathogens

Sample	Concentration [µg/mL]	Growth inhibition against *E. coli* [%]	Growth inhibition against *S. aureus* [%]	Growth inhibition against *S. oralis* [%]	Growth inhibition against *Enterococcus* spp. [%]
Positive control	7.8	–	–	–	–
	15.6	–	–	–	–
	31.2	11.35	11.35	11.35	11.35
	62.5	12.85	12.85	12.85	12.85
	125	47.97	47.97	47.97	47.97
	250	61.88	61.88	61.88	61.88
	500	66.87	66.87	66.87	66.87
	1,000	73.67	73.67	73.67	73.67
CuO+P5C	7.8	–	–	–	–
	15.6	–	–	–	–
	31.2	–	–	–	–
	62.5	–	–	–	–
	125	1.31	2.99	4.55	2.81
	250	40.48	41.11	40.11	42.73
	500	54.33	55.08	53.71	55.39
	1,000	59.76	59.51	59.38	60.94
CuO+P11	7.8	–	–	–	–
	15.6	–	–	–	–
	31.2	–	–	–	–
	62.5	–	–	–	–
	125	1.43	3.93	1.43	9.73
	250	41.98	42.42	40.73	48.03
	500	55.08	55.02	54.46	59.13
	1,000	60.07	61.32	59.51	64.31

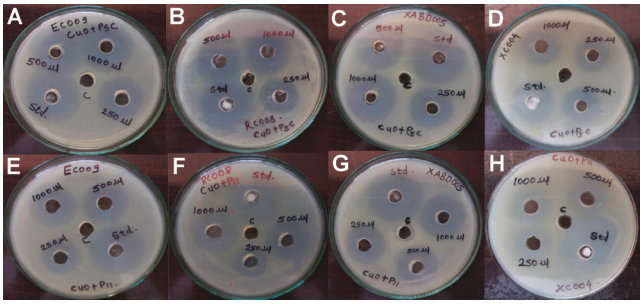

Fig. 10. Antimicrobial activity of CuO+P5C (A–D) and CuO+P11 (E–H) nanoparticles (NPs) against selected endodontic pathogens

A. *E. coli*; B. *S. aureus*; C. *S. oralis*; D. *Enterococcus* spp.

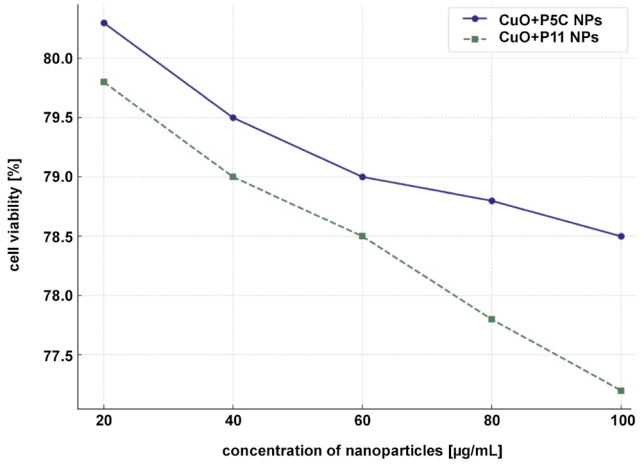

Fig. 11. Cell viability of fibroblast cell lines (L929) following treatment with copper nanoparticles (CuNPs), as determined by the MTT assay

Discussion

The present study demonstrates the successful synthesis and characterization of CuNPs stabilized with *A. xylosoxidans* (P5C) and *P. aeruginosa* (P11) biosurfactants. Fourier transform infrared spectroscopy analysis of nanoparticles provided insights into the functional groups on their surface, which are related to the coating agents used during synthesis. In the present study, the FTIR spectrum of biosurfactant-coated CuNPs revealed key functional groups associated with the biosurfactants. A broad peak at 3,363.40 cm^{-1} corresponds to O–H stretching, indicating hydroxyl groups involved in stabilization. The peak at 1,643.14 cm^{-1} is attributed to C=O stretching vibrations, likely from carbonyl groups in the biosurfactants. A peak at 701 cm^{-1} confirms Cu–O stretching, indicating the presence of copper oxide bonds. Additional peaks at 1,422.58 cm^{-1} and 2,137.72 cm^{-1} correspond to C–H bending vibrations and C≡C stretching, respectively. These findings confirm the successful coating of the nanoparticles with biosurfactants, which play a critical role in enhancing stability and preventing aggregation.

The SEM and TEM structural analyses revealed that the biosurfactant-coated CuNPs were spherical and granular, with particle sizes ranging from 90 nm to 100 nm. While both types of nanoparticles exhibited similar morphology, the granular structure of the CuO+P11 samples suggests subtle differences in stabilization properties. The zeta potential analysis revealed values of –24.1 mV (CuO+P5C) and –12.1 mV (CuO+P11), suggesting moderate stability and a slight variation in surface charge, which may influence their interaction with bacterial membranes and their cytotoxicity profiles. The size range (90–100 nm) and spherical morphology of the synthesized CuNPs align with previous studies reporting nanoparticle dimensions influenced by synthesis conditions and capping agents.[20,31] However, this study uniquely demonstrates the role of biosurfactant coating in enhancing nanoparticle stability and biocompatibility while retaining antimicrobial potency. The variations in size can be attributed to the differences in synthesis conditions, reaction medium compositions and experimental protocols. The size of CuNPs can span from a few nanometers to several hundred nanometers, illustrating the flexibility in manipulating their dimensions for diverse uses in catalysis, biomedicine and bioremediation.[32]

The UV–Vis spectrum of nanoparticles provides critical information about their surface plasmon resonance (SPR), a phenomenon arising from the collective oscillation of conduction electrons when nanoparticles interact with light at a specific wavelength. The observed absorbance peak indicates the successful formation of nanoparticles and is influenced by factors such as particle size, shape, coating, and surface modifications. In this study, the characteristic SPR peaks of biosurfactant-coated CuNPs at 222 nm and 225 nm confirm their formation. Furthermore, UV–Vis spectroscopy is a valuable tool for studying the interaction of nanoparticles with target molecules, as changes in SPR can reveal insights into nanoparticle stability, aggregation and surface interactions.[33]

The antimicrobial activity of CuNPs was evaluated against *E. coli*, *S. aureus*, *S. oralis*, and *Enterococcus* spp., which are commonly implicated in persistent endodontic infections. The CuO+P5C and CuO+P11 nanoparticles demonstrated significant activity, with a MIC value of 125 µg/mL for all bacterial strains. These results confirm that CuNPs effectively inhibit bacterial growth through metal ion release and membrane penetration mechanisms.[34,35] Notably, the highest sensitivity was observed in *E. coli*, likely due to its thinner cell wall structure compared to gram-positive bacteria.

The cytotoxicity analysis on L929 fibroblast cell lines revealed that CuO+P5C and CuO+P11 exhibited minimal toxicity at 100 µg/mL concentrations, with cell viability ranging from nearly 78% to 80%. This result indicates that biosurfactant coating significantly reduces nanoparticle toxicity while improving biocompatibility. These findings align with studies reporting that biosurfactants

provide a natural, eco-friendly capping layer that reduces nanoparticle aggregation and oxidation while enhancing stability and biological safety.[36,37]

The enhanced antimicrobial efficacy of biosurfactant-coated CuNPs can be attributed to several factors. Firstly, biosurfactant-mediated stabilization plays a critical role in preventing nanoparticle oxidation and aggregation, which enhances their stability and promotes effective interaction with microbial cell walls.[38] Secondly, the small size and high surface area of nanoparticles facilitate better penetration through bacterial membranes and enable the generation of reactive copper ions, leading to cellular damage and microbial inhibition.[39] Lastly, the moderately negative zeta potential of the nanoparticles improves their adhesion to bacterial membranes while minimizing excessive cytotoxic effects, thus ensuring a balance between antimicrobial activity and biocompatibility.[40]

The novelty of this study lies in the use of biosurfactants from *A. xylosoxidans* and *P. aeruginosa,* which have not been widely explored for nanoparticle synthesis. These findings open avenues for eco-friendly and cost-effective approaches to nanoparticle-mediated antimicrobial therapies, particularly for endodontic infections, where biofilm formation and microbial persistence pose significant treatment challenges.

Limitations and future directions

While the study demonstrated the potential of biosurfactant-coated CuNPs, several limitations should be acknowledged. The 24-hour cytotoxicity study evaluates only acute effects; thus, extended exposure studies (48–72 h) are needed to determine chronic cytotoxicity. Further research is also required to test the nanoparticles against polymicrobial biofilms commonly present in endodontic infections. In vivo studies are necessary to validate the safety and efficacy of biosurfactant-coated CuNPs for clinical applications.

Conclusions

This study successfully synthesized highly pure and stable biosurfactant-coated CuNPs using biosurfactants derived from *A. xylosoxidans* and *P. aeruginosa* as biostabilizers. Coating with biosurfactants significantly enhanced the stability, morphology and antimicrobial efficacy of the nanoparticles. Characterization analyses confirmed their spherical morphology, with particle sizes ranging from 90 nm to 100 nm. Zeta potential measurements indicated moderate stability and biocompatibility.

CuO+P5C and CuO+P11 demonstrated potent antimicrobial activity against key endodontic pathogens (*E. coli*, *S. aureus*, *S. oralis*, and *Enterococcus* spp.), achieving a MIC of 125 µg/mL. The cytotoxicity evaluation on fibroblast cell lines (L929) revealed minimal toxicity, with nearly 78–80% cell viability maintained at concentrations up to 100 µg/mL, underscoring their excellent biocompatibility.

The eco-friendly and cost-effective use of biosurfactants as stabilizers positions these nanoparticles as a promising alternative to chemical-based antimicrobials for addressing persistent endodontic infections. Future in vivo studies and extended cytotoxicity evaluations are essential to validate their clinical efficacy and ensure their safe application in medical and dental fields.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Elchaghaby MA, Rashad S, Yousry YM. Inhibitory effect of silver nanoparticles synthesized using the chamomile extract against *Streptococcus mutans* cariogenic pathogen. *Dent Med Probl*. 2023;60(3):483–488. doi:10.17219/dmp/152063
2. Lukic D, Karygianni L, Flury M, Attin T, Thurnheer T. Endodontic-like oral biofilms as models for multispecies interactions in endodontic diseases. *Microorganisms*. 2020;8(5):674. doi:10.3390/microorganisms8050674
3. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. *J Endod*. 2008;34(11):1291–1301.e3. doi:10.1016/j.joen.2008.07.028
4. Golob Deeb J, Reddy N, Kitten T, Carrico CK, Grzech-Leśniak K. Viability of bacteria associated with root caries after Nd:YAG laser application in combination with various antimicrobial agents: An in vitro study. *Dent Med Probl*. 2023;60(4):649–655. doi:10.17219/dmp/171690
5. Tomer AK, Saini N, Jain S, Sabharwal G, Guin A. Microflora responsible for failure of RCT: A review. *IJMSDR*. 2022;6(1):56–83. doi:10.32553/ijmsdr.v6i1.908
6. Suresh B, Shailaja S. Endodontic microflora – a review. *J Oral Health Comm Dent*. 2014;8(3):160–165. doi:10.5005/johcd-8-3-160
7. Kumar Y, Singh V, Pandey A, Genwa M, Meena PL. Synthesis, characterization and antibacterial activity of ZnO nanoparticles. *AIP Conf Proc*. 2020;2265(1):030119. doi:10.1063/5.0017120

8. Rojas B, Soto N, Villalba M, Bello-Toledo H, Meléndrez-Castro M, Sánchez-Sanhueza G. Antibacterial activity of copper nanoparticles (CuNPs) against a resistant calcium hydroxide multispecies endodontic biofilm. *Nanomaterials (Basel)*. 2021;11(9):2254. doi:10.3390/nano11092254
9. Varympopi A, Dimopoulou A, Papafotis D, et al. Antibacterial activity of copper nanoparticles against *Xanthomonas campestris* pv. *vesicatoria* in tomato plants. *Int J Mol Sci*. 2022;23(8):4080. doi:10.3390/ijms23084080
10. Marzban A, Mirzaei SZ, Karkhane M, Ghotekar SK, Danesh A. Biogenesis of copper nanoparticles assisted with seaweed polysaccharide with antibacterial and antibiofilm properties against methicillin-resistant *Staphylococcus aureus*. *J Drug Deliv Sci Technol*. 2022;74:103499. doi:10.1016/j.jddst.2022.103499
11. Shakib P, Seyedeh Zahra M, Zeinab S, et al. Biofabrication of copper oxide nanoparticles mediated with *Echium amoenum* petal extract for evaluation of biological functions. *Biomass Conv Bioref*. 2024;14:25651–25661. doi:10.1007/s13399-023-04796-4
12. Pourmadadi M, Holghoomi R, Shamsabadipour A, Maleki-Baladi R, Rahdar A, Pandey S. Copper nanoparticles from chemical, physical, and green synthesis to medicinal application: A review. *Plant Nano Biology*. 2024;8:100070. doi:10.1016/j.plana.2024.100070
13. Ebrahimipour G, Gilavand F, Karkhane M, Kavyanifard A, Teymouri M, Marzban A. Bioemulsification activity assessment of an indigenous strain of halotolerant Planococcus and partial characterization of produced biosurfactants. *Int J Environ Sci Technol*. 2014;11:1379–1386. doi:10.1007/s13762-014-0548-5
14. Gayathiri E, Prakash P, Karmegam N, Varjani S, Awasthi MK, Ravindran B. Biosurfactants: Potential and eco-friendly material for sustainable agriculture and environmental safety – a review. *Agronomy*. 2022;12(3):662. doi:10.3390/agronomy12030662
15. Marzban A, Ebrahimipour G, Danesh A. Bioactivity of a novel glycolipid produced by a halophilic *Buttiauxella* sp. and improving submerged fermentation using a response surface method. *Molecules*. 2016;21(10):1256. doi:10.3390/molecules21101256
16. Bezza FA, Tichapondwa SM, Chirwa EMN. Synthesis of biosurfactant stabilized silver nanoparticles, characterization and their potential application for bactericidal purposes. *J Hazard Mater*. 2020;393:122319. doi:10.1016/j.jhazmat.2020.122319
17. Silva MGC, Medeiros AO, Converti A, Almeida FCG, Sarubbo LA. Biosurfactants: Promising biomolecules for agricultural applications. *Sustainability*. 2024;16(1):449. doi:10.3390/su16010449
18. Ahamed M, Alhadlaq HA, Khan MAM, Karuppiah P, Al-Dhabi NA. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. *J Nanomater*. 2014;2014(1):637858. doi:10.1155/2014/637858
19. Chowdhury MNK, Beg MDH, Khan MR, Mina MF. Synthesis of copper nanoparticles and their antimicrobial performances in natural fibres. *Mater Lett*. 2013;98:26–29. doi:10.1016/j.matlet.2013.02.024
20. Harishchandra BD, Pappuswamy M, PU A, et al. Copper nanoparticles: A review on synthesis, characterization and applications. *Asian Pac J Cancer Biol*. 2020;5(4):201–210. doi:10.31557/apjcb.2020.5.4.201-210
21. Ingle AP, Saxena S, Moharil M, Rai M, Da Silva SS. Biosurfactants in nanotechnology: Recent advances and applications. In: Marcelino PRF, da Silva SS, Lopez AO, eds. *Biosurfactants and Sustainability: From Biorefineries Production to Versatile Applications*. John Wiley & Sons; 2023:173–194. doi:10.1002/9781119854395.ch9
22. Giri AK, Jena B, Biswal B, et al. Green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract and activity against biofilm-producing bacteria. *Sci Rep*. 2022;12(1):8383. doi:10.1038/s41598-022-12484-y
23. Mali SC, Dhaka A, Githala CK, Trivedi R. Green synthesis of copper nanoparticles using *Celastrus paniculatus* Willd. leaf extract and their photocatalytic and antifungal properties. *Biotechnol Rep*. 2020;27:e00518. doi:10.1016/j.btre.2020.e00518
24. Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman AA. Synthesis and antimicrobial activity of copper nanoparticles. *Mater Lett*. 2012;71:114–116. doi:10.1016/j.matlet.2011.12.055
25. Parikh P, Zala D, Makwana BA. Biosynthesis of copper nanoparticles and their antimicrobial activity. *Open Access Library PrePrints*. 2014;1:e067. doi:10.4236/oalib.preprints.1200067
26. Murthy HCA, Desalegn T, Kassa M, Abebe B, Assefa T. Synthesis of green copper nanoparticles using medicinal plant *Hagenia abyssinica* (Brace) *JF. Gmel*. leaf extract: Antimicrobial properties. *J Nanomater*. 2020;2020(1):3924081. doi:10.1155/2020/3924081
27. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomater*. 2008;4(3):707–716. doi:10.1016/j.actbio.2007.11.006
28. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*. 2007;42(4):321–324. doi:10.1016/j.ymeth.2007.01.006
29. Valsalam S, Agastian P, Arasu MV, et al. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus* L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. *J Photochem Photobiol B*. 2019;191:65–74. doi:10.1016/j.jphotobiol.2018.12.010
30. Na I, Kennedy DC. Size-specific copper nanoparticle cytotoxicity varies between human cell lines. *Int J Mol Sci*. 2021;22(4):1548. doi:10.3390/ijms22041548
31. Shehabeldine AM, Amin BH, Hagras FA, et al. Potential antimicrobial and antibiofilm properties of copper oxide nanoparticles: Time–kill kinetic essay and ultrastructure of pathogenic bacterial cells. *Appl Biochem Biotechnol*. 2023;195(1):467–485. doi:10.1007/s12010-022-04120-2
32. Nomoev AV, Khartaeva EC, Yumozhapova NV, et al. Receiving copper nanoparticles: Experiment and modelling. *Solid State Phenom*. 2019;288:140–147. doi:10.4028/www.scientific.net/SSP.288.140
33. Ghosh G, Panicker L. Protein–nanoparticle interactions and a new insight. *Soft Matter*. 2021;17(14):3855–3875. doi:10.1039/D0SM02050H
34. Nisar P, Ali N, Rahman L, Ali M, Shinwari ZK. Antimicrobial activities of biologically synthesized metal nanoparticles: An insight into the mechanism of action. *J Biol Inorg Chem*. 2019;24(7):929–941. doi:10.1007/s00775-019-01717-7
35. Ma X, Zhou S, Xu X, Du Q. Copper-containing nanoparticles: Mechanism of antimicrobial effect and application in dentistry – a narrative review. *Front Surg*. 2022;9:905892. doi:10.3389/fsurg.2022.905892
36. Nagpal M, Mittal A, Vinod. Application of biosurfactants in the green synthesis of inorganic nanoparticles. *BioNanoScience*. 2025;15(1):1–17. doi:10.1007/s12668-024-01628-1
37. Nitschke M, Marangon CA. Microbial surfactants in nanotechnology: Recent trends and applications. *Crit Rev Biotechnol*. 2022;42(2):294–310. doi:10.1080/07388551.2021.1933890
38. El-Baz A, El Kassas RA, Yousseria S, El Enshasy H. Biosurfactant Mediated Synthesis of Nanoparticles and their Applications. In: Sayyed RZ, El-Enshasy HA, Hameeda B, eds. *Microbial Surfactants*. Boca Raton, FL: CRC Press; 2021:287–314.
39. Ermini ML, Voliani V. Antimicrobial nano-agents: The copper age. *ACS Nano*. 2021;15(4):6008–6029. doi:10.1021/acsnano.0c10756
40. Stankic S, Suman S, Haque F, Vidic J. Pure and multi metal oxide nanoparticles: Synthesis, antibacterial and cytotoxic properties. *J Nanobiotechnology*. 2016;14(1):73. doi:10.1186/s12951-016-0225-6

Altered gene expression profile in human gingival keratinocytes treated with *Aggregatibacter actinomycetemcomitans* and their role in the development of head and neck squamous cell carcinoma

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Abstract

Background. Periodontal diseases have been associated with several systemic conditions, including certain types of cancer. Chronic inflammation, a characteristic feature of periodontal disease, has been hypothesized to contribute to carcinogenesis and tumor progression. However, the specific relationship between *Aggregatibacter actinomycetemcomitans* and cancer development remains an area of ongoing research.

Objectives. The aim of the study was to investigate the potential association between *A. actinomycetemcomitans*, a well-established periodontal pathogen, and the development of head and neck squamous cell carcinoma (HNSCC). Additionally, the objective was to examine differentially expressed genes (DEGs) in *A. actinomycetemcomitans*-infected human immortalized gingival keratinocytes (HIGKs) and compare their expression in the HNSCC dataset using computational tools.

Material and methods. The Gene Expression Omnibus (GEO) dataset GSE9723 was used to retrieve the global gene expression profile of human gingival keratinocytes exposed to *A. actinomycetemcomitans*. A computational approach was employed using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System for gene ontology analysis, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) for network analysis and UALCAN (University of ALabama at Birmingham CANcer data analysis) for gene expression and survival analyses.

Results. The GEO2R analysis identified numerous DEGs, of which the top 25 were selected for further evaluation using the HNSCC dataset from The Cancer Genome Atlas (TCGA). Among these genes, *DDIT4* and *RCE1* demonstrated expression patterns consistent with those observed in *A. actinomycetemcomitans*-infected HIGKs. Further analysis revealed that patients presenting with elevated *DDIT4* and *RCE1* expression levels demonstrated a marked reduction in overall survival.

Conclusions. The findings suggest that *DDIT4* and *RCE1* can be considered potential therapeutic targets associated with *A. actinomycetemcomitans*-mediated induction of head and neck cancers. However, further studies are required to validate these observations.

Keywords: gene expression, periodontitis, prognosis, carcinoma, health

Highlights

- GEO2R analysis identified multiple differentially expressed genes (DEGs), with the top 25 further evaluated using The Cancer Genome Atlas (TCGA) head and neck squamous cell carcinoma (HNSCC) dataset.
- *DDIT4* and *RCE1* showed expression patterns consistent with those observed in *Aggregatibacter actinomycetemcomitans*-infected human immortalized gingival keratinocytes (HIGKs).
- Elevated expression levels of *DDIT4* and *RCE1* were associated with significantly reduced overall survival in patients with HNSCC.
- *DDIT4* and *RCE1* may represent potential therapeutic targets involved in *Aggregatibacter actinomycetemcomitans*-mediated head and neck carcinogenesis, although further validation studies are required.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is among the most common types of cancer worldwide, the majority of which are squamous cell carcinomas. It has an annual incidence exceeding 550,000 cases and is responsible for approx. 300,000 deaths each year.[1] In India, oral squamous cell carcinoma (OSCC), a predominant subtype of HNSCC, accounts for 30% of all cancer cases. Patients diagnosed with HNSCC at an early clinical stage exhibit a survival rate of 70–90%; however, 50% of patients diagnosed at advanced stages pass away within 2 years of the initial diagnosis. One of the major clinical challenges associated with HNSCC is the lack of early screening and diagnostic procedures.[2] The oral microbiome harbors diverse forms of microorganisms that adapt to different physiological and pathological conditions.[3] Dysbiosis of the oral microbiota may lead to mild forms of illness, manifesting as cavities, or more serious forms, contributing to changes in normal cells. Head and neck squamous cell carcinoma has been associated with several microorganisms capable of altering host gene expression, including *Haemophilus influenzae*, *Prevotella copri* and *Aggregatibacter actinomycetemcomitans*. They are commensals of the oral microbiota. However, their balance may be disrupted due to habitual practices, systemic diseases and poor oral hygiene, among others.[4] *Aggregatibacter actinomycetemcomitans* is a Gram-negative, facultatively anaerobic, capnophilic bacillus recognized as a major cause of periodontal disease in children and adolescents. It has also been associated with the development of head and neck cancer.[5] Individuals with periodontitis demonstrate an increased risk of extraoral *A. actinomycetemcomitans* infections. Nevertheless, colonies of *A. actinomycetemcomitans* have also been observed in patients with good oral health and in edentulous individuals due to the organism's ability to adhere to epithelial cells and tooth surfaces.[6] Furthermore, *Aggregatibacter actinomycetemcomitans* has been reported to enhance bacterial internalization via phagocytosis while simultaneously limiting excessive inflammatory responses by downregulating interleukin (IL)-1 expression and reactive oxygen species (ROS) generation in macrophages.[7]

Teshima et al. investigated several periodontal pathogens, including *Aggregatibacter actinomycetemcomitans* Y4, a microaerophilic organism isolated primarily from subgingival dental plaque.[8,9] It was identified for its ability to induce double-strand breaks (DSBs) in the DNA. Notably, the accumulation of DSBs persisted even after the inhibition of apoptosis, revealing an independent mechanism. These findings suggest a possible association between certain periodontal pathogens, genome instability and an elevated risk of carcinogenesis, thereby highlighting the intricate interplay between periodontal health and oral cancer susceptibility.[8,9] Although only limited evidence is currently available regarding the role of bacterial pathogens in carcinogenesis, researchers have ventured into the exploration of bacteria-mediated induction of tumorigenesis. Computational approaches have been used for several decades to reveal the role of gene families,[10] gene networks[11] and epigenetic components[12] in the development of cancer. The present study aimed to examine the possible association between *A. actinomycetemcomitans* and the initiation of head and neck tumors. The study is supported by data generated with the use of computational approaches.

Material and methods

Sample dataset

The Gene Expression Omnibus (GEO) dataset GSE9723 was used in the present study. The dataset comprised 4 samples with *A. actinomycetemcomitans*-infected human immortalized gingival keratinocytes (HIGKs) (GSM245733, GSM245734, GSM245735, GSM245736) and 4 Sham-infected HIGK samples (GSM245729, GSM245730, GSM245731, GSM245732).[13] The Sham-infected HIGKs served as controls. The HIGKs infected with *A. actinomycetemcomitans* were considered the test group (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9723). An adjusted p-value <0.05 was deemed statistically significant. As the analysis yielded an exhaustive list of differentially expressed genes (DEGs), only the top 25 DEGs were selected for further analysis (Table 1, Fig. 1).

Table 1. Top 25 differentially expressed genes (DEGs) identified in the GSE9723 dataset (Sham-infected human immortalized gingival keratinocytes (HIGKs) vs. *A. actinomycetemcomitans*-infected HIGKs)

Gene	Protein encoded	Adjusted *p*-value	logFC
FNDC3A	fibronectin type III domain-containing 3A	0.002	–3.3616190
DDIT4	DNA damage inducible transcript 4	0.002	2.0923394
PPP1R10	protein phosphatase 1 regulatory subunit 10	0.002	–3.6486112
MLXIPL	MLX-interacting protein-like	0.002	2.5103228
TNFAIP3	TNF alpha induced protein 3	0.002	2.4146422
PPP1R3C	protein phosphatase 1 regulatory subunit 3C	0.002	2.1541815
PRDM12	PR/SET domain 12	0.002	1.6067603
ADM	adrenomedullin	0.002	2.1584096
NUAK2	NUAK family kinase 2	0.002	1.7663268
HES1	hes family bHLH transcription factor 1	0.005	2.3627212
BTN3A3	butyrophilin subfamily 3 member A3	0.005	3.0071925
BHLHE40	bHLH family member e40	0.005	1.6711381
TCF3	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	0.005	4.7351758
HILPDA	hypoxia inducible lipid droplet-associated	0.005	1.9366609
PMEPA1	prostate transmembrane protein, androgen induced 1	0.007	–1.4403405
EIF5	eukaryotic translation initiation factor 5	0.007	–1.2266054
RNF19A	ring finger protein 19A, RBR E3 ubiquitin protein ligase	0.007	–2.7650566
NFIL3	nuclear factor, IL-3 regulated	0.007	1.5161582
INHBC	inhibin beta C subunit	0.007	1.6599916
HCAR3	hydroxycarboxylic acid receptor 3	0.007	1.5312788
HK2	hexokinase 2	0.007	1.5176530
MOGAT2	monoacylglycerol O-acyltransferase 2	0.007	2.6203199
LUC7L3	LUC7 like 3 pre-mRNA splicing factor	0.007	–1.1864130
C10orf10	chromosome 10 open reading frame 10	0.007	1.6163247
RCE1	Ras converting CAAX endopeptidase 1	0.007	1.9778521

MLX – Max-like protein X; TNF – tumor necrosis factor; bHLH – basic helix-loop-helix; IL – interleukin; FC – fold change.

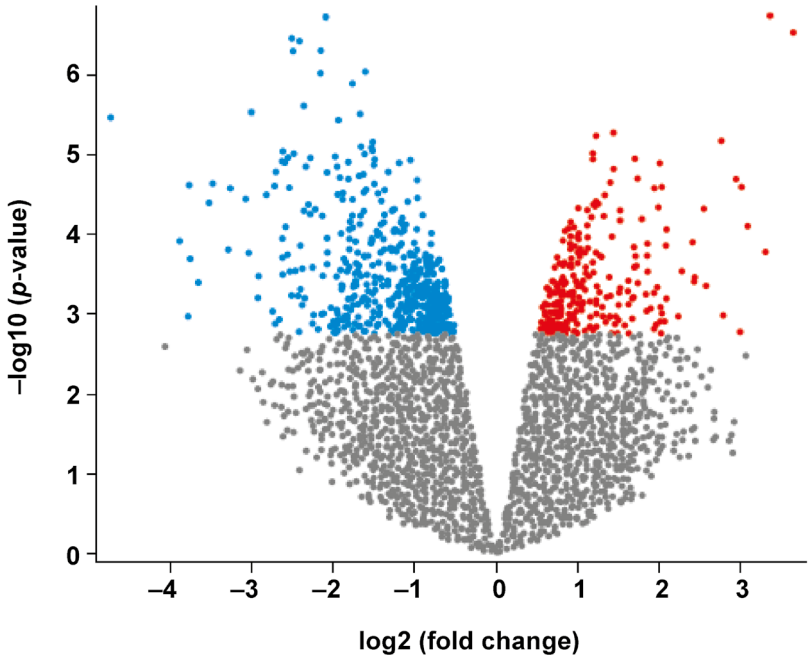

Fig. 1. Volcano plot illustrating upregulated (red) and downregulated (blue) differentially expressed genes (DEGs) in *Aggregatibacter actinomycetemcomitans*-infected human immortalized gingival keratinocytes (HIGKs)

Gene expression and survival analysis

The expression levels of the top 25 DEGs retrieved from the previous analysis were investigated using the UALCAN database (http://ualcan.path.uab.edu). The analysis included 520 primary tumor HNSCC samples and 44 normal tissue samples. Gene expression levels were measured in transcripts per million (TPM), a standardized unit for RNA sequencing (RNA-seq) data normalization. Significant differences between the groups were determined using box-and-whisker plots generated from the TPM values. The Kaplan–Meier analysis was used to demonstrate overall survival of patients with HNSCC. The high-expression and low/medium-expression groups were compared to assess the effect of changes in gene expression on the overall survival of patients.[14]

Gene ontology analysis

The gene ontology analysis was performed using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System, v. 16.0 (https://pantherdb.org). The analysis provided information regarding molecular pathways and functions, biological processes and subcellular localization of the identified genes. A user-defined query of the top 25 genes was submitted as a batch for functional classification of genes. Additionally, classification based on pathways was conducted to identify potential pathways associated with these genes.[15,16]

Protein–protein interaction analysis

The protein–protein interactions between the top 25 DEGs were analyzed using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) tool, v. 10.0. STRING is a bioinformatics resource that provides information on protein–protein interactions, including direct physical interactions and functional associations. The nodes represent proteins interacting in a specific network, whereas edges indicate protein–protein interactions, including physical, enzymatic and genetic interactions.[17]

Statistical analysis

Gene expression data from multiple datasets was analyzed using the GEO2R tool. GEO2R uses the DESeq2 and Limma packages from the R programming environment to compare groups of samples within a GEO series and identify DEGs. The results are presented in the form of a table and graphic plots. The UALCAN portal was used to analyze gene expression profiles by comparing expression levels between different groups via a Perl script accompanied by a Comprehensive Perl Archive Network (CPAN) module. Survival plots were generated with the use of the survival and survminer packages in R. Differences between the groups were assessed using the log-rank test. The survival package was exclusively used for survival analysis, including the generation of survival curves, hypothesis testing and survival modeling. The use of the survminer package improved the visualization of Kaplan–Meier plots and forest plots, allowing for clearer representation and interpretation of complex survival data.[14]

Results

GEO analysis

Affymetrix-based microarray analysis revealed numerous DEGs. These DEGs constituted a group of genes exclusively activated or repressed upon exposure to *A. actinomycetemcomitans*. The volcano plot demonstrated numerous upregulated (red) and downregulated (blue) DEGs in the HIGKs infected with *A. actinomycetemcomitans* (Fig. 1). Among the identified DEGs, the top 25 genes were curated based on adjusted *p*-values (Table 1). This subset included 19 upregulated genes and 6 downregulated genes, which were subsequently probed in the HNSCC dataset derived from The Cancer Genome Atlas (TCGA).

Gene expression and survival analysis

The top 25 genes exhibiting differential gene expression in *A. actinomycetemcomitans*-infected HIGKs were investigated for their expression profiles in the HNSCC dataset using the UALCAN platform. Genes with similar expression patterns in *A. actinomycetemcomitans*-infected cells and the HNSCC dataset were selected for further analysis (Table 2). Among the analyzed genes, *DDIT4* and *RCE1* demonstrated similar expression patterns in *A. actinomycetemcomitans*-infected HIGKs and the HNSCC dataset (Fig. 2,3). Both *DDIT4* ($p < 0.001$) and *RCE1* ($p < 0.001$) were significantly upregulated in the HNSCC patients and positively correlated with reduced survival. In both cases, increased expression led to poor prognosis in patients (*DDIT4*: $p = 0.0018$; *RCE1*: $p = 0.021$).

Gene ontology analysis

The gene enrichment analysis of the top 25 DEGs provided insight into the pathways associated with these genes. Although the DEGs did not form clusters, individual genes were correlated with 10 different pathways, of which the Cadherin, Notch, transforming growth factor beta (TGF-β), Toll receptor, and Wnt signaling pathways were linked to carcinogenesis (Fig. 4).

Protein–protein interaction analysis

The protein–protein interaction analysis revealed findings similar to those obtained from the gene ontology analysis. Five genes, namely *HES1* (hairy and enhancer

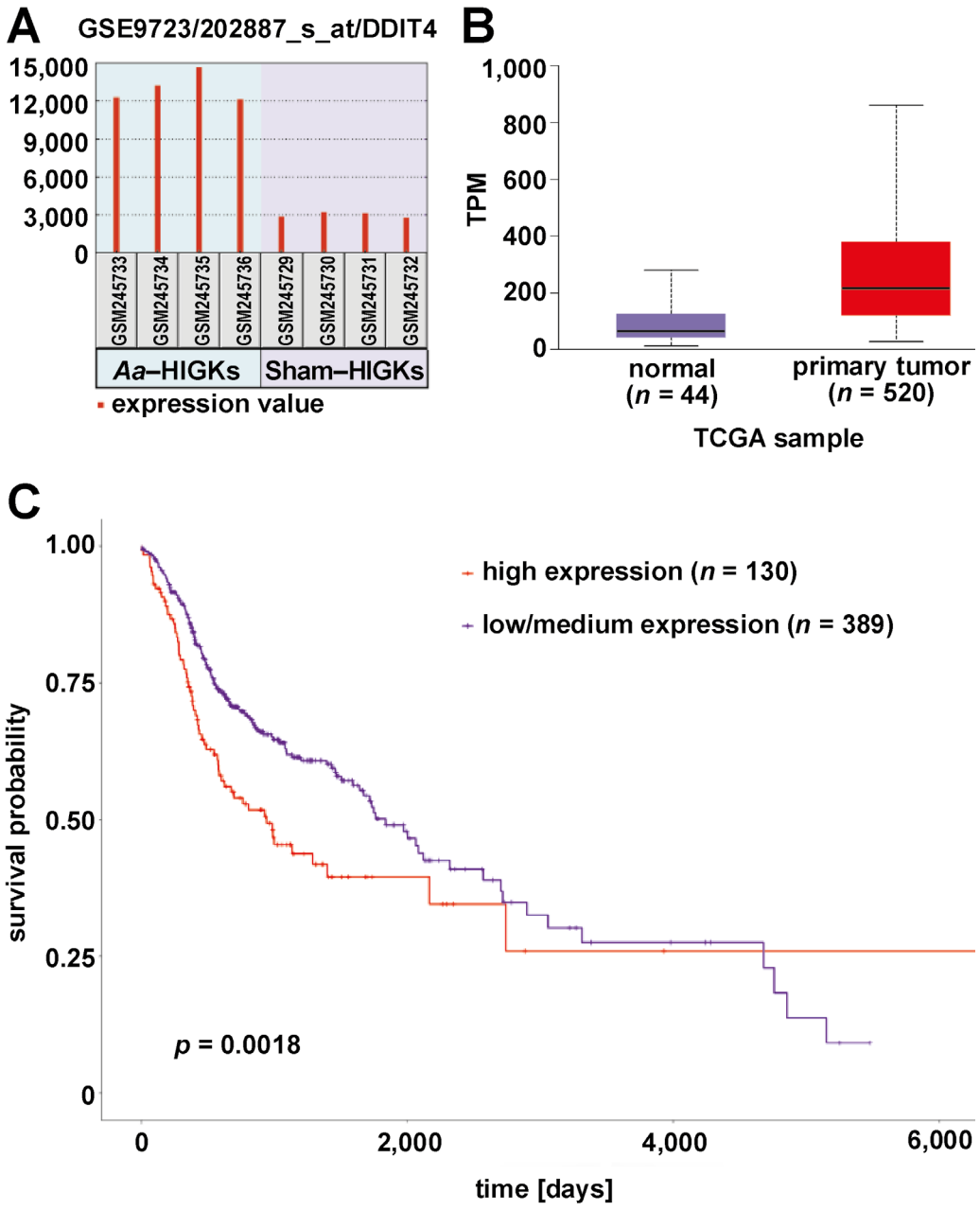

Fig. 2. Expression profile of *DDIT4*

A. Histogram illustrating *DDIT4* expression in *A. actinomycetemcomitans*-infected HIGKs; B. Box-and-whisker plot showing *DDIT4* expression profile in the head and neck squamous cell carcinoma (HNSCC) dataset. Comparison between normal tissues and primary HNSCC tumor tissues revealed significant upregulation of transcript levels in the test group ($p < 0.001$); C. Kaplan–Meier plot demonstrating survival probability in HNSCC patients according to different expression profiles. A statistically significant difference in survival was observed ($p = 0.0018$), and elevated *DDIT4* expression was associated with poor prognosis.

Aa – Aggregatibacter actinomycetemcomitans; TPM – transcripts per million; TCGA – The Cancer Genome Atlas.

of split 1), *TCF3* (transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)), *BHLHE40* (basic helix-loop-helix family member e40), *NFIL3* (nuclear factor, IL-3 regulated), and *MLXIPL* (MLX-interacting protein-like) formed an interaction cluster. The remaining genes appeared as independent units. The text mining process identified an interaction between *BHLHE40* and *MLXIPL*, and the other 3 genes showed experimentally determined interactions (Fig. 5).

Discussion

The microbiome, defined as the collection of microorganisms inhabiting the human body, plays a crucial role in maintaining good health. However, dysbiotic shifts in the microbiome have been linked to several diseases, including autoimmune, neurodegenerative and metabolic diseases such as cancer. Interestingly, the microbiome also presents promising opportunities for the identification

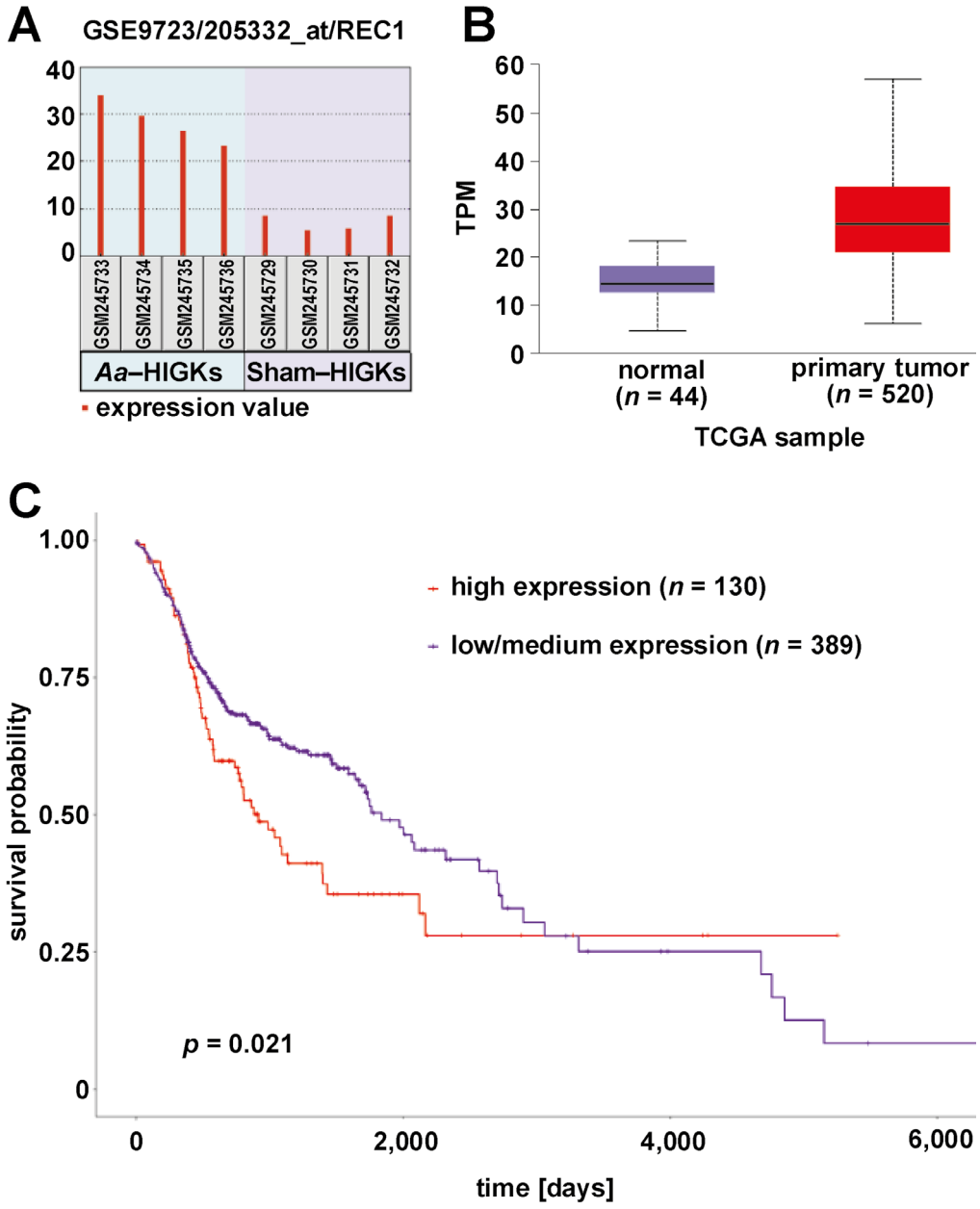

Fig. 3. Expression profile of *RCE1*

A. Histogram illustrating *RCE1* expression in *A. actinomycetemcomitans*-infected HIGKs; B. Box-and-whisker plot showing *RCE1* expression profile in the HNSCC dataset. Comparison between normal tissues and primary HNSCC tumor tissues revealed significant upregulation of transcript levels in the test group ($p < 0.001$); C. Kaplan–Meier plot demonstrating survival probability in HNSCC patients according to different expression profiles. A statistically significant difference in survival was observed ($p = 0.021$), and elevated *RCE1* expression was associated with poor prognosis.

of microbiome-associated markers that may facilitate the early detection of individuals at high risk of disease development. Recent studies have highlighted the role of the oral microbiome in cancer development, with certain oral bacteria, such as *Tannerella forsythia* and *Porphyromonas gingivalis*, playing important roles. By identifying significant differences in the oral microbiome of cancer patients, researchers may improve strategies for early identification of high-risk individuals, improved prevention and treatment.[18] A meta-analysis was conducted to systematically assess the impact of periodontal bacterial infections on cancer incidence and prognosis. A total of 39 studies involving 7,184 participants were included in the analysis. The study results indicated a positive association between periodontal bacterial infections and cancer incidence.[19] A study by Handfield et al. evaluated the gene expression profile in HIGKs co-infected with *P. gingivalis* and *A. actinomycetemcomitans*.[13] The gene ontology analysis revealed approx. 55 apoptosis-associated genes, among which *TP53* emerged as the major node of the network.[13] This scenario mimics the oral environment in which the cells have undergone some dysplastic

Table 2. Expression profile of differentially expressed genes (DEGs) in *A. actinomycetemcomitans*-infected human immortalized gingival keratinocytes (HIGKs) and the head and neck squamous cell carcinoma (HNSCC) dataset (The Cancer Genome Atlas (TCGA))

Gene	Gene expression	Gene expression in HNSCC	*p*-value	Survival (*p*-value)
FNDC3A	downregulated	insignificant	0.090	0.87
DDIT4#	upregulated	upregulated	<0.001*	0.002*
PPP1R10	downregulated	upregulated	<0.001*	0.26
MLXIPL	upregulated	downregulated	0.012*	0.15
TNFAIP3	upregulated	upregulated	<0.001*	0.43
PPP1R3C	upregulated	downregulated	<0.001*	0.34
PRDM12	upregulated	upregulated	<0.001*	0.78
ADM	upregulated	upregulated	<0.001*	0.42
NUAK2	upregulated	downregulated	0.019*	0.59
HES1	upregulated	insignificant	0.914	0.56
BTN3A3	upregulated	upregulated	<0.001*	0.67
BHLHE40	upregulated	downregulated	0.030*	0.98
TCF3	upregulated	upregulated	<0.001*	0.27
HILPDA/C7orf68	upregulated	upregulated	<0.001*	0.10
PMEPA1	downregulated	upregulated	<0.001*	0.015*
EIF5	downregulated	upregulated	<0.001*	0.62
RNF19A	downregulated	upregulated	<0.001*	0.029*
NFIL3	upregulated	upregulated	0.017*	0.47
INHBC	upregulated	upregulated	<0.001*	0.066
HCAR3/GPR109B	upregulated	upregulated	0.046*	0.75
HK2	upregulated	upregulated	0.046*	0.75
MOGAT2	upregulated	downregulated	0.190	0.092
LUC7L3	downregulated	upregulated	<0.001*	0.96
C10orf10	upregulated	downregulated	0.063	0.56
RCE1#	upregulated	upregulated	<0.001*	0.021*

genes demonstrating similar differential expression profiles; * statistically significant ($p < 0.05$, Welch's test).

changes, and the exposure of such cells to bacteria or T cells regulates key pathways associated with carcinogenesis. In this context, the HIGK cell line transfected with the *HPV16 E6/E7* gene may serve as an excellent model for studying the effects of bacterial exposure on transformed cells. Notably, the majority of oropharyngeal cancer cases are associated with *HPV16*.

Recent studies have investigated the influence of oral pathogens on the development and diversity of oral tumors. In one study, oral cancer cells were exposed to different pathogens to better understand this topic.[20] The researchers treated the cells with human defensins, which are antimicrobial peptides of the innate immune system, and subsequently analyzed cell proliferation, the expression of oncogenic relevant defensin genes, and epidermal growth factor receptor (EGFR) signaling after stimulation. The results showed that exposure to *P. gingivalis* in combination with human α-defensins increased cell proliferation, while exposure to *A. actinomycetemcomitans* led to cell death. However, the outcomes of both pathogens on oncogenic relevant defensin gene expression and EGFR signaling differed. Despite the differences in primary proliferation, both pathogens demonstrated opposite effects on tumor cell proliferation. The study also hypothesized that the antimicrobial peptides could serve as EGFR-binding ligands, thereby regulating the behavior of oral tumor cells.[20] A study of 361 pancreatic adenocarcinoma patients and 371 controls demonstrated that oral pathogens (*P. gingivalis* and *A. actinomycetemcomitans*) were associated with an increased risk of pancreatic cancer. At the same time, the phylum Fusobacteria and the genus *Leptotrichia* were linked to a lower risk of disease development. These findings further support the potential role of oral microbiota in the development of pancreatic cancer.[21]

A comprehensive review by Ungureanu et al. highlighted the complex relationship between periodontal disease and pancreatic cancer.[22] Periodontal infections and the associated chronic inflammation have been linked to the development of various systemic conditions, including pancreatic cancer. Recent studies have found that poor oral health, a common manifestation of periodontitis, may increase the risk of pancreatic cancer by 50%, representing a potential risk factor for disease development. Certain

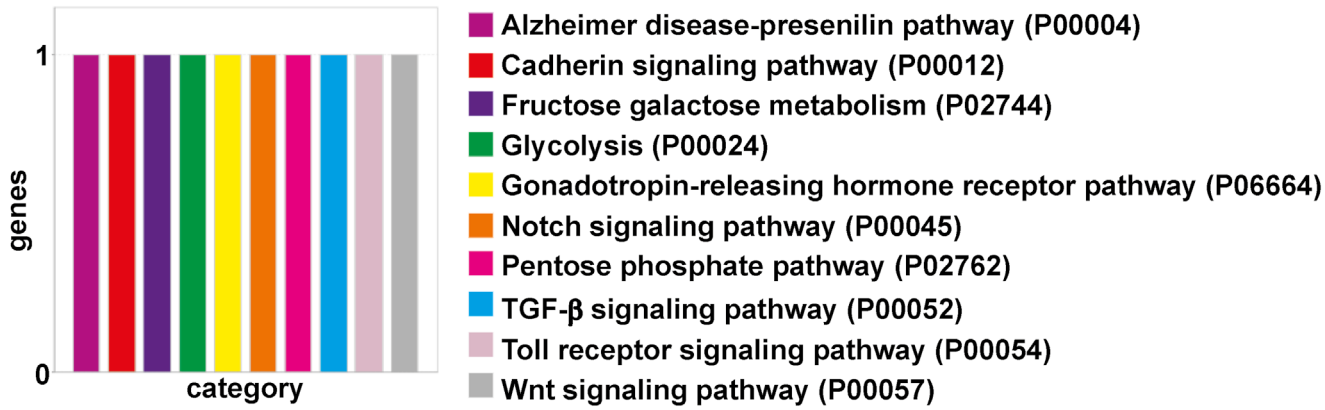

Fig. 4. Molecular pathways associated with the differentially expressed genes (DEGs) identified through gene ontology analysis using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System

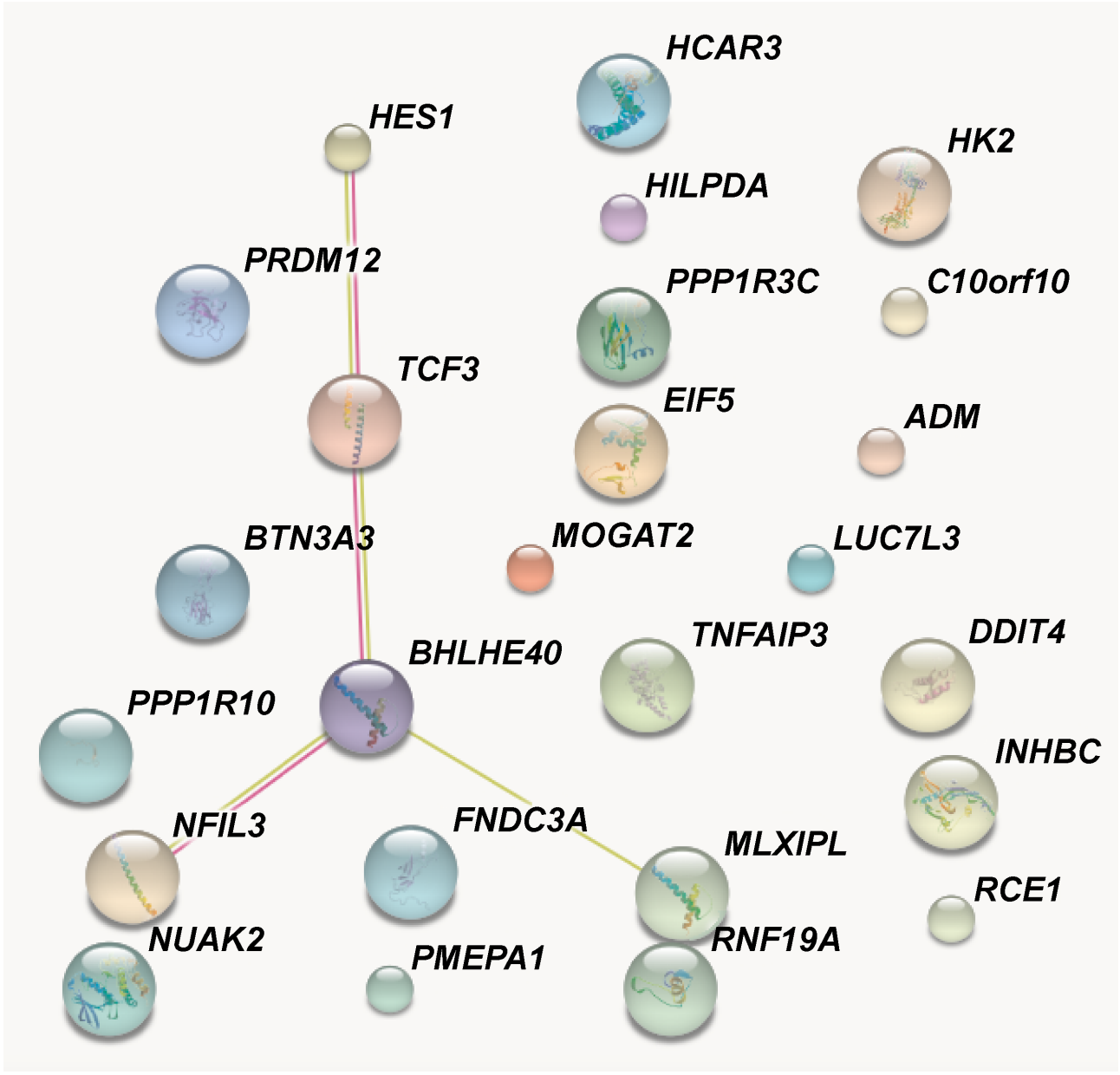

Fig. 5. Protein–protein interaction analysis of differentially expressed genes (DEGs) identified in *A. actinomycetemcomitans*-infected human immortalized gingival keratinocytes (HIGKs)

Green lines indicate interactions established through text mining approach, whereas pink lines represent experimentally determined interactions.

oral bacteria, including *P. gingivalis* and *A. actinomycetemcomitans*, may elevate the risk of pancreatic cancer, while other beneficial microorganisms, such as *Leptotrichia* and Fusobacteria, may decrease the risk of the disease. Researchers have observed changes in the oral cavity, gut and pancreatic tissue microbiomes in patients with pancreatic cancer. These observations underscore the importance of maintaining oral health and implementing appropriate periodontal treatment to reduce the incidence of pancreatic cancer.[22] Reports from various experimental studies confirm the association between periodontal pathogens and various forms of cancer.

The present study is the first to correlate an array of DEGs identified in *A. actinomycetemcomitans*-infected HIGKs with the expression pattern in primary tumor samples of HNSCC patients. Among numerous DEGs, the top 25 genes were selected for further investigation. Two of these, namely *DDIT4* and *RCE1*, were significantly upregulated in both datasets. The overexpression of these genes in the HNSCC dataset resulted in a poor prognosis, based on the observed *p*-values (Fig. 2C, Fig. 3C). A study conducted by Tajik et al. aimed to investigate the function of DDIT4 in pancreatic tumors and its clinical significance.[23] The authors demonstrated elevated expression of DDIT4 in tumor samples, which was directly proportional to the tumor grade and lymphovascular invasion. Immunohistochemical analysis confirmed high DDIT4 protein levels in pancreatic tumors. Nuclear expression was linked to aggressive features, whereas membranous expression was associated with less aggressive behavior in pancreatic ductal adenocarcinoma (PDAC). Although no correlation was found between DDIT4 and patient survival, DDIT4 appeared to be highly sensitive as a diagnostic marker.[23]

Epithelial–mesenchymal transition (EMT) plays a critical role in oral cancer progression. During EMT, epithelial cells acquire mesenchymal characteristics, promoting invasion and metastasis.[24] An experimental study aimed to investigate the role of *DDIT4* in cervical cancer metastasis. *DDIT4* is a gene that is induced by hypoxia. It is associated with poor prognosis and lymph node metastasis in patients with early-stage cervical cancer. In vitro experiments demonstrated that the reduction in *DDIT4* levels inhibited cell migration and invasion, which was linked to reduced expression of EMT-related proteins and decreased activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. In addition, *DDIT4* was found to promote tumor progression in a mouse model. These results suggest that *DDIT4* may serve as a prognostic indicator and promote metastasis in cervical cancer through the EMT and NF-kB pathways.[25] The protein–protein interaction and gene ontology analyses conducted in the present study revealed potential connections with the EMT, inflammatory networks and unique pathways related to tumorigenesis, such as Wnt, Notch and TGF-β signaling pathways.

A recent study has shed light on the importance of *DDIT4* in the phenotype of triple-negative breast cancer (TNBC). Using RNA-seq and data mining, the authors identified the overexpression of *DDIT4*, which has been linked to poor prognosis, resistance to neoadjuvant chemotherapy, and a negative impact on the immune microenvironment. Further analysis of *DDIT4* and its hub genes (*ADM*, *ENO1*, *PLOD1*, *CEBPB*) showed associations with apoptosis, cell cycle and EMT pathways, indicating the potential of *DDIT4* as a prognostic biomarker and a therapeutic target for improving TNBC treatment strategies.[26] These reports are consistent with the observations of the present study and provide further evidence supporting the role of *DDIT4* in the development of head and neck cancers.

The other gene identified in the present study, *RCE1*, encodes the Ras converting CAAX endopeptidase 1, a crucial end-proteolytic enzyme involved in cellular processes, including carcinogenesis. An earlier study investigated the expression of *RCE1* in colorectal carcinoma (CRC) tissues and evaluated its prognostic significance. Immunohistochemical analysis of 244 CRC specimens revealed reduced *RCE1* expression in tumor tissues compared to adjacent non-tumor tissues. Lower *RCE1* expression independently predicted poor overall and disease-free survival. Mechanistically, *RCE1* influenced tumor invasion through the p38 pathway, and *RCE1* knockdown enhanced the invasiveness of CRC cells. These findings suggested that *RCE1* functions as a tumor suppressor in CRC, impacting invasion and predicting unfavorable patient outcomes.[27] Another investigation involving 216 hepatocellular carcinoma (HCC) tissues, 216 adjacent non-tumorous tissues and 20 normal liver tissues revealed decreased *RCE1* expression in HCC, which correlated with early recurrence markers and low alpha-fetoprotein levels. Survival analysis identified reduced *RCE1* expression as a predictor of poor outcomes. Functional studies demonstrated the role of *RCE1* in suppressing HCC proliferation, migration and invasion. In contrast, in vivo studies confirmed its impact on tumor growth and metastasis via the P38 signaling pathway and EMT induction.[28] Although these studies established the tumor-suppressive function of *RCE1*, several other publications portrayed the oncogenic functions of *RCE1* in other malignancies. A study evaluating RCE1 mRNA and protein expression in prostate cancer (PC) and benign prostatic hyperplasia (BPH) tissues revealed markedly higher levels of the gene. The expression of *RCE1* in PC was correlated with clinicopathologic features, including Gleason score, T class and distant metastasis. Immunohistochemical analysis detected *RCE1* in 93.24% of PC tissues, and higher *RCE1* levels were associated with substantially shorter survival time. Therefore, *RCE1* has been established as an independent prognostic biomarker for PC. Similar findings were observed in the present study, emphasizing the need for in-depth investigation of the molecular mechanisms

involving *RCE1* in head and neck cancer.[29] The mechanisms through which *A. actinomycetemcomitans* induces the expression of these genes remain to be elucidated. In recent studies, epigenetic mechanisms have been linked to the stages of carcinogenesis.[30] The pan-genomic modifications in the DNA, such as methylation, histone modification and non-coding RNA-mediated regulation of gene expression, are key drivers of cancer-related pathways. Further investigation of the epigenetic components is warranted to better understand the host–pathogen interactions contributing to disease progression.

Limitations

The present study has several limitations in addition to its strengths. First, the study design was based primarily on computational analyses. Therefore, the findings require validation using experimental approaches and in vivo models. Second, gene expression profiles are influenced by numerous factors, including exposure to carcinogens, lifestyle habits, infectious diseases, inflammatory conditions, age, sex, tumor stage, and ethnic background. Consequently, controlled experimental settings are necessary to establish a definitive association between microbial pathogens and cancer development. Third, although the study demonstrated the relationship between candidate gene expression and patient survival, epigenetic mechanisms may exert additional effects on the prognosis of HNSCC patients, which should be explored further. Finally, the cell line used in the present investigation was an immortalized HIGK line. Although a previous study has reported that HIGKs behave like primary gingival epithelial cells (GECs) under experimental conditions,[13] it is worthwhile to replicate these findings using normal fibroblasts to determine potential deviations in gene expression profiles. This experimental design could eliminate the possibility of differential expression due to transfection with viral components that ensure the immortalization of cells.

Conclusions

Microbial dysbiosis and dysplastic changes in host tissues may contribute to the malignant transformation of cells. Although not an independent factor for cancer development, microbial components may directly damage genetic material or indirectly activate several crucial pathways, leading to carcinogenesis. Recent advancements in oral and gut microgenomics have created new opportunities for improving the outcomes of patients with cancer. By integrating the 2 fields, researchers hope to gain a deeper understanding of the complex interactions between the oral and gut microbiota, paving the way for the development of effective therapeutic strategies for cardiovascular, cerebrovascular, metabolic, inflammatory, and autoimmune conditions. The present study is the first of its kind to provide insight into the putative genetic components related to *A. actinomycetemcomitans*-mediated development of HNSCC.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Prabhash K, Babu G, Chaturvedi P, et al. Indian clinical practice consensus guidelines for the management of squamous cell carcinoma of head and neck. *Indian J Cancer*. 2020;57(Supplement):S1–S5. doi:10.4103/0019-509X.278971
3. Girija ASS, Ganesh PS. Functional biomes beyond the bacteriome in the oral ecosystem. *Jpn Dent Sci Rev*. 2022;58:217–226. doi:10.1016/j.jdsr.2022.05.002
4. Panda M, Rai AK, Rahman T, et al. Alterations of the salivary microbial community associated with oropharyngeal and hypopharyngeal squamous cell carcinoma patients. *Arch Microbiol*. 2020;202(4):785–805. doi:10.1007/s00203-019-01790-1
5. Yang HW, Asikainen S, Doğan B, Suda R, Lai CH. Relationship of *Actinobacillus actinomycetemcomitans* serotypes b to aggressive periodontitis: Frequency in pure cultured isolates. *J Periodontol*. 2004;75(4):592–599. doi:10.1902/jop.2004.75.4.592
6. Turner E, Hastie T, Sundaresan PD. Pulmonary *Aggregatibacter actinomycetemcomitans* infection masquerades as malignancy in a patient with periodontitis. *BMJ Case Rep*. 2021;14(2):e239636. doi:10.1136/bcr-2020-239636
7. Lee HA, Park MH, Song Y, Na HS, Chung J. Role of *Aggregatibacter actinomycetemcomitans*-induced autophagy in inflammatory response. *J Periodontol*. 2020;91(12):1682–1693. doi:10.1002/JPER.19-0639
8. Teshima R. Investigation of DNA double-strand breaks induced in host cells following infection with genotoxic bacteria. *Methods Mol Biol*. 2020;2119:111–122. doi:10.1007/978-1-0716-0323-9_10
9. Teshima R, Hanada K, Akada J, Kawano K, Yamaoka Y. *Aggregatibacter actinomycetemcomitans* infection causes DNA double-strand breaks in host cells. *Genes Cells*. 2018;23(4):264–273. doi:10.1111/gtc.12570

10. Aditya J, Smiline Girija AS, Paramasivam A, Priyadharsini JV. Genetic alterations in Wnt family of genes and their putative association with head and neck squamous cell carcinoma. *Genomics Inform*. 2021;19(1):e5. doi:10.5808/gi.20065
11. Jaikumarr Ram A, Girija As S, Jayaseelan VP, et al. Overexpression of BASP1 indicates a poor prognosis in head and neck squamous cell carcinoma. *Asian Pac J Cancer Prev*. 2020;21(11):3435–3439. doi:10.31557/APJCP.2020.21.11.3435
12. Jayaseelan VP, Arumugam P. Exosomal microRNAs targeting *TP53* gene as promising prognostic markers for head and neck squamous cell carcinoma. *Glob Med Genet*. 2022;9(4):277–286. doi:10.1055/s-0042-1758204
13. Handfield M, Mans JJ, Zheng G, et al. Distinct transcriptional profiles characterize oral epithelium–microbiota interactions. *Cell Microbiol*. 2005;7(6):811–823. doi:10.1111/j.1462-5822.2005.00513.x
14. Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: An update to the integrated cancer data analysis platform. *Neoplasia*. 2022;25:18–27. doi:10.1016/j.neo.2022.01.001
15. Mi H, Thomas P. PANTHER pathway: An ontology-based pathway database coupled with data analysis tools. *Methods Mol Biol*. 2009;563:123–140. doi:10.1007/978-1-60761-175-2_7
16. Mi H, Ebert D, Muruganujan A, et al. PANTHER version 16: A revised family classification, tree-based classification tool, enhancer regions and extensive API. *Nucleic Acids Res*. 2021;49(D1):D394–D403. doi:10.1093/nar/gkaa1106
17. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607–D613. doi:10.1093/nar/gky1131
18. Muszyński D, Kudra A, Sobocki BK, et al. Esophageal cancer and bacterial part of gut microbiota – A multidisciplinary point of view. *Front Cell Infect Microbiol*. 2022;12:1057668. doi:10.3389/fcimb.2022.1057668
19. Xiao L, Zhang Q, Peng Y, Wang D, Liu Y. The effect of periodontal bacteria infection on incidence and prognosis of cancer: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2020;99(15):e19698. doi:10.1097/MD.0000000000019698
20. Hoppe T, Kraus D, Novak N, et al. Oral pathogens change proliferation properties of oral tumour cells by affecting gene expression of human defensins. *Tumour Biol*. 2016;37(10):13789–13798. doi:10.1007/s13277-016-5281-x
21. Fan X, Alekseyenko AV, Wu J, et al. Human oral microbiome and prospective risk for pancreatic cancer: A population-based nested case–control study. *Gut*. 2018;67(1):120–127. doi:10.1136/gutjnl-2016-312580
22. Ungureanu BS, Gheorghe DN, Nicolae FM, et al. Could there be an interplay between periodontal changes and pancreatic malignancies? *World J Clin Cases*. 2023;11(3):545–555. doi:10.12998/wjcc.v11.i3.545
23. Tajik F, Fattahi F, Rezagholizadeh F, et al. Nuclear overexpression of DNA damage-inducible transcript 4 (DDIT4) is associated with aggressive tumour behaviour in patients with pancreatic tumours. *Sci Rep*. 2023;13(1):19403. doi:10.1038/s41598-023-46484-3
24. Krisanaprakornkit S, Iamaroon A. Epithelial–mesenchymal transition in oral squamous cell carcinoma. *ISRN Oncol*. 2012;2012:681469. doi:10.5402/2012/681469
25. Lin X, Yoshikawa N, Liu W, et al. DDIT4 facilitates lymph node metastasis via the activation of NF-κB pathway and epithelial–mesenchymal transition. *Reprod Sci*. 2023;30(9):2829–2841. doi:10.1007/s43032-023-01230-y
26. Chen X, Li Z, Liang M, et al. Identification of DDIT4 as a potential prognostic marker associated with chemotherapeutic and immunotherapeutic response in triple-negative breast cancer. *World J Surg Oncol*. 2023;21(1):194. doi:10.1186/s12957-023-03078-7
27. Shi B, Zhou X, He L, Liang M, Luo Y, Jiang P. Reduced RCE1 expression predicts poor prognosis of colorectal carcinoma. *BMC Cancer*. 2017;17(1):414. doi:10.1186/s12885-017-3393-3
28. Tu M, Wang X, Chen P, et al. RCE1 deficiency enhances invasion via the promotion of epithelial–mesenchymal transition and predicts poor prognosis in hepatocellular carcinoma. *Am J Transl Res*. 2020;12(11):7236–7248. PMID:33312363.
29. Huang L, Li M, Wang D, et al. Overexpressed Rce1 is positively correlated with tumour progression and predicts poor prognosis in prostate cancer. *Hum Pathol*. 2016;47(1):109–114. doi:10.1016/j.humpath.2015.08.021
30. Paramasivam A, George R, Priyadharsini JV. Genomic and transcriptomic alterations in m6A regulatory genes are associated with tumorigenesis and poor prognosis in head and neck squamous cell carcinoma. *Am J Cancer Res*. 2021;11(7):3688–3697. PMID:34354868.

Original paper

Biological evaluation of gelatin-based hemostatic agents – cytocompatibility and irritation assessment

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. The effective management of hemostasis is essential in surgical practice. Topical hemostatic agents become indispensable when conventional methods are insufficient or impractical. However, the wide range of commercially available products with similar compositional bases presents clinicians with numerous choices and potentially variable performance profiles. Notably, to the best of the authors' knowledge, no previous study has comprehensively compared the biological functionality of these commercially available materials. Addressing this knowledge gap is essential for improving the precision and effectiveness of hemostatic interventions, ultimately contributing to better patient outcomes and advancing healthcare practice.

Objectives. This study aimed to conduct a comprehensive evaluation of the biological response to clinically available gelatin-based hemostatic agents, namely Hemospon®, Clinix®, Gelatamp®, and Octocolagen®. The evaluation encompassed in vitro studies to characterize the response of human fibroblasts upon exposure, together with an in vivo irritation assessment using the hen's egg test – chorioallantoic membrane (HET-CAM) assay, thereby providing a comprehensive understanding of their biological effects.

Material and methods. Human fibroblasts were cultured and exposed to the leachables extracted from the hemostatic agents, prepared according to the ISO 10993-12:2021 guidelines, at concentrations of 50%, 25% and 12.5%. The cultures were characterized in terms of cellular viability (the live/dead assay and flow cytometry) and metabolic activity at multiple time points. In addition, irritation potential was evaluated using the HET-CAM assay.

Results. Data analysis revealed that the fibroblast cultures exposed to the leachables from the 4 hemostatic sponges proliferated actively throughout the culture period, with minimal evidence of cell death or significant impairment of metabolic activity. Furthermore, no irritant effects were observed in the HET-CAM assay.

Conclusions. Overall, despite minor variations in the biological response, the comprehensive analysis supports the overall biocompatibility of commercially available gelatin-based hemostatic sponges. These findings advance the current understanding of the biological safety of these materials, further reinforcing their clinical suitability and highlighting their potential to improve surgical healing outcomes.

Keywords: cytotoxicity, hemostatics, gelatin sponges, biological assays, chorioallantoic membrane

Highlights

- Four commercially available gelatin-based hemostatic sponges – Hemospon®, Clinix®, Gelatamp®, and Octocolagen® – showed an overall favorable biological profile.
- Human fibroblasts exposed to sponge leachables maintained high viability, with no significant cytotoxic response detected by live/dead staining or flow cytometry.
- Metabolic activity was preserved over 7 days, with some materials showing increased activity at later time points, supporting sustained fibroblast functionality.
- None of the tested sponges induced irritation in the HET-CAM assay, with all materials receiving a score of 0, comparable to the negative control.
- Despite minor material-dependent differences, the findings reinforce the cytocompatibility, non-irritant behavior and clinical suitability of gelatin-based hemostatic agents.

Introduction

The management of hemostasis remains a critical concern in surgical practice. The challenges associated with bleeding complications are further amplified in patients with altered hemostasis resulting from the underlying pathological conditions or ongoing pharmacological therapies. Beyond compromising surgical precision, such complications may impair wound healing and ultimately pose serious, potentially life-threatening risks.[1] In this context, topical hemostatic agents assume a pivotal role. These agents are particularly valuable in situations where conventional methods, including pressure, electrocautery and suture ligatures, are insufficient or impractical.[2] By enhancing and accelerating the coagulation process, topical hemostatic agents provide an effective adjunctive intervention that not only minimizes adverse effects, but also promotes rapid and efficient hemostasis, representing a significant advancement in the pursuit of optimal surgical outcomes.[3]

Topical hemostatic agents are broadly classified into active, non-active, and hybrid categories, each characterized by distinct mechanisms of action, efficacy, safety profiles, and formulations.[4] Active hemostatic agents, such as those containing fibrinogen and thrombin, act directly within the coagulation cascade, and are therefore classified as adhesive hemostatics because of their ability to seal tissues.[2,5] Non-active hemostatic agents, including collagen, gelatin and regenerated oxidized cellulose, are regarded as passive hemostatic agents. These materials mechanically promote platelet adhesion, aggregation and activation, ultimately leading to the formation of a stable clot.[2,5] A major advantage of mechanical agents is their simplicity and immediate availability for use directly from the package, without the need for specialized storage conditions or pre-application preparation.[6] This characteristic makes them particularly suitable for the management of minor bleeding events.[7] Hybrid hemostatic agents combine the properties of both active and non-active materials; examples include formulations that integrate thrombin with mechanical hemostatic matrices. Commonly referred to as flowable hemostatics, these agents provide versatile options for hemostasis management.[2,8,9]

Within the category of non-active hemostatic agents, a variety of products have historically been employed, including bone wax and cellulose-, collagen-, and gelatin-based materials. Bone wax, primarily used to mechanically occlude vascular channels in bone, effectively controls bleeding, but does not actively participate in the hemostatic process. Moreover, its insoluble and non-resorbable nature is associated with several drawbacks, including an increased risk of inflammation and infection.[10–12] Oxidized cellulose products, another important class of non-active agents, present limitations related to the low-pH microenvironment they generate, which may inactivate biologically active hemostatic agents such as thrombin. Consequently, their combined use with other agents is restricted, while their limited resorbability often necessitates post-surgical removal.[13,14] Microfibrillar collagen, although effective, demonstrates relatively high immunogenicity due to its xenogeneic origin, and is also more costly than its denatured derivative, gelatin, which is characterized by lower immunogenic potential.[15,16]

Against this background, gelatin-based hemostatic agents have emerged as a preferred option because of their favorable biological and handling properties. These agents are derived from denatured xenogeneic collagen through processes that disrupt its native triple-helical structure into single strands by means of thermal denaturation or physical and chemical degradation.[17] Gelatin-based materials exhibit remarkable hygroscopicity, enabling them to absorb large quantities of blood and other fluids, and to expand up to 200% of their original volume.[7,8] In addition, gelatin matrices are associated with minimal tissue reaction, undergo disintegration within approx. 1 week in the oral environment and are completely resorbed within 4–6 weeks, thereby offering a highly favorable profile for postoperative healing and recovery.[18,19]

Despite the widespread use of gelatin-based hemostatic agents, a significant knowledge gap persists, particularly concerning their comparative efficacy. The large number of commercially available products sharing similar compositional foundations provides clinicians with a broad range of options that may nevertheless differ in perfor-

mance characteristics.[20] A clear understanding of the clinical effectiveness, advantages and limitations of hemostatic products derived from the same material is essential to support appropriate clinical selection and use, ultimately contributing to improved patient outcomes.[21] Addressing this knowledge gap is therefore crucial for enhancing the precision and overall effectiveness of hemostatic interventions in surgical practice.

Therefore, the present study aimed to provide a comprehensive evaluation of the biological activity of clinically available gelatin-based hemostatic agents. Through in vitro investigations focused on the functional activity of human fibroblasts, together with an in vivo irritation assessment using the hen's egg test – chorioallantoic membrane (HET-CAM) assay, this work seeks to generate valuable insights into the biological responses elicited by these materials. Such findings are fundamental for supporting informed clinical decision making, and may contribute significantly to the advancement and optimization of hemostatic agents in surgical practice.

Material and methods

In the present study, gelatin-based hemostatic sponges from 4 different manufacturers were evaluated, namely Hemospon® (Technew, Rio de Janeiro, Brazil), Clinix® (Biomaterials, Pisa, Italy), Gelatamp® (Roeke, Langenau, Germany), and Octocolagen® (Laboratorios Clarben, Madrid, Spain). The leachables obtained from these materials were employed for the in vitro assays, whereas the sponges themselves were directly applied in the in vivo irritation assay.

Preparation of leachables from hemostatic sponges

The leachables were prepared from the hemostatic sponges in accordance with the ISO 10993-12:2021 guidelines.[22] Briefly, the sponges were immersed in alpha-Minimum Essential Medium (α-MEM; Gibco, Waltham, USA) and incubated for 24 h at 37°C under constant agitation (100 rpm). Following incubation, the leachables were collected and stored at –20°C until further use.

Establishment of cell cultures

Primary human gingival fibroblasts (AG09319; Coriell Institute for Medical Research, Camden, USA) were cultured in a growth medium consisting of α-MEM supplemented with 10% fetal bovine serum (FBS), 100 UI/mL penicillin, 100 UI/mL streptomycin, and 2.5 µg/mL amphotericin B (all from Gibco). The cell cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2 until reaching approx. 70% confluence. The culture medium was renewed twice weekly. At the selected confluence, the cells were washed twice with phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, USA), and subsequently incubated with trypsin solution (0.05% trypsin in 0.25% ethylenediaminetetraacetic acid (EDTA); Sigma-Aldrich) for 5 min at 37°C to promote cell detachment. Trypsin was then neutralized, and the resulting cell suspension was centrifuged at 1,200 rpm for 10 min. The obtained cell pellet was resuspended in the growth medium, and the cells were seeded at a density of 10^4 cells/cm^2 under the previously described culture conditions. After 24 h of incubation, the culture medium was replaced with the hemostatic sponge leachables diluted in α-MEM to final concentrations of 50%, 25% and 12.5%. The cells cultured in the growth medium alone, without hemostatic leachables, served as the negative control.

Analysis of cell viability by the live/dead assay

Cell viability was evaluated using the live/dead viability assay, following 24 h of exposure to the hemostatic sponge leachables. Briefly, cells were washed twice with PBS and incubated for 10 min at 37°C, protected from light, in a solution containing 2 µL/mL calcein AM (BioLegend, San Diego, USA) and 50 µL/mL propidium iodide (PI; BD Biosciences, Franklin Lakes, USA). Fluorescence images of viable and non-viable cells were immediately acquired using the CELENA® S digital imaging system (Logos Biosystems, Gunpo, South Korea) equipped with green (488 nm) and red (594 nm) filters. All experiments were performed in quintuplicate.

Five randomly selected images from each experimental condition were analyzed using ImageJ, v. 1.8 (National Institutes of Health – NIH) to quantitatively determine live and dead cell events. Channel separation was performed to isolate the fluorescence signals corresponding to viable (green) and non-viable (red) cells. Subsequently, image thresholding was applied using the MaxEntropy method, and the images were converted into a binary format. The "Analyze Particles" function was then used to detect, segment and quantify cellular events.

The percentage of cell death was calculated by dividing the number of events detected in the dead cell channel by the total number of events identified in both channels, and multiplying the result by 100.

Assessment of cell viability by flow cytometry

Cell viability was further evaluated by flow cytometry, following 24 h of exposure to the hemostatic sponge leachables. Briefly, the cells were stained using the Annexin V-FITC (fluorescein isothiocyanate) apoptosis detection kit (640914; BioLegend), according to the manufacturer's instructions, and subsequently analyzed by flow cytometry using the FACSCalibur™ cytometer (BD Biosciences).

During early apoptosis, caspase activation and other proteolytic events lead to cytoskeletal cleavage and the loss of membrane rigidity, enabling the translocation of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, where it is detected by Annexin V (Annexin V-FITC$^+$/PI$^-$; early apoptotic cells). In later stages, the loss of membrane integrity results in impaired exclusion of PI, corresponding to Annexin V-FITC$^+$/PI$^-$ populations indicative of late apoptosis and/or necrosis. Viable cells remain unstained (Annexin V-FITC$^+$/PI$^-$).

Data acquisition and analysis were performed using FlowJo, v. 10.9 (BD Biosciences). All experiments were conducted in quintuplicate.

Evaluation of metabolic activity

Metabolic activity was quantitatively assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on days 3 and 7 of culture, based on the reduction of MTT to a purple formazan product by metabolically active cells. Briefly, at each time point, the cells were incubated for 3 h at 37°C in a humidified atmosphere containing 5% CO_2 in air, with 5 mg/mL MTT stock solution (Sigma-Aldrich). Following incubation, the resulting formazan crystals were solubilized in 100 µL of dimethyl sulfoxide (DMSO; Panreac, Barcelona, Spain), and absorbance was measured at 550 nm using the Synergy HT microplate reader (BioTek, Winooski, USA). All experiments were performed in quintuplicate.

In vivo assessment of the irritation potential – HET-CAM assay

The irritation potential of the hemostatic sponges was evaluated directly using the in vivo HET-CAM assay, in accordance with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) guidelines.[23] Briefly, freshly laid fertilized chicken eggs were incubated at 37°C under 60% humidity in the Octagon Advance incubator (Brinsea, Sommerset, UK), with automatic rotation set at 1-hour intervals. On day 8 post-fertilization, a small window was created in the eggshell under aseptic conditions, and a fragment of each hemostatic sponge (1 mm^3) was carefully placed onto the CAM of the chicken embryo. The chorioallantoic membrane was subsequently examined by visual inspection and documented using the Stemi 305 stereomicroscope equipped with the Axiocam 208 camera (Zeiss, Oberkochen, Germany), at contact times of 0.5, 2 and 5 min.

Irritation was assessed based on the presence of lysis, hemorrhage or coagulation. A scoring system according to the Luepke method,[24] ranging from 0 (no reaction) to 21 (strong reaction), was used for irritation grading. A 0.9% NaCl solution served as the negative control, while 0.5 M NaOH was used as the positive control, in accordance with the ICCVAM recommendations. All experiments were performed in quintuplicate.

Statistical analysis

All quantitative data is presented as mean ± standard deviation (M ±SD), unless otherwise specified. Statistical analyses were performed using the GraphPad Prism 8 software (GraphPad, Boston, USA), employing the one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. A p-value <0.05 was considered statistically significant.

Results

Analysis of cell viability by the live/dead assay

Cell viability following exposure to different concentrations (50%, 25% and 12.5%) of hemostatic sponge leachables was evaluated by live/dead double staining after a 24-hour incubation period. Under control conditions, the cultures exhibited a predominance of viable cells, as indicated by the extensive green fluorescence, with only sporadic dead cells observed throughout the culture. In comparison, the cultures exposed to the leachables demonstrated a higher frequency of cell death events. Nevertheless, despite this trend, all experimental groups maintained high levels of cell viability, comparable to those observed in the controls (Fig. 1).

Analysis of cell viability by flow cytometry

Complementing the live/dead assay, cell viability was further evaluated by flow cytometry to distinguish viable cells from apoptotic and necrotic populations. The control cultures exhibited a high viability of approx. 99%. Similarly, the cultures exposed to the sponge leachables also maintained high viability levels, ranging from 94% to 98%, with no significant differences relative to the controls. Nevertheless, the cultures exposed to Clinix (95.00% viable cells, 1.07% early apoptotic cells, 2.64% late apoptotic cells, and 1.30% necrotic cells) and Gelatamp (93.80% viable cells, 0.59% early apoptotic cells, 2.17% late apoptotic cells, and 3.48% necrotic cells) demonstrated comparatively higher proportions of late apoptotic and necrotic cells than the cultures exposed to Hemospon (98.20% viable cells, 0.20% early apoptotic cells, 0.56% late apoptotic cells, and 1.02% necrotic cells) and Octocolagen (97.00% viable cells, 0.40% early apoptotic cells, 1.30% late apoptotic cells, and 1.26% necrotic cells) (Fig. 2).

Analysis of metabolic activity

The metabolic activity of the cultures exposed to the hemostatic sponge leachables was assessed using the MTT assay. Under control conditions, the cells demonstrated a progressive increase in metabolic activity from

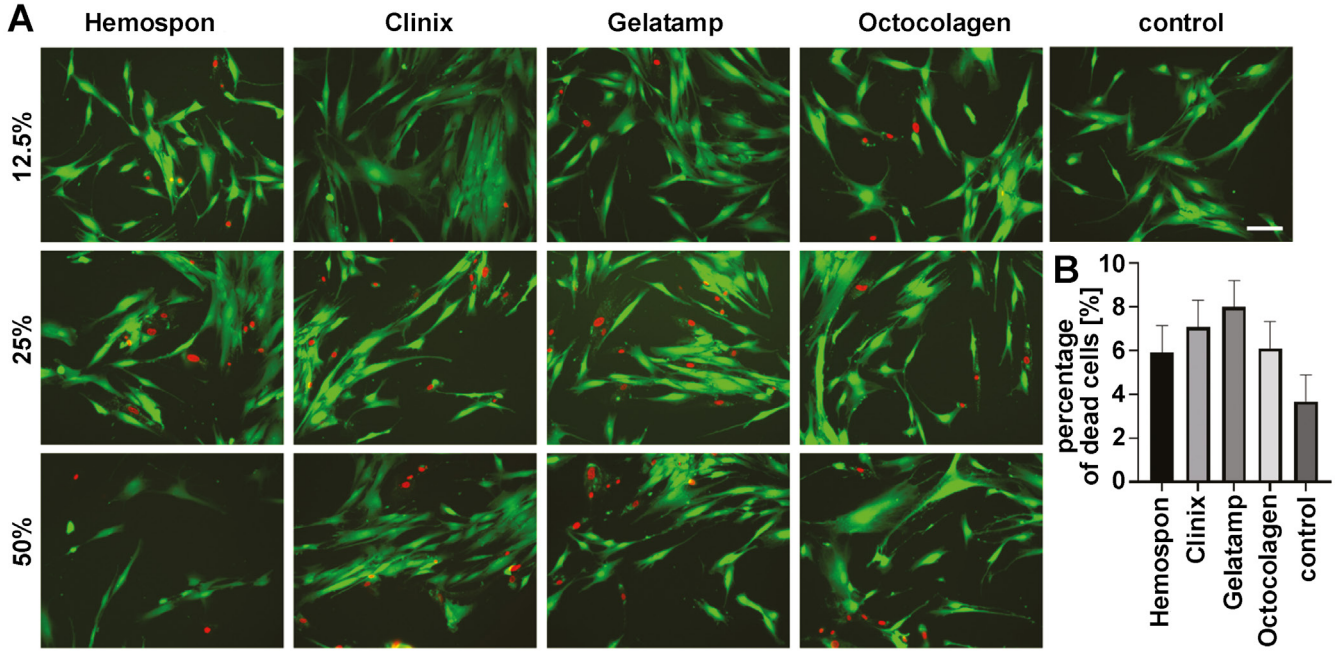

Fig. 1. Cell viability – the live/dead assay

A – representative fluorescence images for the live/dead double staining assay, upon 24 h of exposure to different concentrations (50%, 25% and 12.5%) of the hemostatic sponge leachables (Hemospon, Clinix, Gelatamp, and Octocolagen). Viable cells are depicted in green, while dead cells are marked in red (scale bar = 100 µm); B – quantitative evaluation of dead cells exposed to a concentration of 50% of the hemostatic sponge leachables upon image analysis.

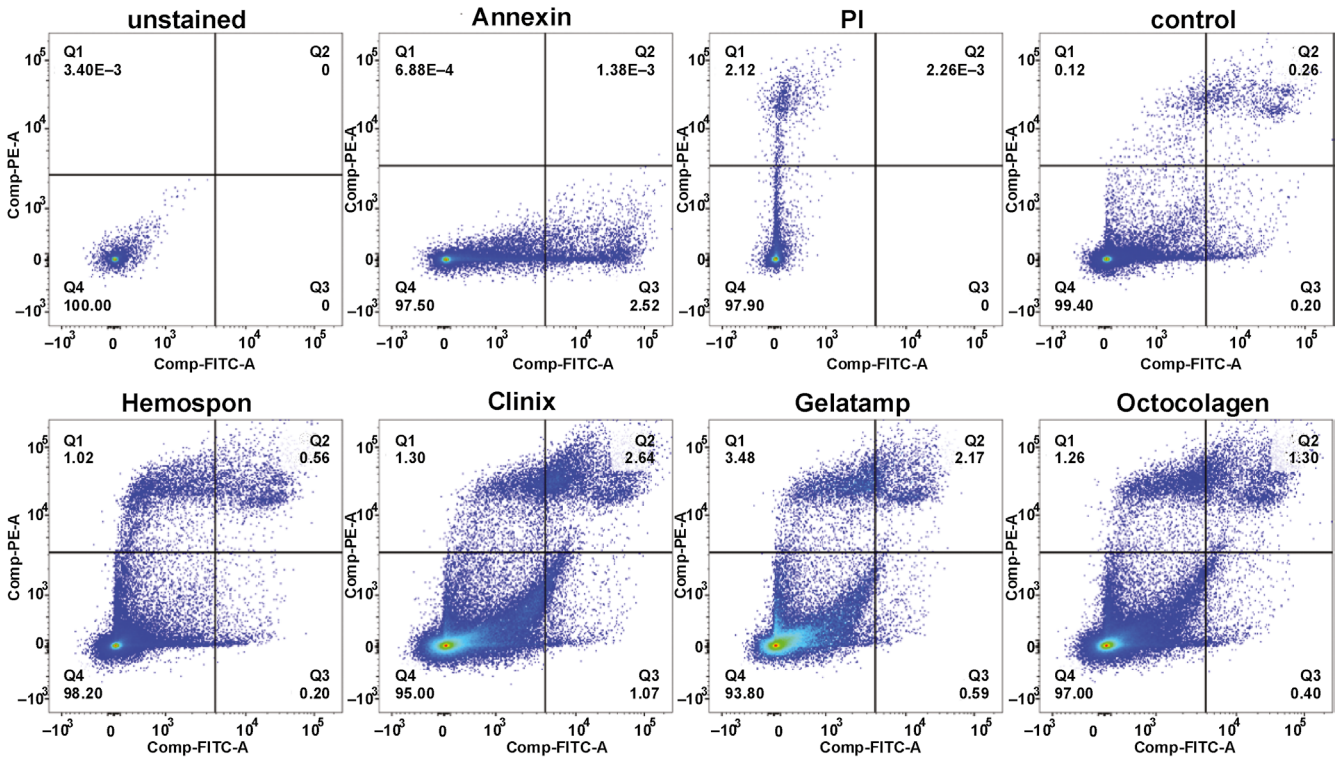

Fig. 2. Representative graphs for the flow cytometric analysis of cell viability, upon 24 h of exposure to a concentration of 50% of the hemostatic sponge leachables (Hemospon, Clinix, Gelatamp, and Octocolagen)

Comp-FITC-A – compensated FITC-channel fluorescence area, corresponding to Annexin V-FITC signal; Comp-PE-A – compensated PE-channel fluorescence area, used for PI detection; PI – propidium iodide.

day 3 to day 7 of culture. On day 3, the cultures exposed to the highest leachable concentration exhibited a tendency toward reduced metabolic activity across all tested materials, an effect that became less pronounced at lower concentrations. By day 7, the leachables from Hemospon induced significantly higher metabolic activity levels, a trend that was also observed for higher concentrations of the Gelatamp and Octocolagen leachables (Fig. 3).

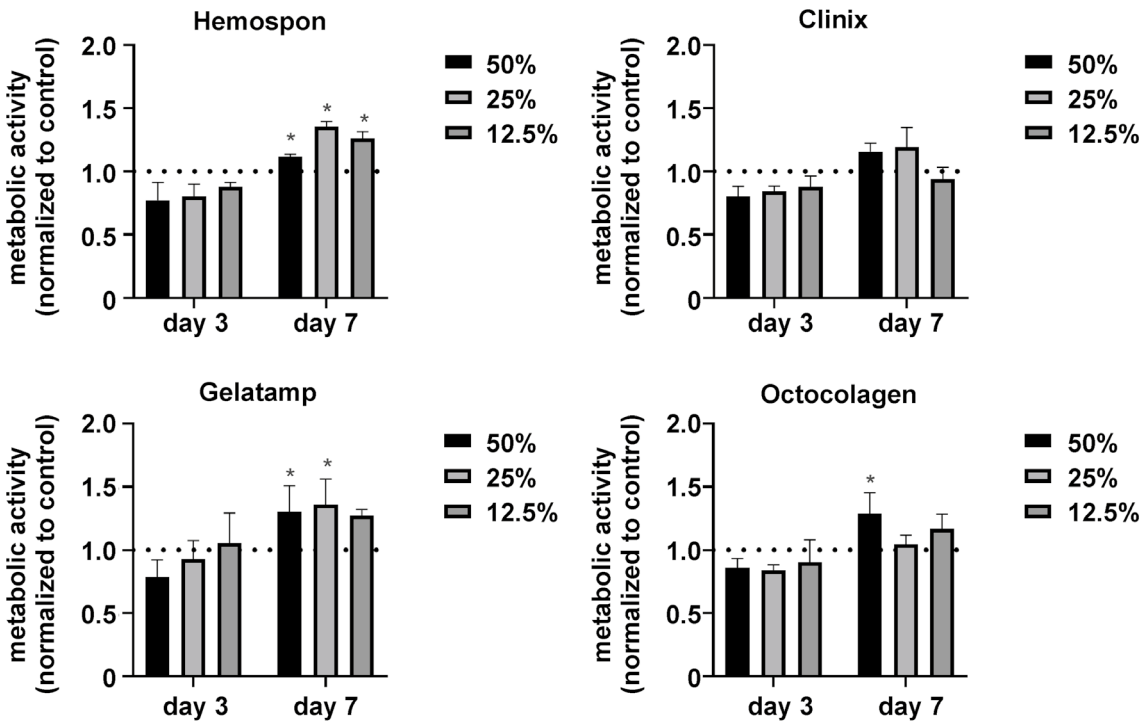

Fig. 3. Metabolic activity of the human fibroblastic cultures exposed to different concentrations (50%, 25% and 12.5%) of the hemostatic sponge leachables (Hemospon, Clinix, Gelatamp, and Octocolagen)

* statistically significant difference with regard to the control ($p < 0.05$).

Analysis of the irritation potential – in vivo HET-CAM assay

The in vivo HET-CAM assay was performed to evaluate the irritation potential of the hemostatic sponges, based on the occurrence of lysis, hemorrhage or coagulation. The test solutions were quantitatively graded according to the Luepke method (Tables 1 and 2). The negative control (0.9% NaCl) produced no visible reaction throughout the 5-minute application period, and was therefore assigned a score of 0, corresponding to the absence of irritation.

Table 1. Numerical scores for the hen's egg test – chorioallantoic membrane (HET-CAM) assay

Effect	Time points		
	0.5 min	2 min	5 min
Lysis	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

Table 2. Irritation assessment based on the cumulative scores for the hen's egg test – chorioallantoic membrane (HET-CAM) assay

Cumulative score	Irritation
0.0–0.9	none
1.0–4.9	slight
5.0–8.9	moderate
9.0–21.0	severe

In contrast, the positive control (0.5 M NaOH) induced a severe hemorrhagic response, resulting in a score of 19, indicative of severe irritation. Regarding the hemostatic sponges, none of the tested materials induced visible signs of irritation within the duration of the assay. Consequently, all materials received a score of 0, similarly to the negative control (Table 3, Fig. 4).

Discussion

Surgical bleeding can significantly affect both patient outcomes and healthcare costs, with inadequate hemorrhage control being closely associated with adverse outcomes and increased morbidity.[20] In this context, gelatin-based hemostatic agents represent a valuable class among non-active hemostatic materials, helping to overcome

Table 3. Scores obtained from the in vivo hen's egg test – chorioallantoic membrane (HET-CAM) irritation assay

Sample	Score
NaOH (0.5 M)	19
NaCl (0.9%)	0
Hemospon	0
Clinix	0
Gelatamp	0
Octocolagen	0

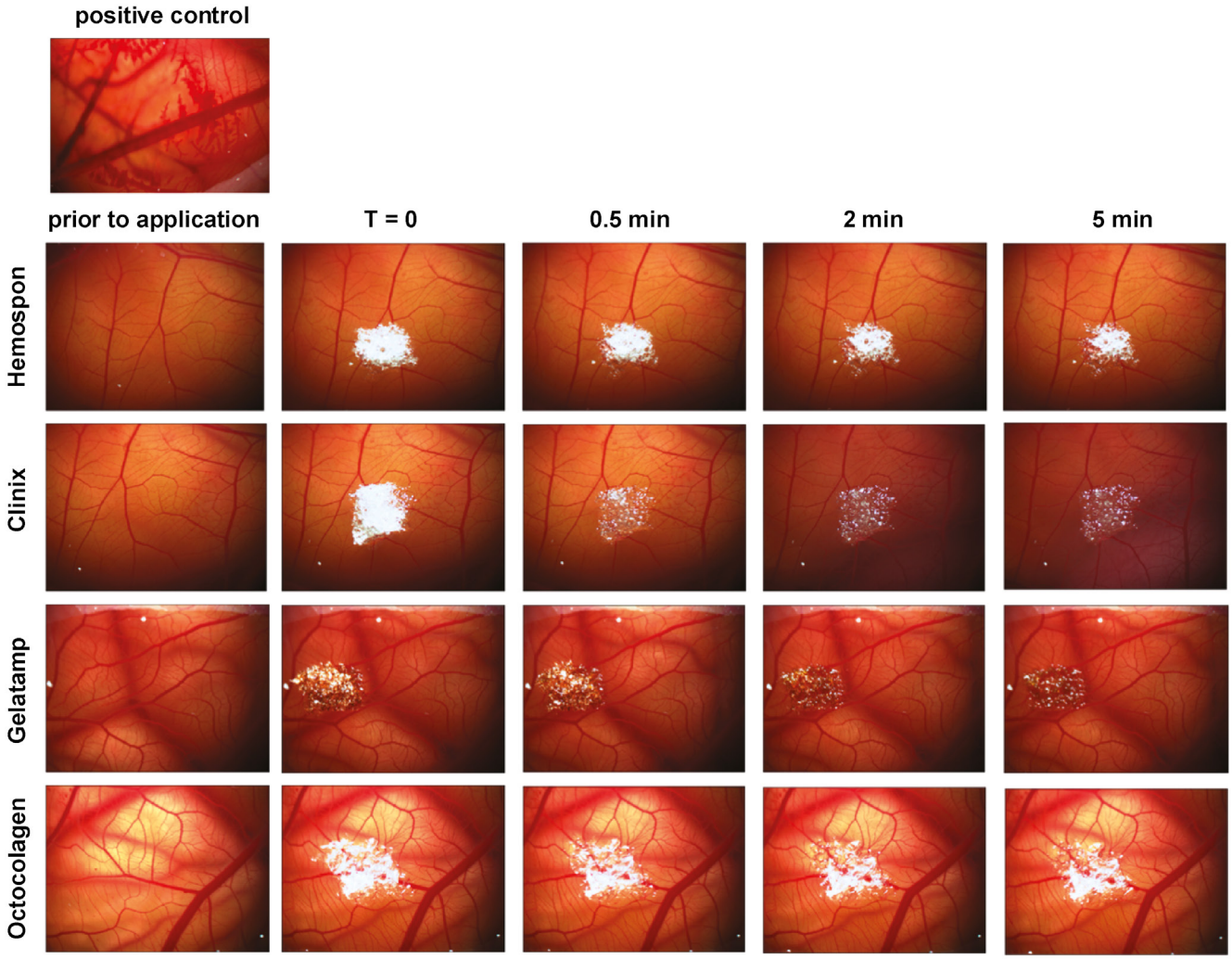

Fig. 4. In vivo hen's egg test – chorioallantoic membrane (HET-CAM) assay for irritation assessment

Macroscopic features of the CAM vasculature prior to application (first column), immediately upon placement (T = 0), and after 0.5, 2 and 5 min of exposure to the hemostatic sponges (Hemospon, Clinix, Gelatamp, and Octocolagen).

clinical challenges while contributing to improved therapeutic outcomes and more cost-effective healthcare practices. Their high absorbency, minimal tissue reactivity and gradual resorption profile are particularly advantageous for achieving effective hemostasis and supporting postoperative healing.[25] Despite their widespread clinical use, an important knowledge gap remains regarding the comparative biological performance of commonly used gelatin-based hemostatic agents. Accordingly, the present study aimed to biologically characterize 4 commercially available lyophilized porcine gelatin sponges – Hemospon, Clinix, Gelatamp, and Octocolagen – through cytocompatibility and irritation assays.

Human fibroblast cultures represent a relevant model for evaluating the biological response to materials intended for tissue contact. As the predominant cellular component of connective tissues, fibroblasts play a fundamental role in maintaining tissue structure and integrity, while also contributing significantly to tissue repair and regenerative processes during healing.[26,27] To assess fibroblast functionality, live/dead staining and flow cytometry analyses were performed at early culture time points, followed by metabolic activity evaluation at later stages of culture.

Viability assays demonstrated the absence of significant cytotoxic effects in the cultures exposed to the hemostatic sponge leachables when compared with the control condition at early culture time points. Although the exposed cultures exhibited a slightly greater number of dead cells relative to the control, these differences were not statistically significant, as confirmed by both the live/dead assay and flow cytometry. The evaluation of cell viability is a critical component of cytotoxicity assessment, with fluorescence-based microscopic and flow cytometric methods being widely recognized as reliable and effective approaches that demonstrate high correlation between datasets.[28] These findings are consistent with those reported by Sezer et al., who evaluated different gelatin-based hemostatic agents using human umbilical vein endothelial cells (hUVECs), and observed no significant differences among groups with respect to cell death.[29]

Similarly, Awad et al. reported high viability levels (>95%) in human adipose-derived stem cells (hADSCs) exposed to a gelatin sponge.[30] In addition, diluted extracts from a gelatin-based hemostatic agent were shown not to significantly affect the viability of rodent fibroblasts, following 24 h of exposure.[31]

Regarding the assessment of metabolic activity at later culture time points, the MTT assay revealed a consistent pattern across all 4 tested materials throughout the 7-day evaluation period. On day 3, the cultures exposed to more diluted extracts exhibited no significant differences relative to the control condition, whereas a tendency toward reduced MTT values was observed, particularly at higher extract concentrations. Nevertheless, these values remained above the 70% threshold established by ISO 10993-5:2009, below which a material or extract is considered cytotoxic.[32] By day 7, the cultures exposed to all extract concentrations demonstrated metabolic activity levels comparable to or exceeding those of the control group. These findings further support the preservation of adequate cellular functionality in the presence of the sponge leachables. The initial reduction tendency observed in the MTT values may be associated with cumulative exposure within the in vitro culture environment, potentially resulting in concentrations exceeding those expected under clinical conditions, where continuous dilution and clearance by biological fluids would occur following application. At the later culture stage, however, a tendency toward increased metabolic activity became evident, corroborating previous findings reported by Sezer et al.[29] and Lan et al.[33] These authors evaluated hUVECs and murine fibroblasts, respectively, following exposure to gelatin-based materials, and reported favorable cytocompatibility without evidence of cytotoxicity.[29,33] This pattern is consistent with the well-documented ability of gelatin-based materials and their degradation products to stimulate cellular activity and support tissue healing processes.[34,35]

Beyond the in vitro cytocompatibility assessment, irritation assessment – an essential component of the biocompatibility and biosafety evaluation of biomaterials and medical devices – was performed using the HET-CAM assay. This method has been widely recognized as a rapid, simple and reliable alternative approach for irritation assessment across different experimental settings.[36–38] Accordingly, the hemostatic agents were evaluated following direct CAM exposure, with no evidence of hyperemia or irritative responses, including vessel lysis, hemorrhage or coagulation, throughout the duration of the assay. These findings are consistent with previous reports, such as the study by Rodrigues da Silva et al., who also employed the CAM assay to evaluate the irritation potential of a gelatin membrane, and observed no signs of hyperemia, hemorrhage or coagulation.[39] Moreover, the extensive use of gelatin sponges in CAM-based studies, extending beyond irritation testing to applications involving angiogenesis assessment, further highlights their role as inert substrate materials capable of serving as platforms for the controlled delivery of cells or bioactive substances under investigation.[40–42] Collectively, these observations reinforce the inert and non-irritant nature of gelatin-based sponges, and further support their suitability for a wide range of biomedical applications.

Conclusions

This comprehensive study characterized the biological response to 4 commercially available gelatin-based hemostatic materials, with particular emphasis on their effects on human fibroblast functionality and irritation potential. The findings demonstrated high cytocompatibility, consistent cellular responses and sustained metabolic activity, in agreement with the well-established capacity of gelatin-based materials to support tissue healing processes. The irritation assessment further confirmed the biocompatibility of these gelatin sponges, highlighting their inert nature and their inability to induce alterations in a well-established vascular tissue model. Although minor differences were observed among the tested materials regarding the biological response, the overall favorable biocompatibility profile reinforces the safety and effectiveness of commercially available gelatin-based hemostatic agents. Collectively, these findings support their suitability for clinical applications and their potential contribution to improved surgical outcomes.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Ruangchainicom N, Mahardawi B, Sakdejayont W. Topical hemostatic agents from an oral-surgery perspective. *J Oral Maxillofac Surg Med Pathol*. 2021;33(3):249–255. doi:10.1016/j.ajoms.2020.11.007
2. Slezak P, Heher P, Monforte X, et al. Efficacy of topical hemostatic agents: A comparative evaluation of two gelatin/thrombin-based hemostatic matrices in a porcine kidney surgical model. *J Invest Surg*. 2019;32(7):646–653. doi:10.1080/08941939.2018.1447619
3. Tompeck AJ, Reham Gajdhar AU, Dowling M, et al. A comprehensive review of topical hemostatic agents: The good, the bad, and the novel. *J Trauma Acute Care Surg*. 2020;88(1):e1–e21. doi:10.1097/TA.0000000000002508
4. Bracey A, Shander A, Aronson S, et al. The use of topical hemostatic agents in cardiothoracic surgery. *Ann Thorac Surg*. 2017;104(1):353–360. doi:10.1016/j.athoracsur.2017.01.096
5. Chiara O, Cimbanassi S, Bellanova G, et al. A systematic review on the use of topical hemostats in trauma and emergency surgery. *BMC Surg*. 2018;18(1):68. doi:10.1186/s12893-018-0398-z
6. Spotnitz WD, Burks S. State-of-the-art review: Hemostats, sealants, and adhesives II: Update as well as how and when to use the components of the surgical toolbox. *Clin Appl Thromb Hemost*. 2010;16(5):497–514. doi:10.1177/1076029610363589
7. Vyas KS, Saha SP. Comparison of hemostatic agents used in vascular surgery. *Expert Opin Biol Ther*. 2013;13(12):1663–1672. doi:10.1517/14712598.2013.848193
8. Masci E, Santoleri L, Belloni F, et al. Topical hemostatic agents in surgical practice. *Transfus Apher Sci*. 2011;45(3):305–311. doi:10.1016/j.transci.2011.10.013
9. Kamoh A, Swantek J. Hemostasis in oral surgery. *Dent Clin North Am*. 2012;56(1):17–23. doi:10.1016/j.cden.2011.06.004
10. Sawan A, Elhawary Y, Amer MZ, Rahman MA. Controversial role of two different local haemostatic agents on bone healing. *J Am Sci*. 2010;6(12):155–163.
11. Scarano A, Leo L, Lorusso F, et al. Topical hemostatic agents in oral surgery: A narrative review. *Eur Rev Med Pharmacol Sci*. 2023;27(3 Suppl):135–140. doi:10.26355/eurrev_202304_31332
12. Wellisz T, An YH, Wen X, Kang Q, Hill CM, Armstrong JK. Infection rates and healing using bone wax and a soluble polymer material. *Clin Orthop Relat Res*. 2008;466(2):481–486. doi:10.1007/s11999-007-0067-5
13. Kumar S. Local hemostatic agents in the management of bleeding in oral surgery. *Asian J Pharm Clin Res*. 2016;9(3):35–41.
14. Vezeau PJ. Topical hemostatic agents. *Oral Maxillofac Surg Clin North Am*. 2016;28(4):523–532. doi:10.1016/j.coms.2016.06.007
15. Yazdimamaghani M, Vashaee D, Assefa S, et al. Hybrid macroporous gelatin/bioactive-glass/nanosilver scaffolds with controlled degradation behavior and antimicrobial activity for bone tissue engineering. *J Biomed Nanotechnol*. 2014;10(6):911–931. doi:10.1166/jbn.2014.1783
16. Peter M, Ganesh N, Selvamurugan N, et al. Preparation and characterization of chitosan–gelatin/nanohydroxyapatite composite scaffolds for tissue engineering applications. *Carbohydr Polym*. 2010;80(3):687–694. doi:10.1016/j.carbpol.2009.11.050
17. Irfan NI, Mohd Zubir AZ, Suwandi A, Haris MS, Jaswir I, Lestari W. Gelatin-based hemostatic agents for medical and dental application at a glance: A narrative literature review. *Saudi Dent J*. 2022;34(8):699–707. doi:10.1016/j.sdentj.2022.11.007
18. Imani R, Rafienia M, Emami SH. Synthesis and characterization of glutaraldehyde-based crosslinked gelatin as a local hemostat sponge in surgery: An in vitro study. *Biomed Mater Eng*. 2013;23(3):211–224. doi:10.3233/BME-130745
19. Chanu NR, Bhattacharya K, Marbaniang D, Pal P, Ray S, Mazumder B. Evaluation of a novel melatonin-loaded gelatin sponge as a wound dressing. *J Vasc Nurs*. 2022;40(1):2–10. doi:10.1016/j.jvn.2021.09.004
20. Shander A, Kaplan LJ, Harris MT, et al. Topical hemostatic therapy in surgery: Bridging the knowledge and practice gap. *J Am Coll Surg*. 2014;219(3):570–579e4. doi:10.1016/j.jamcollsurg.2014.03.061
21. Cziperle DJ. Avitene™ microfibrillar collagen hemostat for adjunctive hemostasis in surgical procedures: A systematic literature review. *Med Devices (Auckland)*. 2021;14:155–163. doi:10.2147/MDER.S298207
22. International Organization for Standardization (ISO). ISO 10993-12:2021. Biological evaluation of medical devices. Part 12: Sample preparation and reference materials. 2021. https://www.iso.org/standard/75769.html. Accessed December 15, 2023.
23. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM-recommended test method protocol: Hen's egg test – chorioallantoic membrane (HET-CAM) test method. 2010. https://ntp.niehs.nih.gov/sites/default/files/iccvam/docs/protocols/ivocular-hetcam.pdf. Accessed December 15, 2023.
24. Luepke NP. Hen's egg chorioallantoic membrane test for irritation potential. *Food Chem Toxicol*. 1985;23(2):287–291. doi:10.1016/0278-6915(85)90030-4
25. Nepal A, Tran HDN, Nguyen NT, Ta HT. Advances in haemostatic sponges: Characteristics and the underlying mechanisms for rapid haemostasis. *Bioact Mater*. 2023;27:231–256. doi:10.1016/j.bioactmat.2023.04.008
26. Li B, Wang JHC. Fibroblasts and myofibroblasts in wound healing: Force generation and measurement. *J Tissue Viability*. 2011;20(4):108–120. doi:10.1016/j.jtv.2009.11.004
27. Vijayashree RJ, Sivapathasundharam B. The diverse role of oral fibroblasts in normal and disease. *J Oral Maxillofac Pathol*. 2022;26(1):6–13. doi:10.4103/jomfp.jomfp_48_22
28. Kummrow A, Frankowski M, Bock N, Werner C, Dziekan T, Neukammer J. Quantitative assessment of cell viability based on flow cytometry and microscopy. *Cytometry A*. 2013;83(2):197–204. doi:10.1002/cyto.a.22213
29. Sezer UA, Kocer Z, Sahin İ, Aru B, Demirel GY, Sezer S. Oxidized regenerated cellulose cross-linked gelatin microparticles for rapid and biocompatible hemostasis: A versatile cross-linking agent. *Carbohydr Polym*. 2018;200:624–632. doi:10.1016/j.carbpol.2018.07.074
30. Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials*. 2004;25(16):3211–3222. doi:10.1016/j.biomaterials.2003.10.045
31. Cenni E, Ciapetti G, Stea S, Corradini A, Carozzi F. Biocompatibility and performance in vitro of a hemostatic gelatin sponge. *J Biomater Sci Polym Ed*. 2000;11(7):685–699. doi:10.1163/156856200743959
32. International Organization for Standardization (ISO). ISO 10993-5:2009. Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity. 2009. https://www.iso.org/standard/36406.html. Accessed December 21, 2023.
33. Lan G, Lu B, Wang T, et al. Chitosan/gelatin composite sponge is an absorbable surgical hemostatic agent. *Colloids Surf B Biointerfaces*. 2015;136:1026–1034. doi:10.1016/j.colsurfb.2015.10.039
34. Dekoninck S, Blanpain C. Stem cell dynamics, migration and plasticity during wound healing. *Nat Cell Biol*. 2019;21(1):18–24. doi:10.1038/s41556-018-0237-6
35. Wang L, Mao L, Qi F, et al. Synergistic effect of highly aligned bacterial cellulose/gelatin membranes and electrical stimulation on directional cell migration for accelerated wound healing. *Chem Eng J*. 2021;424:130563. doi:10.1016/j.cej.2021.130563
36. Dreesmann L, Ahlers M, Schlosshauer B. The pro-angiogenic characteristics of a cross-linked gelatin matrix. *Biomaterials*. 2007;28(36):5536–5543. doi:10.1016/j.biomaterials.2007.08.040
37. Zwadlo-Klarwasser G, Görlitz K, Hafemann B, Klee D, Klosterhalfen B. The chorioallantoic membrane of the chick embryo as a simple model for the study of the angiogenic and inflammatory response to biomaterials. *J Mater Sci Mater Med*. 2001;12(3):195–199. doi:10.1023/a:1008950713001
38. Öztürk AA, Kıyan HT. Treatment of oxidative stress-induced pain and inflammation with dexketoprofen trometamol loaded different molecular weight chitosan nanoparticles: Formulation, characterization and anti-inflammatory activity by using in vivo HET-CAM assay. *Microvasc Res*. 2020;128:103961. doi:10.1016/j.mvr.2019.103961
39. Rodrigues da Silva F, Silva RO, De Castro Oliveira HM, et al. Gelatin-based membrane containing usnic acid-loaded liposomes: A new treatment strategy for corneal healing. *Biomed Pharmacother*. 2020;130:110391. doi:10.1016/j.biopha.2020.110391
40. Fu J, Chen F, Chai H, Gao L, Lv X, Yu L. Lyophilized gelatin@non-woven scaffold to promote spheroids formation and enrich cancer stem cell incidence. *Nanomaterials (Basel)*. 2022;12(5):808. doi:10.3390/nano12050808
41. Chen Y, Li L, Chen L, et al. Gellan gum-gelatin scaffolds with Ca^{2+} crosslinking for constructing a structured cell cultured meat model. *Biomaterials*. 2023;299:122176. doi:10.1016/j.biomaterials.2023.122176
42. Koivisto JT, Gering C, Karvinen J, et al. Mechanically biomimetic gelatin–gellan gum hydrogels for 3D culture of beating human cardiomyocytes. *ACS Appl Mater Interfaces*. 2019;11(23):20589–20602. doi:10.1021/acsami.8b22343

Effect of chia seeds (*Salvia hispanica* L.) on the regeneration of salivary glands in a streptozotocin (STZ)-induced diabetic rat model

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Abstract

Background. Diabetes mellitus (DM) is a complex condition, with salivary gland malfunction being one of its complications. The management of the disease presents numerous side effects. Consequently, botanical products have been proposed as a promising alternative for the development of safe and effective treatments.

Objectives. The aim of the study was to investigate the antioxidant activity of chia seeds (*Salvia hispanica* L.) as a potential therapeutic tool for alleviating the deleterious effects of DM in albino rats.

Material and methods. The study sample comprised 66 male albino rats. Group I ($n = 6$) received a saline solution, group II ($n = 30$) was subjected to diabetic induction via a single intraperitoneal injection of streptozotocin (STZ), and group III ($n = 30$) was subjected to diabetic induction and treated with 250 mg/kg/day of powdered chia seeds for 2 weeks prior to the administration of STZ and until the end of the study. The rats were euthanized 45 days after the induction of DM. The parotid and mandibular mucosal glands were extracted and prepared for histological (hematoxylin and eosin (H&E)), immunohistochemical (vascular endothelial growth factor (VEGF)) and comet assay analyses.

Results. Marked degenerative changes were evident in the acini and duct architecture of the parotid and mandibular mucosal glands in group II. These findings were confirmed histologically, immunohistochemically and via the comet assay. In contrast, in group III, treatment with chia seeds resulted in the regeneration of salivary gland tissues.

Conclusions. Chia seeds demonstrated antioxidant properties against DM-induced salivary gland dysfunction in albino rats.

Keywords: diabetes mellitus, regeneration, *Salvia hispanica* L., salivary glands, antioxidant properties

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Highlights

- Diabetes causes severe structural damage in parotid and mandibular glands of the rats through oxidative DNA damage.
- Chia seed supplementation effectively attenuates these alterations, demonstrating pronounced antioxidant activity against diabetes-induced oxidative stress.
- The findings support the potential use of chia seeds as a dietary adjunct for improving salivary gland histology and function in diabetic patients.
- Further studies are required to determine optimal dosage, long-term safety and the specific bioactive compounds responsible for the observed protective effects.

Introduction

Diabetes mellitus (DM) is a metabolic condition characterized by high blood glucose levels and disruption of carbohydrate, lipid and protein metabolism.[1] It is a disorder of the endocrine system in which a decrease in the production of insulin results in elevated blood glucose levels.[2] In addition, uncontrolled DM results in end organ damage affecting the genitourinary, cardiovascular and neurological systems.[3]

Salivary glands are a set of major and minor glands that secrete saliva into the oral cavity through the duct system. Rats have multiple minor glands and 3 pairs of major salivary glands, namely the parotid, submandibular and sublingual glands.[4]

The production of saliva is necessary to maintain the equilibrium of the oral cavity and overall health.[5] In diabetic conditions, salivary glands are exposed to deleterious effects, leading to reduced saliva production, destruction of the periodontium, salivary hypofunction, and oral mucous membrane injury.[6] An association has been established between complications of diabetes and salivary malfunction. Xerostomia (dry mouth due to a deficient amount of saliva) and polydipsia (pathological thirst) are frequently observed in diabetic subjects as a result of diminished salivary secretion.[7] Many authors have stated that the decreased salivary flow rate in diabetic patients is caused by increased urination, which leads to a reduction in extracellular fluid volume, necessary for saliva production by the salivary glands.[8]

Recent studies have demonstrated that hyperglycemia occurs as a consequence of DM. The condition produces oxidative and cellular impairments, which in turn negatively affect the normal structure of organs and tissues.[9] Constant elevation of blood sugar levels leads to long-term oxidative stress that triggers the excessive production of reactive oxygen species (ROS). The occurrence of diabetic complications and their progression are mediated by ROS.[10] Reactive oxygen species are highly reactive chemical molecules that, when significantly elevated under stressful conditions, lead to profound damage to cellular structures, especially when antioxidant systems are compromised.[11]

Various oxidative stress markers, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), protein carbonyl (PC), 4-hydroxynonenal–protein adduct (4-HNE), oxidized and/or malondialdehyde (MDA)-modified low-density lipoprotein (LDL) cholesterol (oxy-LDL/MDA-LDL) and 8-isoprostanes (8-isoP), have been investigated in streptozotocin (STZ)-induced DM rats to assess oxidative damage in the salivary glands. The results indicated that the 3 major salivary glands are subjected to increased oxidative stress regardless of disease duration. However, the parotid glands showed the greatest extent and diversity of oxidative injury.[11]

At present, salivary gland dysfunction is treated by stimulation of salivary secretion, either through oral receptors using ascorbic acid, by stimulating mastication (e.g., chewing gum), or by pharmacological stimulation using pilocarpine. Nevertheless, all of these remedies cause several complications and provide only short-time relief; therefore, effective long-term therapy is still needed.[12]

Another treatment approach for DM relies on oral hypoglycemic agents or insulin. Besides being costly, these drugs cause several side effects, such as decreased blood sodium levels, jaundice, nausea, headaches, vomiting, and increased body weight.[13] In low-, middle- and high-income countries, populations often rely on botanical products for health maintenance, because they are frequently considered a safe and effective approach to managing chronic diseases. Lately, a variety of botanical products have been accepted for the treatment of DM and its complications.[14]

Over the last few years, medicinal plants have emerged as promising therapeutic agents with hypoglycemic effects for DM treatment.[15] Shukla et al. examined a variety of herbal remedies for the treatment of DM, including aloe vera, eucalyptus, garlic, and ginger.[16] Chia (*Salvia hispanica* L.), which belongs to the genus *Salvia*,[17] is a plant native to northern Guatemala and southern Mexico. It was used as a nutritional food source by pre-Columbian populations.[18] Chia is distinguished by its high content of alpha-linolenic acid (omega-3), fiber, proteins, vitamins, minerals, and phytonutrients, including phenolic compounds.[19] These constituents contribute to improvements

in biological markers associated with non-communicable diseases, exhibiting anti-inflammatory,[20] antioxidant,[21] hypotensive,[22] hypocholesterolemic,[23] and hypoglycemic properties.[24]

Chia may assist in the prevention of cardiovascular diseases, inflammatory and neurological disorders, as well as diabetes, as stated by Vuksan et al., who proposed that a high-fiber diet may contribute to the management of diabetes.[25] The study was carried out on 20 patients with diabetes, who were given bread made with chia flour and additional whole seeds to be sprinkled on foods at home. The total consumption of chia seeds was 37 g/day. The study results demonstrated a small reduction in blood glucose as well as reduced levels of systemic inflammation, measured by C-reactive protein (CRP). Notably, blood became less susceptible to coagulation, which constitutes a risk factor that may lead to heart attacks and strokes.[25]

Objectives

This study aimed to assess the therapeutic efficacy of chia seeds in ameliorating salivary gland dysfunction caused by DM in diabetic rats.

Material and methods

A total of 66 mature male albino rats, weighing 200–250 g, were utilized in the current research. The animals were housed in separate cages and provided with a standard rodent diet and water. Prior to the study, the rats were administered chia seeds for 14 days. The animals were divided into the following 3 groups:

- group I (control group; n = 6) – administered a saline solution;
- group II (n = 30) – subjected to diabetic induction via a single intraperitoneal injection of STZ without additional treatment;
- group III (n = 30) – subjected to diabetic induction via a single intraperitoneal injection of STZ and treated with 250 mg/kg/day of chia seed flour for 2 weeks prior to diabetic induction and until the end of the study.[26]

The study design is presented in Fig. 1.

Parotid and mandibular mucosal gland assessment

The rats were euthanized by an overdose of halothane 45 days after diabetic induction.[27] The parotid and

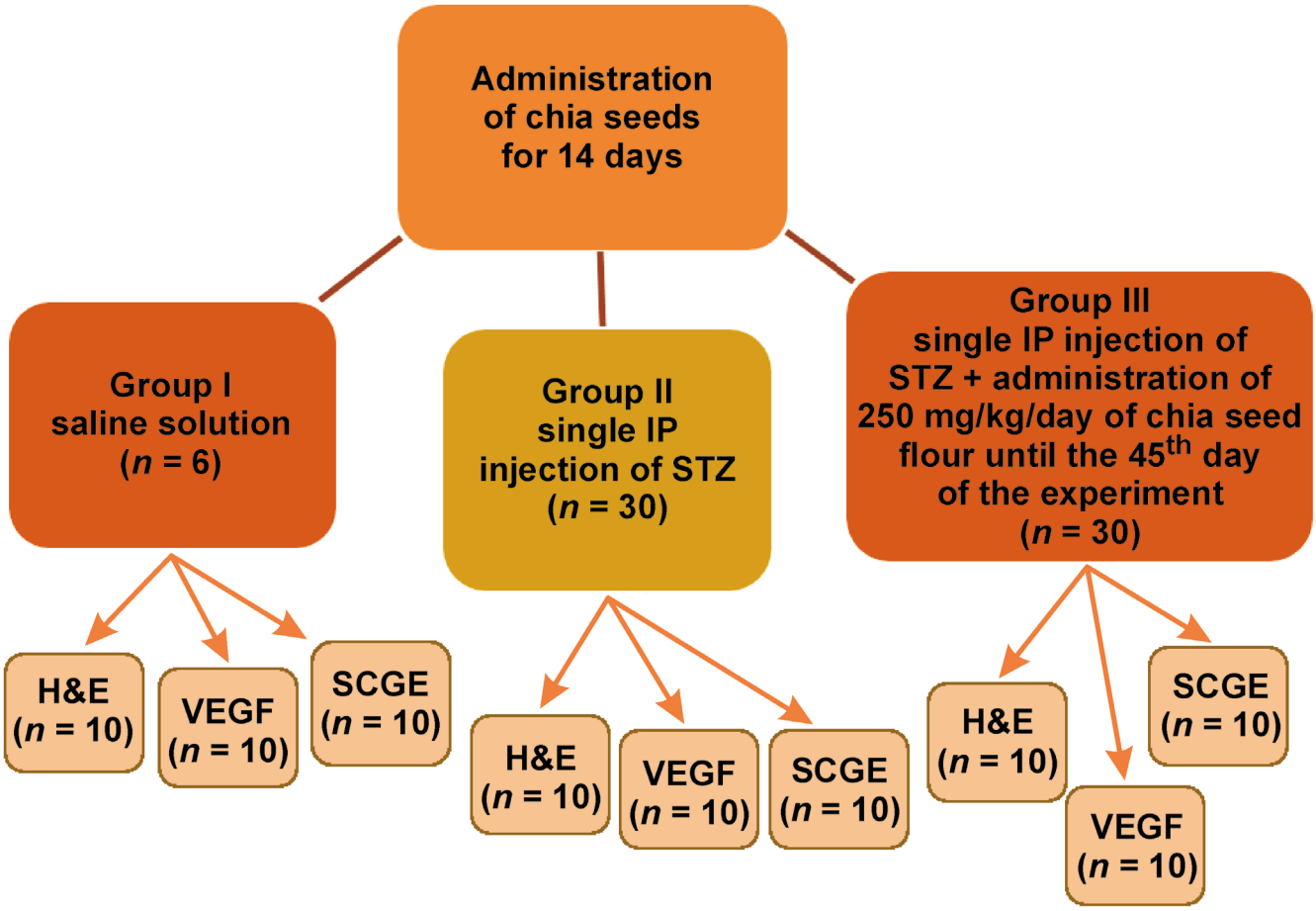

Fig. 1. Flowchart of the study design

STZ – streptozotocin; H&E – hematoxylin and eosin; VEGF – vascular endothelial growth factor; SCGE – single-cell gel electrophoresis; IP – intraperitoneal.

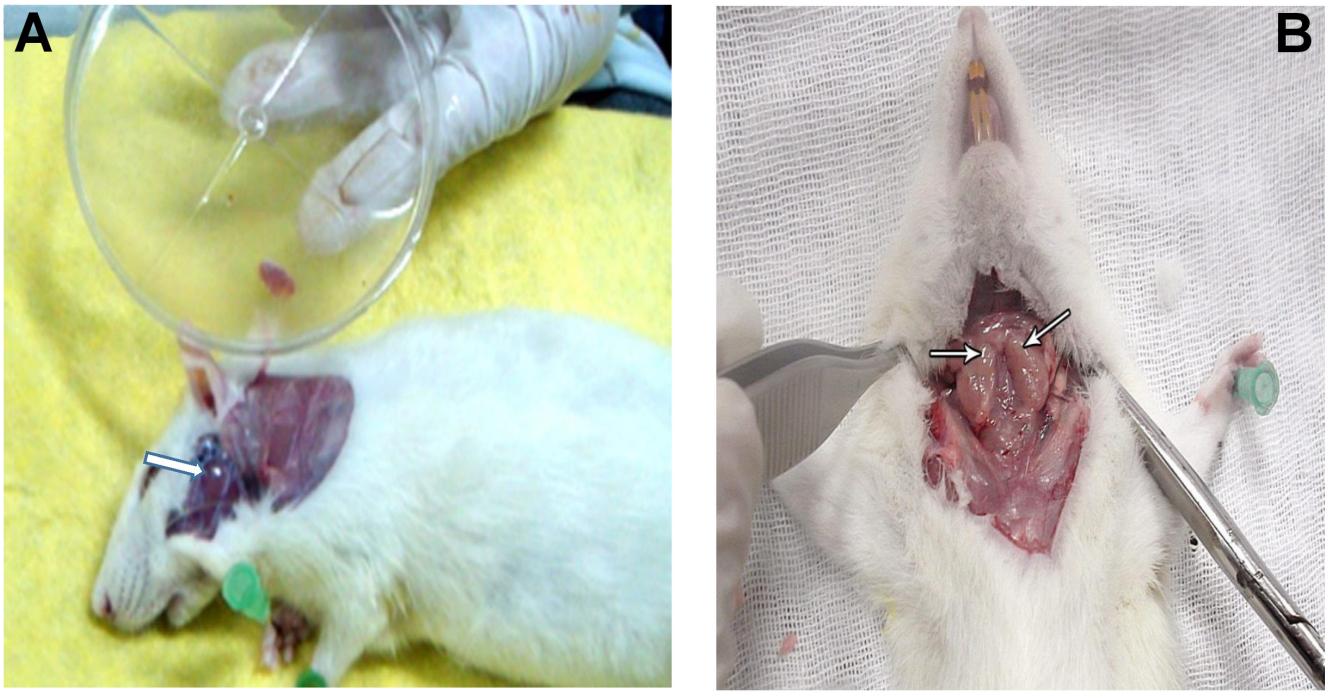

Fig. 2. Location of the parotid gland (A) and mandibular mucosal salivary gland (B)

mandibular mucosal glands were removed (Fig. 2) and divided as follows:

- 2 rats from group I, 10 rats from group II and 10 rats from group III were used for histological examination by hematoxylin and eosin (H&E) staining. The excised glands were fixed in 10% neutral-buffered formalin for 24 h, immersed in paraffin and sectioned at 5 µm;
- 2 rats from group I, 10 rats from group II and 10 rats from group III were used for immunohistochemical examination of vascular endothelial growth factor (VEGF). The excised glands were preserved in 10% neutral-buffered formalin for 24 h, immersed in paraffin and sectioned at 5 µm. Subsequently, they were immunostained using the avidin–biotin method. The sections were stained with rabbit anti-rat VEGF polyclonal antibodies (Sigma-Aldrich, St. Louis, USA);
- 2 rats from group I, 10 rats from group II and 10 rats from group III were used for single-cell gel electrophoresis (comet assay). The excised glands were stored in 1 mL of saline solution in Eppendorf tubes at –180°C.[28,29]

Histological staining

Hematoxylin and eosin staining was performed as a routine histological procedure. For microscopic analysis, the excised glands were fixed in 10% formalin for 24 h. The specimens were washed thoroughly under running water, dehydrated through a graded series of alcohol, cleared in xylene, and embedded in paraffin wax according to standard techniques.[30] After embedding in paraffin, 5 µm-thick sections were cut and stained with H&E.

These histological sections were investigated under a light microscope (Olympus CH2; Olympus Optical Co., Ltd., Tokyo, Japan).

Immunohistochemical evaluation

The sectioned glands were subjected to immunostaining using the avidin–biotin technique. The sections were stained with rabbit anti-rat VEGF polyclonal antibodies (Sigma-Aldrich).

Five non-overlapping fields from each slide were digitized using a digital camera (Olympus Optical Co., Ltd.) mounted on a microscope (Olympus CH2). The images were analyzed on an Intel Core i3-based computer using VideoTesT-Morphology software (Video Test, Saint Petersburg, Russia) with a specific built-in routine for immunostain quantification. The targeted, positively immunostained areas were automatically selected and separated from the original image based on the stain hue range. The selected area was thresholded and defined as a region of interest. Subsequently, a 3D histogram was constructed, and the integrated density of the area was calculated.[31]

Diabetes induction

Experimental diabetes was induced in rats from groups II and III after overnight fasting[32] by a single intraperitoneal injection of STZ (STZ: 45 mg/kg body weight in 0.1 mL citrate buffer, pH 4.5). Three days after the injection, the presence of diabetes was confirmed by hyperglycemia (non-fasting blood glucose level >300 mg/dL) using

Table 1. Blood glucose levels measured at 3 and 45 days after diabetic induction across the groups

Time period	Group I	Group II	Group III	ANOVA	
				F	*p*-value
3 days after diabetic induction	90.56 ±73.70^{Aa}	384.25 ±32.74^{Ba}	293.33 ±35.02^{AB}	20.08	<0.001*
45 days after diabetic induction	105.83 ±19.64^{Aa}	518.25 ±35.14^{a}	195.50 ±40.99^{A}	341.80	<0.001*

Data expressed as mean ± standard deviation (*M ±SD*); * statistically significant ($p < 0.05$); ANOVA – analysis of variance. The same capital letters indicate statistically significant differences between the groups. The same lowercase letters indicate statistically significant differences between day 3 and day 45 within the groups.

a blood glucose meter.[33] The blood glucose levels were monitored at 3 and 45 days after induction, and the results are presented in Table 1.

Comet assay

The comet assay,[28,29] also referred to as single-cell gel electrophoresis, is a rapid and highly sensitive fluorescence microscopy-based technique for the assessment of DNA damage and repair in individual nucleated cells. The method is based on the measurement of the ability of denatured, cleaved DNA fragments to migrate out of the cell under the influence of an electric field, whereas undamaged DNA migrates more slowly and remains within the nucleoid. The evaluation of the DNA "comet" tail shape and migration pattern allows for the assessment of DNA damage. In this assay, cells are immobilized in a layer of low-melting-point agarose, followed by gentle cell lysis, and treated with alkaline solution to unwind and denature the DNA and hydrolyze sites of damage. The samples are then submitted to electrophoresis, stained with a fluorescent DNA-intercalating dye, and visualized using epifluorescence microscopy.[35]

Chia administration

Group III received a daily dose of 250 mg/kg of chia seeds (PLANTON, New Delhi, India).[26] The doses used in animal studies vary from 3.0% to 41.3% for chia seeds, and from 0.15% to 10.00% for chia oil, as reported by Enes et al.[34] Freshly prepared seeds were minced and immersed in water 1 day prior to administration via a stomach tube. The administration was commenced 2 weeks before the induction of diabetes and continued until the end of the experiment.[26]

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). The results are presented as mean ± standard deviation (*M ±SD*). Differences between the groups were assessed using one-way analysis of variance (ANOVA). Subsequently, a post hoc least significant difference (LSD) test was performed. Values were considered statistically significant at $p < 0.05$.

The DNA fragmentation pattern was estimated using a computer-assisted image analysis system (Comet Assay IV™; Perceptive Instruments). A total of 100 haphazardly selected cells per slide were recorded. DNA damage was assessed by evaluating the percentage of DNA in the comet tail relative to the total cellular DNA content.

Results

Clinical observations

A total of 8 rats were excluded from the study, as 5 rats died within 24 h after diabetes induction, and in group II, 2 rats died 1 week after diabetes induction, and 1 rat died after the 2nd week of diabetes induction. The rats were replaced to maintain group sizes.

Histological evaluation

In group I, the parotid glands revealed a normal architecture of serous acini with pyramidal cells and a rounded basally located nucleus arranged around a small lumen (Fig. 3A). The evaluation of the mandibular mucosal glands revealed the presence of typical mucous acini with oval, basally located nuclei encircling a broader lumen. The intralobular ducts of both glands were lined with columnar cells with centrally placed, large, rounded nuclei (Fig. 3E).

In group II, the examination of parotid sections revealed loss of the normal acinar architecture with signs of deterioration. The nuclei were irregularly arranged, with variable sizes and shapes (Fig. 3B,C). Mandibular mucosal gland sections demonstrated atrophy of the acini, and some showed loss of architecture. The intralobular ducts of both glands revealed degenerative changes and loss of normal structure (Fig. 3F,G).

In group III, the sections of both glands revealed improvement in acinar structure. Additionally, the intralobular ducts re-established their normal architecture (Fig. 3D,H).

Immunohistochemical evaluation

In group I, positive cytoplasmic staining was observed in the cells of both acini and ducts in the parotid and mandibular mucosal glands. In group II, a weak positive reaction was reported. In group III, acinar cells exhibited moderate positivity, which was lower than that reported

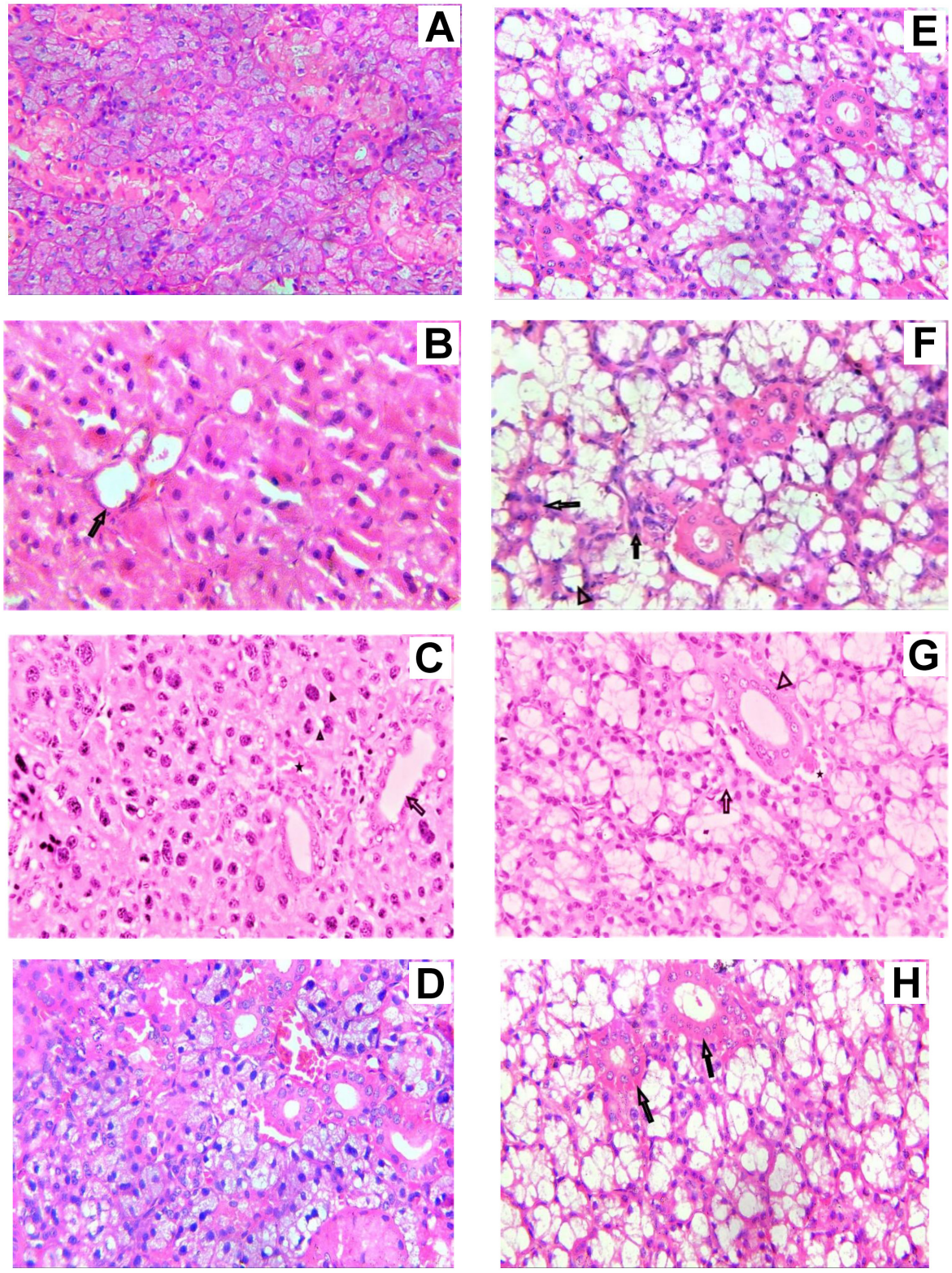

Fig. 3. Photomicrographs of the parotid and mandibular mucosal salivary glands (hematoxylin and eosin (H&E) staining, ×400 magnification)

A. Parotid gland of the control group showing normal architecture of serous acini composed of pyramidal cells with basally located spherical nuclei surrounding a narrow lumen; B. Parotid gland of group II demonstrating loss of normal acinar structure with degenerative changes, irregularly arranged nuclei with variable sizes and shapes, darkly stained nuclei, and thinning of the epithelial lining of the intralobular ducts (arrow); C. Parotid gland of group II showing dilated duct (arrow), dilated blood vessels (star) and intercellular vacuolation (arrowheads); D. Parotid gland of group III demonstrating apparent improvement of the acinar structure and intralobular ducts; E. Mandibular mucosal salivary glands of the control group showing normal mucous acini with oval, basally located nuclei surrounding a wide lumen; F. Mandibular mucosal salivary glands of group II demonstrating acinar atrophy (arrows), reduced acinar size (arrowhead), signs of degeneration in the intralobular ducts, and loss of normal architecture; G. Mandibular mucosal salivary glands of group II demonstrating degeneration of mucous acini (arrow) and dilated duct (arrowhead); H. Mandibular mucosal salivary glands of group III showing improved acinar structure and restoration of intralobular duct architecture (arrows).

in group I. Ductal cells presented stronger positive staining compared to group II (Fig. 4). The expression of VEGF in the parotid and mandibular mucosal glands in all groups is presented in Table 2.

The results of one-way ANOVA and the post hoc LSD test for VEGF expression in the parotid and mandibular mucosal glands in all groups are presented in Table 3 and Table 4.

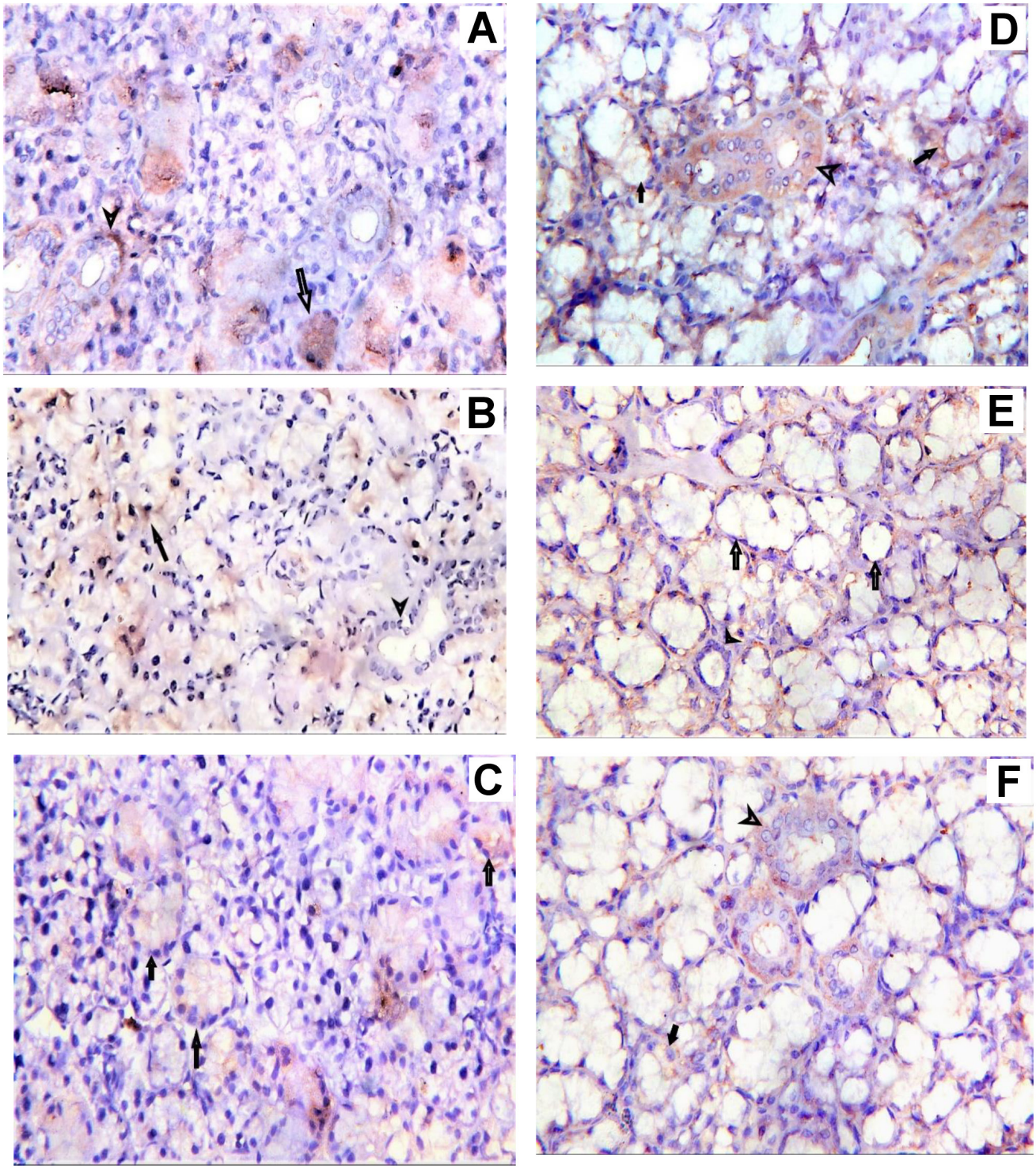

Fig. 4. Representative images of vascular endothelial growth factor (VEGF) immunoreactivity in the parotid and mandibular mucosal salivary glands (×400 magnification)

A. Group I – positive reaction in acinar (arrow) and ductal cells (arrowhead) of the parotid gland; B. Group II – weak positive reaction in acinar (arrow) and ductal cells (arrowhead) of the parotid gland; C. Group III – moderate positive reaction in acinar cells (arrows), lower than in group I; D. Group I – stronger positive reaction in ductal cells (arrowhead) than in acinar cells (arrows) of the mandibular mucosal salivary glands; E. Group II – minimal positive reaction in acinar (arrows) and ductal cells (arrowhead) of the mandibular mucosal salivary glands; F. Group III – increased reaction in acinar (arrow) and ductal cells (arrowhead) of the mandibular mucosal salivary glands.

Table 2. Vascular endothelial growth factor (VEGF) expression in the parotid and mandibular salivary glands across the groups

Group	Gland type	VEGF expression [pg/mL]
Group I	parotid	5.1 ±0.4
	submandibular	3.6 ±0.6
Group II	parotid	1.6 ±1.4
	submandibular	1.5 ±0.6
Group III	parotid	3.2 ±0.5
	submandibular	2.5 ±0.4

Data expressed as *M* ±*SD*.

Table 3. Results of one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) test for vascular endothelial growth factor (VEGF) expression in the parotid glands across the groups

Variable	One-way ANOVA (*p*-value)	Post hoc LSD test		
		comparison		*p*-value
VEGF expression	0.001*	group I	group II	0.001*
			group III	0.001*
		group II	group I	0.001*
			group III	0.004*
		group III	group I	0.001*
			group II	0.004*

* statistically significant ($p < 0.05$).

Comet assay analysis

The comet assay was conducted to examine the degree of cellular DNA damage in the parotid and mandibular mucosal gland cells in all groups. In group I, the majority of cells were observed as uniform discs, characterized by low tail moment, short tail length and a low percentage of tail DNA.

In group II, in both glands, the percentage of tailed cells, tail length, tail moment, and the percentage of tail DNA were significantly increased in comparison to group I. In group III, in both glands, the tail moment, tail length and the percentage of tailed cells were markedly reduced compared to group II (Fig. 5,6).

Table 4. Results of one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) test for vascular endothelial growth factor (VEGF) expression in the mandibular salivary glands across the groups

Variable	One-way ANOVA (*p*-value)	Post hoc LSD test		
		comparison		*p*-value
VEGF expression	0.001*	group I	group II	0.004*
			group III	0.001*
		group II	group I	0.004*
			group III	0.001*
		group III	group I	0.001*
			group II	0.001*

* statistically significant ($p < 0.05$).

The mean values of comet assay parameters were calculated for the parotid and mandibular mucosal glands in all groups, including the percentage of tail DNA, tail moment, tail length, and the percentage of tailed and untailed cells (Fig. 7,8).

The results of comet assay in the parotid and mandibular mucosal glands for the 3 groups are presented in Table 5 and Table 6.

Discussion

The current study investigated the efficacy of chia seeds in restoring the architecture and histological integrity of the parotid and mandibular mucosal glands in rats with STZ-induced diabetes. Streptozotocin is widely used for the experimental induction of diabetes. Streptozotocin-induced diabetes in rats provides a reliable and well-established model for studying the deleterious effects of diabetes on multiple systems.[36]

The diabetic status induced by the administration of STZ was confirmed by elevated levels of serum glucose, nitric oxide and MDA. The administration of a single dose of STZ initiates an autoimmune process leading to the destruction of the Langerhans islet beta cells, resulting in the development of clinical diabetes within a short period (2–4 days) and the induction of oxidative stress.[37]

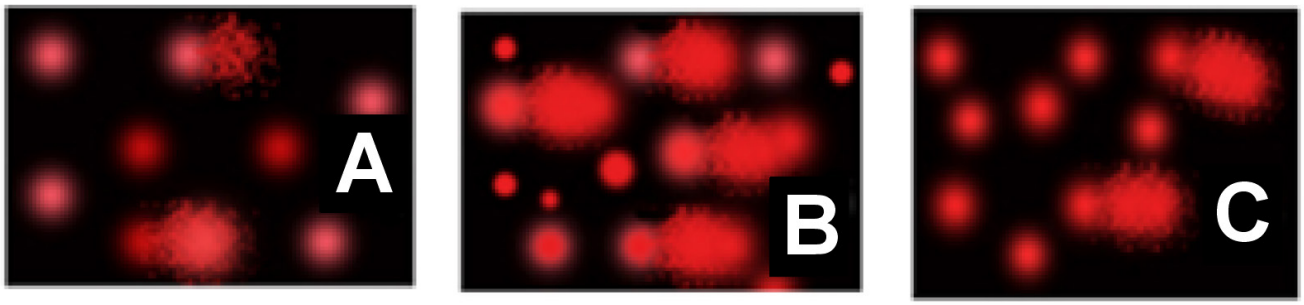

Fig. 5. Representative comet assay images of acinar cells from the parotid salivary glands

A. Group I; B. Group II; C. Group III.
The dark, round areas represent intact DNA without migration, whereas the brighter comet-shaped areas adjacent to the nucleus represent DNA damage.

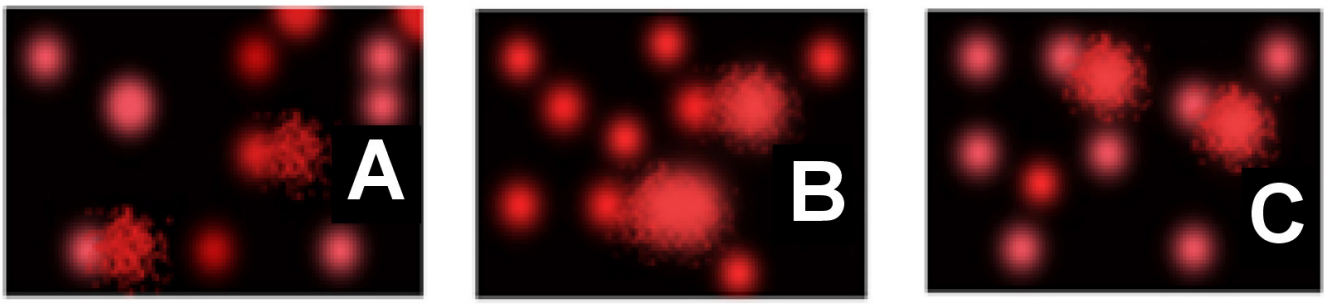

Fig. 6. Representative comet assay images of acinar cells from the mandibular mucosal salivary glands

A. Group I; B. Group II; C. Group III.
The dark, round areas represent intact DNA without migration, whereas the brighter comet-shaped areas adjacent to the nucleus represent DNA damage.

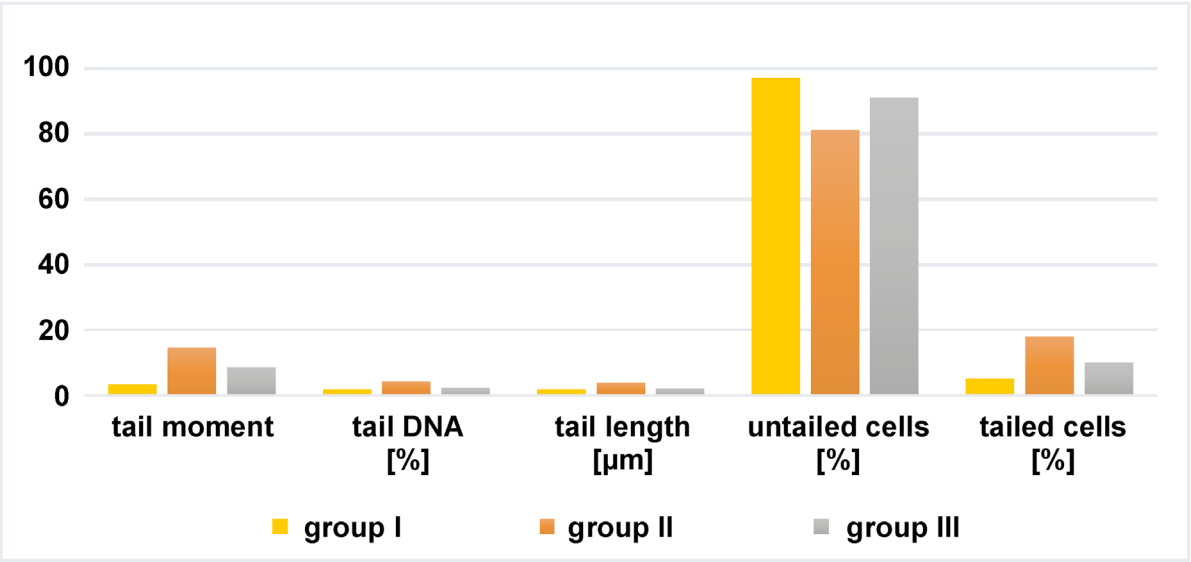

Fig. 7. Column charts showing mean values of comet assay parameters in the parotid salivary glands across all groups

Tail moment was calculated based on the tail length and tail DNA.

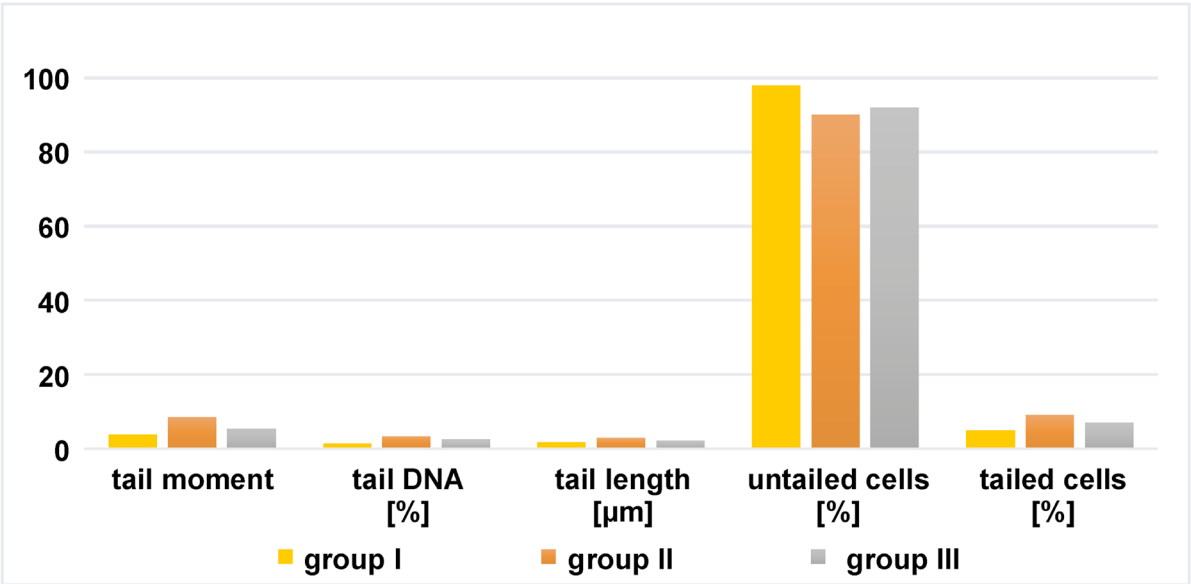

Fig. 8. Column chart showing mean values of comet assay parameters in the mandibular mucosal salivary glands across all groups

Table 5. Comet assay results in the parotid salivary glands across the groups

Variable	Group I	Group II	Group III
Tail moment	3.45 ±0.52	14.53 ±2.65[a]	8.62 ±1.33[ab]
Tail DNA [%]	1.76 ±0.49	4.31 ±0.63[a]	2.37 ±0.34[b]
Tail length [μm]	1.90 ±0.37	3.72 ±0.50[a]	2.05 ±0.45[b]
Untailed cells [%]	97.00 ±13.73	81.00 ±11.50	91.00 ±14.00
Tailed cells [%]	5.00 ±0.63	18.00 ±2.17[a]	10.00 ±1.52[ab]

Data expressed as *M* ±*SD*; [a] statistically significant compared to group I (control group); [b] statistically significant compared to group II (one-way ANOVA followed by Tukey's post hoc test). Tail moment was calculated based on the tail length and tail DNA.

Table 6. Comet assay results in the mandibular salivary glands across the groups

Variable	Group I	Group II	Group III
Tail moment	3.72 ±0.82	8.53 ±2.65[a]	5.32 ±1.33[ab]
Tail DNA [%]	1.36 ±0.49	3.31 ±0.21[a]	2.47 ±0.30[b]
Tail length [μm]	1.81 ±0.17	2.82 ±0.70[a]	2.05 ±0.45[b]
Untailed cells [%]	98.00 ±13.73	90.03 ±11.50	92.00 ±34.00
Tailed cells [%]	5.00 ±0.43	9.00 ±1.17[a]	7.00 ±1.52[ab]

Data expressed as *M* ±*SD*; [a] statistically significant compared to group I (control group); [b] statistically significant compared to group II (one-way ANOVA followed by Tukey's post hoc test).

In the current study, the parotid gland specimens in group II demonstrated loss of normal acinar architecture with degenerative changes. The nuclei were irregularly arranged, with variable sizes and shapes. The intralobular ducts revealed deterioration and loss of normal structure. These findings are consistent with results of study by Cui et al., who reported serous acinar cell distortion and disintegration with reduced secretory granules, vacuolization and leukocyte invasion in the salivary glands of diabetic rats.[38] In the present study, the observed changes in blood vessels may be attributed to the inflammatory processes associated with DM, as stated by Ror et al.[39]

In group II, the mandibular mucosal gland sections demonstrated acinar atrophy with loss of normal architecture, as well as deterioration and structural disruption of the intralobular ducts, which is in agreement with the findings of Stewart et al., who confirmed that diabetes is associated with hypofunction and atrophy of the parotid and mandibular mucosal salivary glands in diabetic rats due to reduced nitric oxide synthase activity, which is important for the proper functioning of salivary glands.[40] These findings may be explained by the deleterious effects of chronic hyperglycemia, which promote oxidative stress.[41] Oxidative stress results from multiple pathways, including glucose autoxidation, activation of the polyol pathway, and the formation of advanced glycation end-products (AGEs).[42] Other circulating factors reported to be elevated during the course of diabetics, namely free fatty acids and leptin, are also associated with the increased production of ROS.[43]

Krippeit-Drews et al. explained the mechanisms of oxidative stress-induced cell injury through both indirect and direct pathways.[44] Indirect pathways include the release of calcium from intracellular stores and its influx across the plasma membrane. The increased number of cytosolic calcium ions activates enzymes responsible for cellular damage, including phospholipases, proteases, endonucleases, and adenosine triphosphatases (ATPases), thereby accelerating ATP reduction. This ATP depletion causes structural disruption of protein synthesis. Moreover, ATP reduction impairs the activity of the plasma membrane energy-dependent sodium pump, leading to intracellular aggregation of Na^+ and loss of K^+, resulting in cell enlargement and dilation of the endoplasmic reticulum.[44] Hyperglycemia may also impair the function of sodium–glucose cotransporters and aquaporins, affecting cellular water balance.[45]

Conversely, direct pathways include peroxidation of polyunsaturated membrane lipids, which are susceptible to attack by oxygen-derived free radicals. Lipid radical interactions generate peroxides that compromise the integrity of cellular and nuclear membranes. This permits oxygen radicals to attack chromatin, leading to DNA disintegration. Additionally, free radicals react with thymine in nuclear and mitochondrial DNA, causing single-strand breaks. This DNA damage contributes to cellular aging and apoptosis.[46]

Whole chia seeds were administered in the form of flour since the particle size directly influences digestion and metabolic processes.[47] Furthermore, whole seed flour retains all structural components of the grain, including endosperm, bran, germ, and coat.[48] Moreover, grinding enhances the bioaccessibility of bioactive antioxidants, such as phenolic acids, thereby increasing their potential health benefits.[49]

In group III, which was subjected to diabetic induction and treated with chia seeds, the parotid and mandibular mucosal glands showed improvement in acinar architecture and intralobular duct structure. These findings indicate that chia seeds might have an ameliorative effect on salivary glands affected by diabetes-induced degeneration. Moreover, Enes et al. reported similar findings regarding the effects of chia seeds on glucose metabolism in both in vitro and in vivo studies.[34] The authors demonstrated that chia flour and chia oil decreased adiposity; however, only chia oil was able to improve glucose tolerance and restore the energy fuel system in the liver of rats fed a high-fat high-fructose (HFHF) diet. In addition, chia hydroalcoholic protein extract (CHPE) decreased the mRNA expression levels of gluconeogenic enzymes.[34]

In addition, de Paula Dias Moreira et al. stated that the inclusion of chia flour or chia oil in an unbalanced diet rich in saturated fat and fructose promoted beneficial effects on liver health by reducing metabolic disturbances through modulation of different metabolic pathways.[50] Chia flour and chia oil restored antioxidant system activity and enhanced fatty acid oxidation. Furthermore, chia flour modulated lipogenesis, whereas chia oil improved blood glucose levels, triglyceride (TG) levels and body weight.[50]

Chia seeds contain a wide range of antioxidant compounds, including vitamins C and E, carotenoids, phenolic compounds, and polyphenols such as flavonoids.[51] The flavonoid group with antioxidant activity includes flavones, flavonols, isoflavones, catechins, and chalcones. Micronutrients present in plants, such as vitamins A, C and E, folic acid, carotenoids, anthocyanins, and polyphenols, can scavenge free radicals and may serve as natural alternatives to synthetic antioxidants.[52]

Furthermore, chia is one of the most notable natural sources of omega-3 fatty acids, which inhibit the production of prostaglandins and pro-inflammatory cytokines, thereby modulating the inflammatory response and indirectly reducing cellular damage.[53] Additionally, the high levels of α-linolenic acid support the function of the epithelial barrier by maintaining the integrity of intercellular junction proteins.[54]

In a study conducted by Enes et al., the extraction of chia seeds into chia oil was shown to significantly reduce blood glucose levels.[55] The consumption of chia seeds has been associated with the restoration of lipids and membrane fatty acid translocase (FAT/CD36) in the plasma membrane, as well as with enhanced mitochondrial function and beta-oxidation.[56] The distribution of lipids may improve glucose tolerance by reducing body adiposity, an effect that has been associated with α-lipoic acid. Alpha-lipolic acid acts as an activator of adenosine monophosphate-activated protein kinase (AMPK). The activation of AMPK reduces blood glucose levels by promoting the phosphorylation of Akt substrate of 160 kDa (AS160), a protein active in glucose transporters, thereby enhancing insulin-dependent glucose uptake.[57]

A study conducted by Hassan Mahmoud et al. further supported the antioxidant activity of chia seeds.[26] The authors investigated the impact of low-level laser therapy (LLLT) and chia seeds on the alleviation of photoreceptor aberrations in experimental diabetic retinopathy (DR) and reported that the association between LLLT and the antioxidant action of chia seeds accelerated the recovery of retinal photoreceptors. They demonstrated that in DR, the production of ROS is elevated during the early stages of diabetes, leading to structural damage to mitochondria in photoreceptors. Additionally, persistent hyperglycemia results in the excessive production of ROS, continuous mitochondrial damage and elevated oxidative stress. Chia seeds reduced oxidative stress and hastened photoreceptor layer (PRL) mitigation, likely due to their antioxidant properties.[26]

Vascular endothelial growth factor has a pleiotropic role in tissue repair via angiogenesis, re-epithelialization and maintenance of extracellular matrix balance.[58] In group II, the expression of VEGF in acinar and ductal cells was reduced compared to groups I and III, which may be attributed to the adverse effects of diabetes on vascular function, including increased endothelial permeability to macromolecules and impaired endothelial proliferation.[59] Radwan et al. also investigated the antidiabetic effect of bee venom compared with bone marrow mesenchymal stem cells on the rat tongue using VEGF as an indicator of regeneration. Comparable results were obtained, as VEGF expression in the diabetic group was lower than in the treated groups.[60] Diabetes also disrupts the balance between cell proliferation and apoptosis.[61] Studies on cutaneous wound healing in diabetic models have shown delayed re-epithelialization, absence of growth factors and decreased angiogenesis.[62]

The alkaline comet assay is utilized as a conventional technique for the identification of DNA damage in genotoxicity testing and human biomonitoring.[63] Furthermore, it is an accepted method in ecotoxicology and environmental monitoring for the study of various animal and plant species.[64] Hyperglycemia in DM increases the production of ROS, thus enhancing endothelial cell apoptosis in vivo and in vitro, promoting free radical formation and reducing antioxidant capacity due to glycation and lipid oxidation reactions.[65] Oxidative modifications to DNA occur due to alterations of nucleotide bases and sugars, as well as the formation of cross-links, leading initially to base oxidation and fragmentation, followed by single- and double-strand breaks, interstrand and intrastrand cross-links, and eventually DNA–protein cross-links and sugar fragmentation products.[66]

In the current study, group II of both glands presented increased DNA damage, as evidenced by greater DNA tail length, higher percentage of tail DNA, and increased tail moment (defined as the product of tail length and the fraction of total DNA in the tail) compared to group I. In group III, DNA damage was markedly reduced following treatment with chia seeds compared to group II, indicating that the extent of oxidative stress correlates with the size of comet tail. These findings confirm the ability of chia seeds to ameliorate the deleterious effects of DM on salivary glands, as reflected by the improvement in acinar and ductal architecture. Moreover, the phenolic compounds present in chia seeds include flavonols and phenolic acids, such as myricetin, quercetin, kaempferol, and caffeine acid.[67] These compounds act as primary and synergistic antioxidants, contributing to the overall antioxidant activity of chia seeds. Quercetin, in particular, is a powerful antioxidant that inhibits the oxidation of fats, proteins and DNA, and exhibits stronger antioxidant activity compared to other flavonoids.[68]

Limitations

The present research was conducted using an experimental animal model. Although such model provides invaluable insights, findings of the study cannot be directly extrapolated to humans. Moreover, the study did not include the monitoring of blood glucose levels before, during and after the administration of chia seeds.

Conclusions

The immunohistochemical and genotoxic results obtained from the present study demonstrated that diabetes induction leads to significant structural changes in the parotid and mandibular mucosal glands in rats, primarily through oxidative DNA damage. Treatment with chia seeds ameliorated these changes, likely due to their antioxidant properties. Chia seeds showed promising effects in reducing oxidative stress. However, further studies are required, including investigations with different treatment durations and dosages, to support the use of chia as a dietary supplement in the management of DM. Further research should also evaluate long-term safety, identify bioactive compounds, and elucidate the underlying mechanisms. Additionally, studies should assess the effects of chia seeds on salivary gland function (flow rate and composition) to better understand their potential clinical relevance. The interactions between the various bioactive compounds of chia seeds, as well as the specific compound or synergistic effect responsible for their observed health benefits, remain unclear.

Ethics approval and consent to participate

The study was approved by the Institutional Animal Care at Faculty of Medicine, Mansoura University, Egypt (approval No. A 11070898). The research was conducted in accordance with the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines and regulations (https://arriveguidelines.org). The maintenance and care of the experimental animals adhered to the International Guiding Principles for Biomedical Research Involving Animals.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Bakianian Vaziri P, Vahedi M, Mortazavi H, Abdollahzadeh S, Hajilooi M. Evaluation of salivary glucose, IgA and flow rate in diabetic patients: A case–control study. *J Dent (Tehran)*. 2010;7(1):13–18. PMID:21998770.
2. Soares MSM, Batista-Filho MMV, Pimentel MJ, Passos IA, Chimenos-Küstner E. Determination of salivary glucose in healthy adults. *Med Oral Patol Oral Cir Bucal*. 2009;14(10):e510–e513. doi:10.4317/medoral.14.e510
3. Park J, Jang HJ. Anti-diabetic effects of natural products an overview of therapeutic strategies. *Mol Cell Toxicol*. 2017;13(1):1–20. doi:10.1007/s13273-017-0001-1
4. Attia MA, Hassan SS. Expression of cytokeratin, actin and proliferating cell nuclear antigen (PCNA) in parotid gland of normal and alloxan-induced diabetic dogs. (Histological and immunohistochemical study). *Cairo Dent J*. 2015;31(2):2661–2674.
5. Wang B, Du J, Zhu Z, Ma Z, Wang S, Shan Z. Evaluation of parotid salivary glucose level for clinical diagnosis and monitoring type 2 diabetes mellitus patients. *Biomed Res Int*. 2017;2017:2569707. doi:10.1155/2017/2569707
6. Peralta I, Marrassini C, Barreiro Arcos ML, Cremaschi G, Alonso MR, Anesini C. *Larrea divaricata* Cav. aqueous extract and nordihydroguaiaretic acid modulate oxidative stress in submandibular glands of diabetic rats: A buccal protective in diabetes. *BMC Complement Altern Med*. 2019;19:227. doi:10.1186/s12906-019-2636-z
7. Mauri-Obradors E, Estrugo-Devesa A, Jané-Salas E, Viñas M, López-López J. Oral manifestations of diabetes mellitus. A systematic review. *Med Oral Patol Oral Cir Bucal*. 2017;22(5):e586–e594. doi:10.4317/medoral.21655
8. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. *Med Oral Patol Oral Cir Bucal*. 2006;11(4):E309–E314. PMID:16816810.
9. Rohani B. Oral manifestations in patients with diabetes mellitus. *World J Diabetes*. 2019;10(9):485–489. doi:10.4239/wjd.v10.i9.485
10. Kaludercic N, Di Lisa F. Mitochondrial ROS formation in the pathogenesis of diabetic cardiomyopathy. *Front Cardiovasc Med*. 2020;7:12. doi:10.3389/fcvm.2020.00012
11. Knaś M, Maciejczyk M, Daniszewska I, et al. Oxidative damage to the salivary glands of rats with streptozotocin-induced diabetes-temporal study: Oxidative stress and diabetic salivary glands. *J Diabetes Res*. 2016;2016:4583742. doi:10.1155/2016/4583742
12. César M, Justo R, Vidal J, Bonel M, Migowski E, Cisne R. The use of drugs for the treatment of xerostomia – brief review. *IJOBAS*. 2016;5(4):238–244. doi:10.14419/IJBAS.V5I4.6708
13. American Diabetes Association. Standards of medical care in diabetes – 2009. *Diabetes Care*. 2009;32(Suppl 1):S13–S61. doi:10.2337/dc09-S013
14. Campbell-Tofte JI, Mølgaard P, Winther K. Harnessing the potential clinical use of medicinal plants as anti-diabetic agents. *Bot Targets Ther*. 2012;2:7–19. doi:10.2147/BTAT.S17302
15. Allen DA, Yaqoob MM, Harwood SM. Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications. *J Nutr Biochem*. 2005;16(12):705–713. doi:10.1016/j.jnutbio.2005.06.007
16. Shukla A, Bukhariya V, Mehta J, et al. Herbal remedies for diabetes: An overview. *Int J Biomed Adv Res*. 2011;2(1):57–68.
17. Gupta P, Geniza M, Elser J, et al. Reference genome of the nutrition-rich orphan crop chia (*Salvia hispanica*) and its implications for future breeding. *Front Plant Sci*. 2023;14:1272966. doi:10.3389/fpls.2023.1272966
18. Ayerza R. The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (*Salvia hispanica* L.) as affected by environmental factors. *J Oleo Sci*. 2009;58(7):347–354. doi:10.5650/jos.58.347

19. da Silva BP, Anunciação PC, Matyelka JCS, Della Lucia CM, Martino HSD, Pinheiro-Sant'Ana HM. Chemical composition of Brazilian chia seeds grown in different places. *Food Chem*. 2017;221:1709–1716. doi:10.1016/j.foodchem.2016.10.115
20. Hamedi A, Jamshidzadeh A, Ahmadi S, Sohrabpour M, Zarshenas MM. *Salvia macrosiphon* seeds and seed oil: Pharmacognostic, anti-inflammatory and analgesic properties. *Res J Pharmacogn*. 2016;3(4):27–37. https://www.rjpharmacognosy.ir/article_33342_a9a7d9989a71bd19ec1969b5127ae616.pdf. Accessed February 1, 2024.
21. Scapin G, Schmidt MM, Prestes RC, Rosa CS. Phenolics compounds, flavonoids and antioxidant activity of chia seed extracts (*Salvia hispanica*) obtained by different extraction conditions. *Int Food Res J*. 2016;23(6):2341–2346. https://www.cabidigitallibrary.org/doi/full/10.5555/20173079231. Accessed February 1, 2016.
22. Toscano LT, da Silva CSO, Toscano LT, de Almeida AEM, Santos AC, Silva AS. Chia flour supplementation reduces blood pressure in hypertensive subjects. *Plant Foods Hum Nutr*. 2014;69(4):392–398. doi:10.1007/s11130-014-0452-7
23. Tavares Toscano L, Tavares Toscano L, Leite Tavares R, da Oliveira Silva CS, Silva AS. Chia induces clinically discrete weight loss and improves lipid profile only in altered previous values. *Nutr Hosp*. 2014;31(3):1176–1182. doi:10.3305/nh.2015.31.3.8242
24. Fonte-Faria T, Citelli M, Atella GC, et al. Chia oil supplementation changes body composition and activates insulin signaling cascade in skeletal muscle tissue of obese animals. *Nutrition*. 2019;58:167–174. doi:10.1016/j.nut.2018.08.011
25. Vuksan V, Whitman D, Sievenpiper JL, et al. Supplementation of conventional therapy with the novel grain Salba (*Salvia hispanica L.*) improves major and emerging cardiovascular risk factors in type 2 diabetes: Results of a randomized controlled trial. *Diabetes Care*. 2007;30(11):2804–2810. doi:10.2337/dc07-1144
26. Hassan Mahmoud AR, Abdelkawi SA, Ghoneim DF, Hassan AA, Morsy ME. Mitigation of photoreceptors abnormalities after low-level laser therapy and chia seeds supplementation in experimental diabetic retinopathy. *Oman J Ophthalmol*. 2022;15(3):347–352. doi:10.4103/ojo.ojo_251_21
27. Alqahtani MS, Hassan SS. Immunohistochemical evaluation of the pathological effects of diabetes mellitus on the major salivary glands of albino rats. *Eur J Dent*. 2023;17(2):485–491. doi:10.1055/s-0042-1749159
28. Dhawan A, Bajpayee M, Pandey AK, Parmar D. Protocol for the single cell gel electrophoresis/comet assay for rapid genotoxicity assessment. ITRC: THE SCGE/COMET ASSAY PROTOCOL. 2009:1–10.
29. Nandhakumar S, Parasuraman S, Shanmugam MM, Ramachandra R, Parkash C, Vishnu Bhat B. Evaluation of DNA damage using single-cell gel electrophoresis (comet assay). *J Pharmacol Pharmacother*. 2011;2(2):107–111. doi:10.4103/0976-500X.81903
30. Bancroft JD, Layton C. The hematoxylin and eosin. In: Suvarna SK, Layton C, Bancroft JD, eds. *Theory and Practice of Histological Techniques*. 7th ed. Philadelphia, PA: Churchill Livingstone; 2013:173–186. doi:10.1016/B978-0-7020-4226-3.00010-X
31. Al-Dissi AN, Haines DM, Singh B, Kidney BA. Immunohistochemical expression of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 in canine simple mammary gland adenocarcinomas. *Can Vet J*. 2010;51(10):1109–1114. PMID:21197202.
32. Belkacemi L, Selselet-Attou G, Hupkens E, et al. Intermittent fasting modulation of the diabetic syndrome in streptozotocin-injected rats. *Int J Endocrinol*. 2012;2012:962012. doi:10.1155/2012/962012
33. Hie M, Shimono M, Fujii K, Tsukamoto I. Increased cathepsin K and tartrate-resistant acid phosphatase expression in bone of streptozotocin-induced diabetic rats. *Bone*. 2007;41(6):1045–1050. doi:10.1016/j.bone.2007.08.030
34. Enes BN, Moreira LPD, Toledo RCL, et al. Effect of different fractions of chia (*Salvia hispanica* L.) on glucose metabolism, in vivo and in vitro. *J Funct Foods*. 2020;71:104026. doi:10.1016/j.jff.2020.104026
35. Balasubramanyam M, Adaikalakoteswari A, Sameermahmood Z, Mohan V. Biomarkers of oxidative stress: Methods and measures of oxidative DNA damage (COMET assay) and telomere shortening. *Methods Mol Biol*. 2010;610:245–261. doi:10.1007/978-1-60327-029-8_15
36. Thrailkill KM, Nyman JS, Bunn RC, et al. The impact of SGLT2 inhibitors, compared with insulin, on diabetic bone disease in a mouse model of type 1 diabetes. *Bone*. 2017;94:141–151. doi:10.1016/j.bone.2016.10.026
37. Turner S, Zettler G, Barreiro Arcos ML, Cremaschi G, Davicino R, Anesini C. Effect of streptozotocin on reactive oxygen species and antioxidant enzymes's secretion in rat submandibulary glands: A direct and an indirect relationship between enzyme activation and expression. *Eur J Pharmacol*. 2011;659(2–3):281–288. doi:10.1016/j.ejphar.2011.03.015
38. Cui F, Hu M, Li R, et al. Insulin on changes in expressions of aquaporin-1, aquaporin-5, and aquaporin-8 in submandibular salivary glands of rats with streptozotocin-induced diabetes. *J Clin Exp Pathol*. 2021;14(2):221–229. PMID:33564354.
39. Ror FN, Col LO, Moura EG, Jaciara MA, Caldeira EJ. N-acetylcysteine, anti-CD4/CD8 antibodies, and physical exercise reduces histopathological damage in salivary glands of spontaneously diabetic mice. *Austin Endocrinol Diabetes Case Rep*. 2018;3(1):1013–1018. https://austinpublishinggroup.com/diabetes-case-reports/fulltext/diabetescrv3-id1013.pdf. Accessed March 1, 2021.
40. Stewart CR, Obi N, Epane EC, et al. Effects of diabetes on salivary gland protein expression of tetrahydrobiopterin and nitric oxide synthesis and function. *J Periodontol*. 2016;87(6):735–741. doi:10.1902/jop.2016.150639
41. Mednieks MI, Szczepanski A, Clark B, Hand AR. Protein expression in salivary glands of rats with streptozotocin diabetes. *Int J Exp Pathol*. 2009;90(4):412–422. doi:10.1111/j.1365-2613.2009.00662.x
42. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. *Free Radic Biol Med*. 2006;40(2):183–192. doi:10.1016/j.freeradbiomed.2005.06.018
43. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J*. 2009;417(1):1–13. doi:10.1042/BJ20081386
44. Krippeit-Drews P, Kramer C, Welker S, Lang F, Ammon HP, Drews G. Interference of H_2O_2 with stimulus-secretion coupling in mouse pancreatic beta-cells. *J Physiol*. 1999;514(Pt 2):471–481. doi:10.1111/j.1469-7793.1999.471ae.x
45. El Sadik A, Mohamed E, El Zainy A. Postnatal changes in the development of rat submandibular glands in offspring of diabetic mothers: Biochemical, histological and ultrastructural study. *PLoS One*. 2018;13(10):e0205372. doi:10.1371/journal.pone.0205372
46. Pizzimenti S, Toaldo C, Pettazzoni P, Dianzani MU, Barrera G. The "two-faced" effects of reactive oxygen species and the lipid peroxidation product 4-hydroxynonenal in the hallmarks of cancer. *Cancers (Basel)*. 2010;2(2):338–363. doi:10.3390/cancers2020338
47. Slavin J. Why whole grains are protective: Biological mechanisms. *Proc Nutr Soc*. 2003;62(1):129–134. doi:10.1079/PNS2002221
48. Arvola A, Lähteenmäki L, Dean M, et al. Consumers' beliefs about whole and refined grain products in the UK, Italy and Finland. *J Cereal Sci*. 2007;46(3):197–206. doi:10.1016/j.jcs.2007.06.001
49. Rosa NN, Dufour C, Lullien-Pellerin V, Micard V. Exposure or release of ferulic acid from wheat aleurone: Impact on its antioxidant capacity. *Food Chem*. 2013;141(3):2355–2362. doi:10.1016/j.foodchem.2013.04.132
50. de Paula Dias Moreira L, Nery Enes VPB de SJ B, Celi Lopes Toledo R, et al. Chia (*Salvia hispanica* L.) flour and oil ameliorate metabolic disorders in the liver of rats fed a high-fat and high fructose diet. *foods*. 2022;11(3):285. doi:10.3390/foods11030285
51. Baynes JW, Dominiczak MH. *Medical Biochemistry*. 4th ed. Elsevier Saunders; 2015;377.
52. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Kader AA. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J Agric Food Chem*. 2002;50(17):4976–4982. doi:10.1021/jf020136b
53. Singh M, Tonk RS. Management of salivary hypofunction. *Pharm Anal Acta*. 2015;6:2. doi:10.4172/2153-2435.1000331
54. Xiao K, Liu C, Qin Q, et al. EPA and DHA attenuate deoxynivalenol-induced intestinal porcine epithelial cell injury and protect barrier function integrity by inhibiting necroptosis signaling pathway. *FASEB J*. 2020;34(2):2483–2496. doi:10.1096/fj.201902298R
55. Enes BN, Moreira LPD, Silva BP, et al. Chia seed (*Salvia hispanica* L.) effects and their molecular mechanisms on unbalanced diet experimental studies: A systematic review. *J Food Sci*. 2020;85(2):226–239. doi:10.1111/1750-3841.15003

56. Gumantara MPB, Oktarlina RZ. Perbandingan monoterapi dan kombinasi terapi sulfonilurea-metformin terhadap pasien diabetes melitus tipe 2. *Majority*. 2017;6(1):55–59. https://www.semanticscholar.org/paper/Perbandingan-Monoterapi-dan-Kombinasi-Terapi-Pasien-Gumantara-Oktarlina/a0feefc0d90d1697a49a94aba3bf2c3fa38ec9e3. Accessed February 1, 2017.
57. Husna F, Suyatna FD, Arozal W, Purwaningsih EH. Model Hewan Coba pada Penelitian Diabetes. *Pharm Sci Res*. 2019;6(3):131–141. doi:10.7454/psr.v6i3.4531
58. Keswani SG, Balaji S, Le LD, et al. Role of salivary vascular endothelial growth factor (VEGF) in palatal mucosal wound healing. *Wound Repair Regen*. 2013;21(4):554–562. doi:10.1111/wrr.12065
59. Perrotti V, Piattelli A, Piccirilli M, Bianchi G, Di Guilio C, Artese L. Vascular endothelial growth factor expression (VEGF) in salivary glands of diabetic rats. *Int J Immunopathol Pharmacol*. 2007;20(1 Suppl 1): 55–60. doi:10.1177/039463200702001s12
60. Radwan IA, Rady D, Ramadan M, El Moshy S. Mechanism underlying the anti-diabetic potential of bee venom as compared to bone marrow mesenchymal stem cells in the diabetic rat tongue. *Dent Med Probl*. 2024;61(1):53–64. doi:10.17219/dmp/152924
61. Desta T, Li J, Chino T, Graves DT. Altered fibroblast proliferation and apoptosis in diabetic gingival wounds. *J Dent Res*. 2010;89(6):609–614. doi:10.1177/0022034510362960
62. Peplow PV, Baxter GD. Gene expression and release of growth factors during delayed wound healing: A review of studies in diabetic animals and possible combined laser phototherapy and growth factor treatment to enhance healing. *Photomed Laser Surg*. 2012;30(11):617–636. doi:10.1089/pho.2012.3312
63. Neri M, Milazzo D, Ugolini D, et al. Worldwide interest in the comet assay: A bibliometric study. *Mutagenesis*. 2015;30(1):155–163. doi:10.1093/mutage/geu061
64. Gajski G, Žegura B, Ladeira C, et al. The comet assay in animal models: From bugs to whales – (part 1 invertebrates). *Mutat Res Rev Mutat Res*. 2019;779:82–113. doi:10.1016/j.mrrev.2019.02.003
65. Prawitasari DS. Diabetes melitus dan antioksidan. *KELUWIH: Jurnal Kesehatan dan Kedokteran*. 2019;1(1):47–51. doi:10.24123/kesdok.V1i1.2496
66. Evans MD, Cooke MS. Factors contributing to the outcome of oxidative damage to nucleic acids. *Bioessays*. 2004;26(5):533–542. doi:10.1002/bies.20027
67. Grancieri M, Martino HSD, de Mejia EG. Chia seed (*Salvia hispanica* L.) as a source of proteins and bioactive peptides with health benefits: A review. *Compr Rev Food Sci Food Saf*. 2019;18(2):480–499. doi:10.1111/1541-4337.12423
68. Vuksan V, Jenkins AL, Brissette C, et al. Salba-chia (*Salvia hispanica* L.) in the treatment of overweight and obese patients with type 2 diabetes: A double-blind randomized controlled trial. *Nutr Metab Cardiovasc Dis*. 2017;27(2):138–146. doi:10.1016/j.numecd.2016.11.124

Original paper

Effect of dynamic fatigue loading on the adaptation and retention of maxillary single dentures reinforced with digitally constructed zirconia and cobalt–chromium frameworks

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Abstract

Background. The impact of dynamic fatigue loading on denture adaptation and retention is clinically important. Zirconia and cobalt–chromium (Co–Cr) frameworks enhance denture strength, but their performance under cyclic loading remains unclear.

Objectives. The aim of the study was to evaluate the adaptation and retention of maxillary single dentures reinforced with digitally constructed Co–Cr or zirconia frameworks after a simulated 1 year of function.

Material and methods. Three types of maxillary single dentures were constructed on maxillary master casts. Group A included maxillary dentures without reinforcement (control group), whereas groups B and C encompassed maxillary dentures reinforced with digitally designed and milled Co–Cr and zirconia frameworks, respectively ($n = 13$/group). Acrylic resin was conventionally processed on stone casts. Initial adaptation was evaluated for each denture using Geomagic software, which allowed for accurate matching and superimposition of the scanned master cast with the standard tessellation language (STL) file of the scanned fitting denture surface obtained with the use of a desktop scanner. Initial retention was evaluated using a universal testing machine. Dynamic fatigue loading of 50 N at a speed of 60 mm/s was applied in the chewing simulator, after which final adaptation and retention values were recorded. Paired *t*-tests were conducted to compare results within each group, and one-way analysis of variance (ANOVA) was performed to compare the 3 groups. When ANOVA revealed significant differences, Tukey's honestly significant difference (HSD) test was used for post hoc pairwise comparisons. The significance level was set at $p \leq 0.05$.

Results. Group C showed the most favorable adaptation after dynamic fatigue loading (0.31 ±0.04 mm), while group B exhibited the least favorable adaptation (0.61 ±0.08 mm). Regarding retention, group C demonstrated the highest values (20.04 ±0.96 N), while group B exhibited the lowest values (9.32 ±0.78 N). Significant differences were observed among the 3 groups for both adaptation and retention.

Conclusions. Maxillary single dentures reinforced with zirconia frameworks may represent a successful alternative to Co–Cr frameworks for prosthetic rehabilitation using digital technology.

Keywords: zirconia, retention, adaptation, Co–Cr, reinforced maxillary single denture

Highlights

- Dynamic fatigue loading significantly reduces the performance of dental frameworks made from zirconia and cobalt–chromium alloys.
- Zirconia and cobalt–chromium exhibit different mechanical behaviors under dynamic stress conditions.
- Design modifications may help reduce the effects of fatigue and improve the durability of dental restorations.
- Consideration of dynamic loading conditions is important for material selection and treatment planning to improve patient outcomes.
- Further research on the fatigue behavior of dental materials is needed to optimize their performance in clinical applications.

Introduction

Maxillary single dentures opposing mandibular natural teeth pose challenges in achieving adequate comfort, retention, function, and esthetics.[1] The most common complication associated with maxillary dentures is fracture, especially in the presence of natural mandibular teeth. The predominant mode of failure is flexural fatigue caused by cyclic deformation due to masticatory forces, typically resulting in a midline fracture of the denture.[2,3]

The most commonly used material for the fabrication of dentures is acrylic resin, specifically polymethyl methacrylate (PMMA). Polymethyl methacrylate has gained widespread acceptance among patients due to its favorable mechanical, biological and esthetic qualities. Compared with metal denture bases, PMMA denture bases are easier to construct and repair.[1] However, PMMA dentures are susceptible to failure due to their low fatigue resistance. Midline fractures are a frequent complication, often resulting from occlusal disharmony, flexure and fatigue of the denture base.[1] Reinforcing materials can be used to enhance the mechanical properties of maxillary dentures. Therefore, the implementation of reinforced denture bases has been proposed as a potential solution.[4–7]

Cobalt–chromium (Co–Cr) alloys are primarily used as metal reinforcement materials to improve the stability and fracture resistance of single dentures. These alloys show excellent mechanical properties, biocompatibility and corrosion resistance.[8] However, their rough surface and susceptibility to internal defects, as well as difficult casting procedures may lead to unexpected complications.[8,9]

Advances in digital technology and the development of computer-aided design and computer-aided manufacturing (CAD/CAM) have enabled the production of Co–Cr prostheses through computer numerical control milling, eliminating casting-induced porosity and manufacturing defects.[10]

Recent trends in prosthetic dentistry favor metal-free restorations. Metal frameworks are heavier and require more complicated fabrication procedures, as well as pose a risk of hypersensitivity due to their alloy composition.[11]

A newer generation of zirconia, ceria-stabilized zirconia/alumina (Ce-TZP/Al_2O_3), is a nanocomposite in which nanometer-sized alumina particles and ceria-stabilized zirconia particles are dispersed within ceria-stabilized zirconia crystal and alumina crystal, respectively.[12,13] Ceria-stabilized zirconia/alumina demonstrates excellent mechanical properties, including high flexural strength, high fracture toughness and high resistance to low-temperature degradation.[14–17] These characteristics exceed those of conventional yttria-stabilized zirconia (Y-TZP), and its elastic modulus is comparable to that of Co–Cr alloys, suggesting potential applicability of Ce-TZP/Al_2O_3 as a denture base material and a possible substitute for metal alloys.[13]

The present study was conducted to assess whether reinforcement of a maxillary single denture with digitally constructed Co–Cr or zirconia frameworks affects denture retention and adaptation. Additionally, the effect of these frameworks after dynamic fatigue loading simulating 1 year of function was assessed using a chewing simulator. The null hypothesis posited that there are no variations between conventional and reinforced maxillary single dentures in terms of adaptation and retention.

Material and methods

Three types of maxillary single dentures were constructed on maxillary master casts obtained from patients requiring maxillary single dentures. The maxillary arch presented a U-shaped residual alveolar ridge without bony or soft tissue undercuts. Group A included conventional acrylic resin maxillary single dentures without reinforcement (control group), group B encompassed maxillary single dentures reinforced with a digitally designed and milled Co–Cr framework, and group C comprised maxillary single dentures reinforced with a digitally designed and milled zirconia framework (Fig. 1).

The study was reviewed and approved by the Research Ethics Committee of the Faculty of Dentistry, Ain Shams University, Cairo, Egypt (approval No. FDASU-RecID041914).



Fig. 1. Three types of maxillary single dentures evaluated in the study

Master cast preparation and maxillary single denture fabrication

Thirteen definitive master casts were obtained from the patients. The geometric center of each arch was determined. The midline of the cast was drawn from the center of the incisive papilla to a point located in the middle of a line connecting the 2 hamular notches. Then, a midpoint was marked on the midline to represent the center of the arch.[18,19] The definitive master casts were fixed on a plate inside a desktop scanner with their labial surfaces facing the interior of the scanner (D850; 3Shape, Copenhagen, Denmark). The scanning procedure followed the software protocol. Subsequently, the casts were duplicated using a silicone base duplication material (REPLISIL 22 N; dent-e-con, Lonsee, Germany) to obtain 39 duplicate stone master casts (n = 13/group), on which the 3 types of maxillary single dentures were constructed.

Acrylic unreinforced maxillary single dentures (group A) were constructed using the conventional method on the duplicate stone master casts and served as reference dentures to standardize the acrylic thickness and tooth positioning during the fabrication of dentures reinforced with Co–Cr and zirconia frameworks (groups B and C).[20] A rubber index of the maxillary teeth and the polished surface of the flanges was prepared for each acrylic denture.

The object replacement character (OBJ) files of the scanned definitive master casts were imported into the digital design software partial module (PartialCAD; exocad GmbH, Darmstadt, Germany) to design the reinforcement frameworks for groups B and C (Fig. 2). The frameworks were virtually relieved with a 0.5-mm thickness wax on the crest of the ridge with tissue stops and on the median palatine raphe. It was designed to cover the palatal area with an extended palatal plate, a major connector and a mesh denture base extending to the crest of the ridge. The framework thickness was set at 1.5 mm.[20] An attachment was placed at the geometric center of the polished surface of the framework, corresponding to the previously marked point on the cast (Fig. 3).

The standard tessellation language (STL) files of the design were imported into the CAM software (iCAM V5 smart; imes-icore GmbH, Eiterfeld, Germany) and milled in both Co–Cr and zirconia frameworks using a 5-axis dry and wet milling machine (CORiTEC 350i; imes-icore GmbH).

For Co–Cr framework milling (group B), the design was nested in a 98 mm × 18 mm pre-sintered Co–Cr blank (MoguCera C Disc; Scheftner, Mainz, Germany) (Fig. 4).

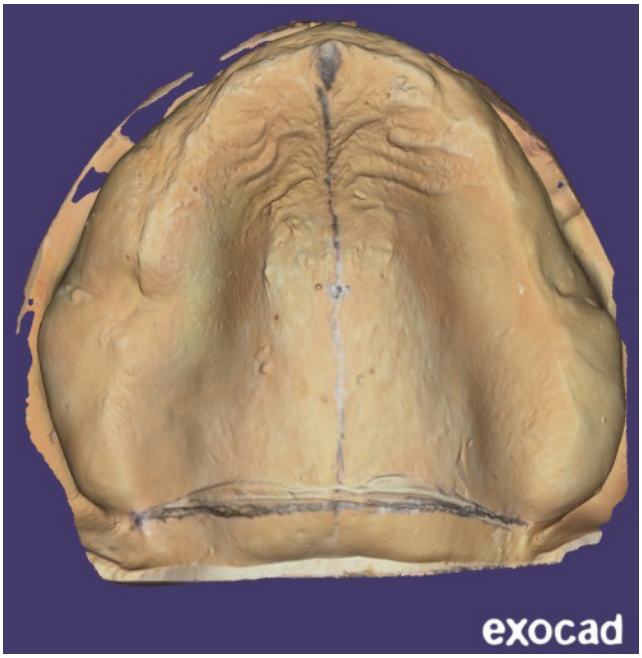


Fig. 2. Scanned definitive master cast

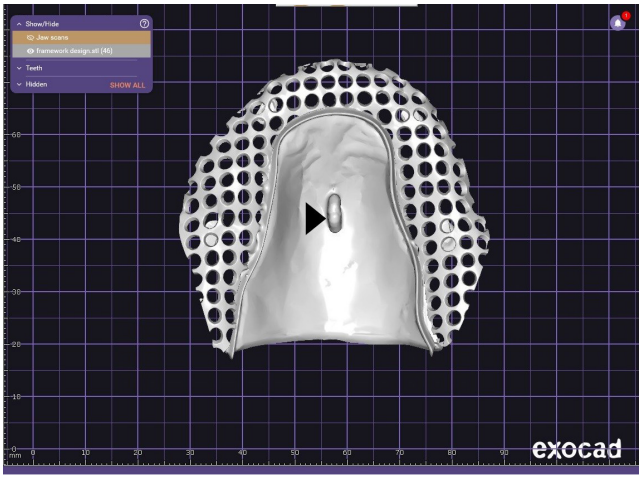


Fig. 3. Standard tessellation language (STL) file showing the framework design
The arrow indicates the attachment point located at the geometric center of the framework surface.

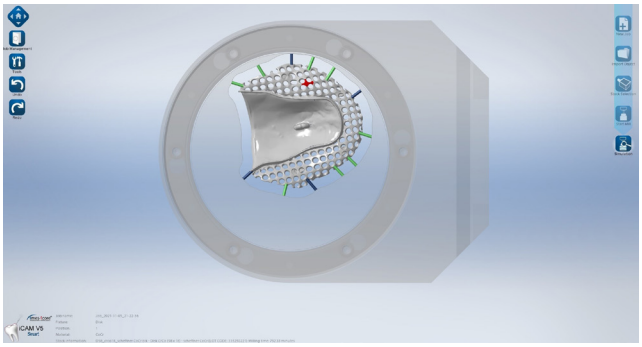


Fig. 4. Framework design nested in a cobalt–chromium (Co–Cr) blank for milling

Dry milling of the Co–Cr disk was performed using burs of different sizes. The average milling time was 752.33 min Polishing was performed with the use of a polishing lathe and cloth brushes.

For zirconia framework milling (group C), the design was nested in a 98 mm × 25 mm zirconia blank (Nacera®; DOCERAM Medical Ceramics GmbH, Dortmund, Germany) with a scale factor of 1.25 (Fig. 5). The zirconia disks were dry milled, with an average milling time of 300.23 min. Subsequently, complete sintering was performed in a furnace at 1,350–1,500°C to achieve their final shape.

With the aid of the rubber index, 2 reinforced maxillary dentures, with the same tooth position as conventional unreinforced dentures, were constructed on duplicate stone master casts. Zirconia and Co–Cr frameworks were fixed to the duplicated master casts using a quick-drying glue to prevent deformation. Teeth were set within the index, and the acrylic resin was carefully packed and processed in a conventional manner.[21]

Construction of resin casts with mucosa simulation

The definitive maxillary master casts were modified to construct resin casts with simulated mucosa, which served as models for the evaluation of retention and dynamic fatigue loading. A vacuum sheet was processed on the cast to fabricate a stent with 3 tissue stops. The cast surface was then reduced by 2 mm to create space for the tissue-simulating mask. The modified cast was duplicated to obtain 3 polyurethane epoxy resin casts (Exakto-Form; bredent medical GmbH & Co. KG, Senden, Germany) using a silicone mold. An adhesive was applied to the epoxy casts, and a silicone mucosa gingival mask with viscoelastic properties similar to those of palatal mucosa (Multisil; bredent medical GmbH & Co. KG)[22] was injected into the stent and pressed onto the epoxy cast to simulate the mucosa. Therefore, for each denture, an epoxy resin master cast with soft tissue simulation was constructed.

Evaluation of initial adaptation

All dentures were lightly coated with antiglare spray before scanning their fitting surfaces using the desktop scanner (D850; 3Shape) to obtain STL files. The STL files were imported into the Meshmixer software (https://meshmixer.org) to invert the fitting surfaces so that they resembled cast surfaces.[23]

The Geomagic software (Geomagic Control X64; Hexagon Manufacturing Intelligence, LLC, Morrisville, USA) was used to evaluate the adaptation of the maxillary denture bases to the scanned master cast. The STL files of the master cast and the inverted fitting surface were imported into the software. Then, the STL file of the denture fitting surface was superimposed onto the STL file

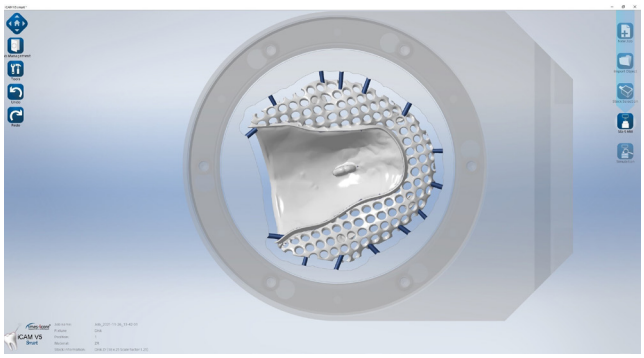
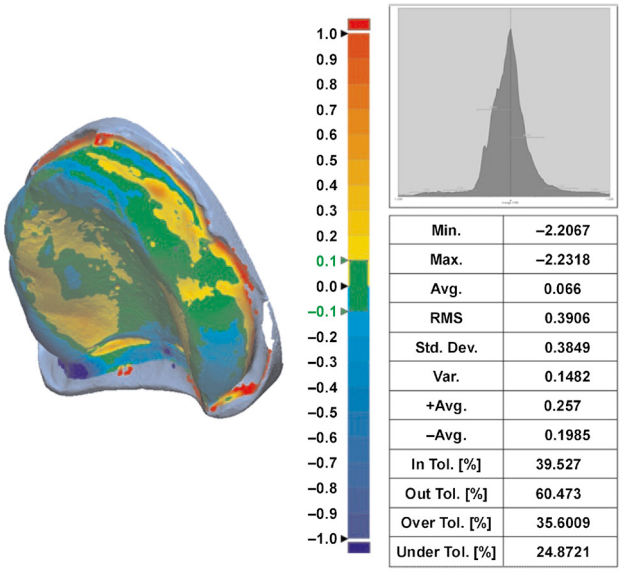


Fig. 5. Framework design nested in a zirconia blank for milling

of the orresponding master cast.[23] Alignment between the reference and measured data was performed automatically using the best-fit alignment option. The 3D Compare function was used to evaluate the adaptation of the measured data. The results were presented as both a color scale and numerical values (Fig. 6). Adaptation was interpreted based on the distance between the fitting surface of the denture base and the reference data.

Evaluation of initial retention

A metallic ring was attached to the geometric center of the polished surface of the denture bases using self-curing acrylic resin. Artificial saliva was prepared according to the Glandosane formula by the Laboratory of the Pharmaceutical Industry at the Faculty of Pharmacy, Ain



Min.	–2.2067
Max.	–2.2318
Avg.	0.066
RMS	0.3906
Std. Dev.	0.3849
Var.	0.1482
+Avg.	0.257
–Avg.	0.1985
In Tol. [%]	39.527
Out Tol. [%]	60.473
Over Tol. [%]	35.6009
Under Tol. [%]	24.8721

Fig. 6. Geomagic software analysis report for denture base adaptation

Cyan-to-blue colors indicate a gap between the denture base and the cast surface, yellow-to-red colors indicate the impingement of the denture base against the cast, and green indicates intimate contact between the two surfaces. Pressure areas are represented by positive deviation values, whereas gap areas are denoted by negative deviation values.
Min. – minimum; Max. – maximum; Avg. – average; RMS – root mean square; Std. Dev. – standard deviation; Var. – variability; In tol. – within tolerance; Under tol. – below tolerance; Over tol. – above tolerance; Out tol. – outside tolerance.

Shams University, Cairo, Egypt, and placed between the cast and the denture. A pressure of 98 N was applied to the dentures for 20 s to spread the artificial saliva uniformly across the entire mucosal surface of the denture base. The epoxy resin cast with mucosa simulation was secured to the lower component of a universal testing machine (Instron 3365; Instron®, Norwood, USA) with the palate facing upward. Tensile force was applied to the attached ring in an upward direction perpendicular to the imaginary occlusal plane (Fig. 7). The crosshead speed of the universal testing machine was set at 50 mm/min. Retention was defined as the maximum traction force required to separate the denture base from the maxillary cast. The test was conducted 10 times, and the mean value of the 10 measurements was recorded as the retention force.

Dynamic fatigue loading

Each denture was seated on its corresponding epoxy resin cast and placed in a chewing simulator (CS-4; SD Mechatronik, Feldkirchen-Westerham, Germany) (Fig. 8). Dynamic cyclic loading was applied by means of a stylus falling at the center of the metal plate that was previously attached to the occlusal surface of the denture at the center of the arch. The applied load was set at 50 N, and the software parameters were as follows: a speed of 60 mm/s; a vertical path of 3 mm; a horizontal path of 0.7 mm; and a frequency of 1.6 Hz. Each denture was subjected to biaxial cyclic loading for a total of 250,000 cycles, which represented 1 year of intraoral function.[21]

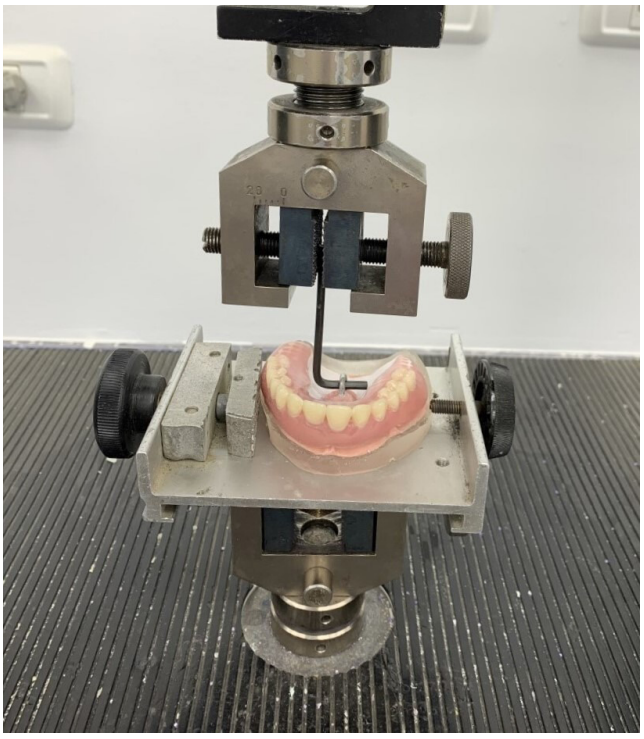


Fig. 7. Universal testing machine used for retention testing (Instron 3365; Instron®, Norwood, USA)

After the chewing simulation, all procedures for measuring adaptation and retention were repeated for the 3 groups as previously described.

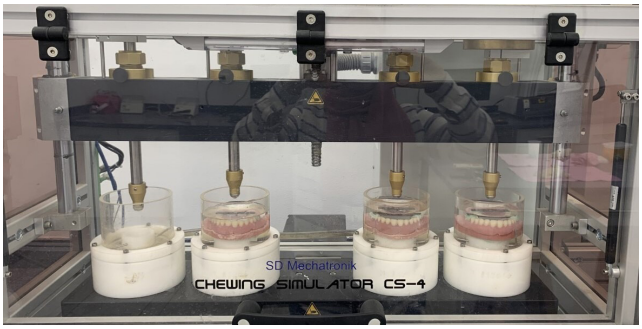


Fig. 8. Chewing simulator used for dynamic fatigue loading (CS-4; SD Mechatronik, Feldkirchen-Westerham, Germany)

Statistical analysis

The sample size was calculated using the G*Power software for Windows (v. 3.1.9.4; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). A sample size of 12 dentures per group was determined to detect significant differences with a statistical power of 95% ($\alpha = 0.05$). The sample size was subsequently increased to 13 dentures per group to compensate for potential dropouts.

The obtained data was recorded, tabulated and statistically analyzed. Paired *t*-tests were applied to compare results within each group, and one-way analysis of variance (ANOVA) was performed to compare the 3 groups. Tukey's honestly significant difference (HSD) test was used for post hoc pairwise comparisons when the results of ANOVA indicated significant differences. The significance level was set at $p \leq 0.05$. Statistical analyses were performed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA).

Results

Regarding the effect of dynamic fatigue loading on the adaptation within each group, the deviation from the scanned master cast increased in group A (conventional acrylic resin) and group B (Co–Cr framework), indicating reduced adaptation. In contrast, group C (zirconia framework) showed less deviation from the scanned master cast, indicating better adaptation. These changes were statistically significant according to the results of the paired *t*-test (Table 1, Fig. 9).

When adaptation was compared between the 3 groups before dynamic fatigue loading, only minimal differences were observed, with group C demonstrating slightly more favorable adaptation. However, these differences were not statistically significant according to ANOVA.

Table 1. Root mean square (RMS) deviation values for denture base adaptation before and after dynamic fatigue loading in the 3 groups

Dynamic fatigue loading	Group A (conventional acrylic resin)		Group B (Co–Cr framework)		Group C (zirconia framework)		*F*-value	*p*-value (ANOVA)
	M	*SD*	*M*	*SD*	*M*	*SD*		
Before loading	0.39	0.06	0.43	0.06	0.38	0.04	3.107	0.057
After loading	0.53[a]	0.07	0.61[b]	0.08	0.31[c]	0.04	63.458	<0.00001*
p-value (paired *t*-test)	<0.0001*		<0.0001*		0.0016*		–	

* statistically significant ($p \leq 0.05$); Co–Cr – cobalt–chromium. Different superscript letters indicate statistically significantly differences between the groups (Tukey's post hoc honestly significant difference (HSD) test). Mean (*M*) and standard deviation (*SD*) values are presented in millimeters [mm].

After dynamic fatigue loading, group C exhibited the best adaptation, while group B demonstrated the least favorable adaptation. These changes were statistically significant based on the outcomes of ANOVA. Tukey's post hoc HSD test revealed significant differences between groups A and B, groups A and C, and groups B and C (Table 1, Fig. 9).

Regarding the effect of dynamic fatigue loading on retention within each group, retention decreased in groups A and B after loading, whereas it increased in group C. These changes were statistically significant based on the results of the paired *t*-test (Table 2, Fig. 10).

Comparison of retention among the 3 groups before and after dynamic fatigue loading revealed that group C achieved the highest retention values, while group B exhibited the lowest retention. These differences were statistically significant according to ANOVA. Tukey's post hoc HSD test indicated significant differences between groups A and B, groups A and C, and groups B and C (Table 2, Fig. 10).

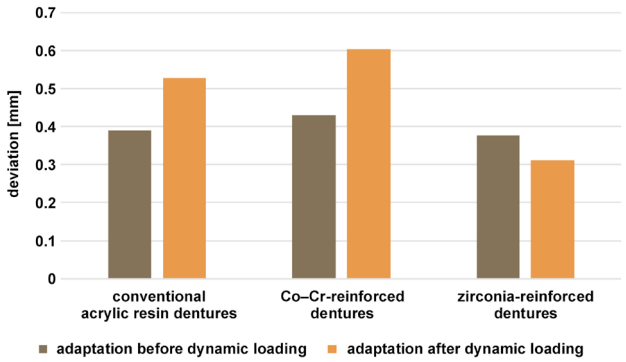


Fig. 9. Bar chart showing the root mean square (RMS) deviation values for denture base adaptation before and after dynamic fatigue loading in the 3 groups

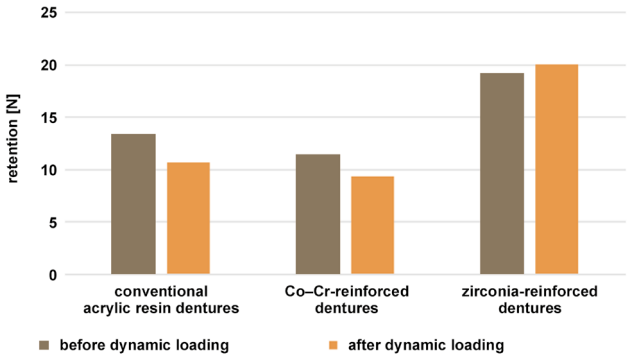


Fig. 10. Bar chart showing the mean retention values before and after dynamic fatigue loading in the 3 groups

Discussion

The present study aimed to evaluate the retention and adaptation of reinforced maxillary dentures fabricated using CAD/CAM with Co–Cr and zirconia frameworks, compared with conventional acrylic resin dentures, before and after dynamic fatigue loading. Adequate denture adaptation contributes to good retention, stability and support.[24]

Table 2. Retention values before and after dynamic fatigue loading

Dynamic fatigue loading	Group A (conventional acrylic resin)		Group B (Co–Cr framework)		Group C (zirconia framework)		*F*-value	*p*-value
	M	*SD*	*M*	*SD*	*M*	*SD*		
Before loading	13.34[a]	1.51	11.37[b]	0.74	19.19[c]	0.88	179.094	<0.0001*
After loading	10.66[a]	0.75	9.32[b]	0.78	20.04[c]	0.96	636.585	<0.00001*
p-value	<0.0001*		<0.0001*		0.0102*		–	

* statistically significant ($p \leq 0.05$). Different superscript letters indicate statistically significant differences between the groups (Tukey's post hoc HSD test). Mean and *SD* values are presented in Newtons [N].

Definitive master casts were obtained from patients to include a diverse range of residual alveolar ridge shapes and forms. These anatomical variations were carefully considered during the design and execution of the cross-over study. However, it is crucial to emphasize that these variations did not introduce bias or significantly influence the study outcomes. A similar approach was reported by Faty et al. and Abd El Galil et al., [23,25] who incorporated casts from different patients to evaluate denture adaptation and retention.

The geometric center of the arch was manually determined on the definitive master cast before scanning and later transferred to the framework design for standardization. While digital application during the design process of both frameworks would provide greater accuracy, transferring this position to the conventional denture was difficult.[23] The geometric center was chosen to measure the retention of the denture base based on previous studies reporting this region as the most reliable for testing the retention of maxillary complete dentures.[26,27]

A desktop scanner (D850; 3Shape) with a camera resolution of 5 MP and an accuracy of 4 μm was used. This open system allows data export to the PartialCAD software.[25] The definitive master cast scans were exported to OBJ files and imported into the PartialCAD module to preserve the drawn data on the cast. A plate design was used to reinforce the maxillary denture rather than a mesh or wire embedded within the acrylic denture base. It was found that the use of metal wire reinforcement inhibits polymerization and thermal shrinkage of the dough resin during denture construction, thereby compromising denture adaptation.[28]

A 5-axis dental milling machine (CORiTEC 350i; imes-icore GmbH) was used to construct the frameworks. This system offers advantages over conventional 3-axis machines. It enables movement in 3 spatial directions, in addition to rotational movement along the 4th axis. Additionally, the milling spindle of this machine can rotate, providing the 5th axis of motion. These combined functionalities facilitate the milling process of complex geometries with subsections.[29] Different bur sizes were used, as large burs provide good durability and an excellent cutting rate, while smaller burs allow accurate reproduction of fine details. Precise cutting is an important factor in the CAD/CAM milling process.[30]

Denture adaptation was evaluated using the Geomagic software, a surface-matching program that enables superimposition of 2 surfaces to determine deviations between them.[24] The calculated mean values might be close to 0, which is non-practical, indicating a perfect fit. Therefore, the root mean square (RMS) values were used to eliminate the influence of positive and negative signs.[31,32]

A gingival mask with viscoelastic properties similar to those of oral mucosa and minimal dimensional change or permanent deformation was used during retention measurements and chewing simulation. To ensure a stable non-movable model surface, the gingival mask was bonded to the underlying model using an adhesive.[33] During chewing simulation, the load was applied to the center of a metal plate. Metal was selected instead of acrylic due to its superior stress distribution properties, as demonstrated in previous studies.[34–36] The simulation consisted of 2,500,000 chewing cycles, representing approx. 1 year of dental function.[37] During the retention measurements and chewing simulation, Glandosane artificial saliva was employed. Artificial saliva simulates oral conditions and influences the biological properties of the materials, while also removing debris resulting from material wear.[38,39] Bayer et al. demonstrated that the absence of saliva can alter frictional wear, consequently affecting retention force values.[40]

Hwang et al. reported an acceptable range for adaptation deviation, ranging between 0.05 mm and 0.30 mm.[41] In the present study, before dynamic fatigue loading, all maxillary single dentures demonstrated acceptable adaptation values, with no statistically significant differences observed between the groups. Hence, all methods may be considered suitable for restoring single maxillary arches. However, subjecting the dentures to dynamic fatigue loading revealed a statistically significant difference among the groups, leading to the rejection of the null hypothesis. Among the tested groups, group C (zirconia framework) exhibited the best adaptation, which may be attributed to the high flexural and bending strength of nanozirconia (900–1,200 MPa).[42] These values surpass those of milled Co–Cr (480 ±20 MPa)[43] and conventional acrylic resin (624.6–825.4 MPa).[44] On the other hand, the adaptation of group A (conventional acrylic resin) decreased after dynamic fatigue loading, Notably, our study demonstrated that group A showed better adaptation than group B (Co–Cr framework). This difference may be attributed to water absorption of acrylic resin during the experimental period.[45,46]

In a study conducted by Amaral et al., the flexural strength of surface-treated Y-TZP disks was evaluated before and after mechanical cycling.[47] The researchers observed a phase transformation from the tetragonal to monoclinic phase, resulting in the creation of a superficial compression layer that enhanced surface toughness and increased initial flexural strength. Regarding Co–Cr alloys, hard milling of Co–Cr blocks minimized the occurrence of casting defects.[48] However, Kim et al. concluded that milled Co–Cr exhibited inferior mechanical properties and was considered the least favorable method for Co–Cr manufacturing.[43] This was attributed to the presence of a large grain size in milled Co–Cr, which adversely affected its mechanical properties, particularly yield strength.

In the present study, all groups demonstrated acceptable retention values. However, zirconia-reinforced dentures (group C) exhibited the highest retention both before and after dynamic fatigue loading. This finding can be explained by the well-established relationship

between complete denture retention and good denture base adaptation, as reported in numerous studies.[4,49,50] Thus, the observed retention results are consistent with the findings related to adaptation.

Limitations

Several limitations of the present study should be acknowledged. Laboratory methods and results may not fully reflect clinical performance. Although the chewing simulator provided a controlled environment for evaluating denture adaptation and retention, it could not fully replicate the complex and dynamic oral conditions experienced by patients in real-life situations. Factors such as variations in salivary flow, oral muscle activity and oral hygiene practices may influence denture behavior in the clinical setting. Therefore, caution should be exercised when extrapolating these findings to clinical practice. Further research, including clinical studies with long-term follow-up, is required validate the results of this laboratory-based investigation.

Conclusions

Maxillary single dentures reinforced with zirconia frameworks can be considered a successful alternative approach to Co–Cr frameworks for prosthetic rehabilitation using digital technology.

Ethics approval and consent to participate

The study was reviewed and approved by the Research Ethics Committee of the Faculty of Dentistry, Ain Shams University, Cairo, Egypt (approval No. FDASU-RecID041914).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Ulla MS, DL S, TV S, K B. Management of maxillary single denture fractures: An enigma. *Int J Appl Dent Sci*. 2021;7(3):86–88. doi:10.22271/oral.2021.v7.i3b.1284
2. Bhandari S. Outcome of single maxillary complete dentures opposing mandibular teeth: A need to introspect on the prosthodontic treatment protocol. *J Indian Prosthodont Soc*. 2016;16(1):15–19. doi:10.4103/0972-4052.167941
3. Williamson R, reviewer. *J Prosthodont*. 2005;14(2):144–146. Review of: Baskar RM, Davenport J. *Prosthetic Treatment of the Edentulous Patient, 4th Edition*. doi:10.1111/j.1532-849x.2005.00030_2.x
4. Mohammed EH, Elddamony E, Mohamed S. Evaluation of metal base adaptation and clinical retention of upper complete dentures with a CO–CR metal palate constructed by two different approaches. *Egypt Dent J*. 2022;68(4):3771–3782.
5. Abdulrahim R, Yanikoğlu N. Evaluation of fracture resistance for autopolymerizing acrylic resin materials reinforced with glass fiber mesh, metal mesh and metal wire materials: An in vitro study. *Open J Stomatol*. 2022;12(2):33–41. doi:10.4236/ojst.2022.122004
6. Altaie SF. Tribological, microhardness and color stability properties of a heat-cured acrylic resin denture base after reinforcement with different types of nanofiller particles. *Dent Med Probl*. 2023;60(2):295–302. doi:10.17219/dmp/137611
7. Machado-Santos L, Baroudi K, Silikas N, et al. Physical analysis of an acrylic resin modified by metal and ceramic nanoparticles. *Dent Med Probl*. 2023;60(4):657–664. doi:10.17219/dmp/171844
8. Williams RJ, Bibb R, Eggbeer D, Collis J. Use of CAD/CAM technology to fabricate a removable partial denture framework. *J Prosthet Dent*. 2006;96(2):96–99. doi:10.1016/j.prosdent.2006.05.029
9. Ponto-Wolska M, Wagner L. Assessment of the influence of selected electrolytic polishing process parameters on the surface roughness of casts made of the CoCrMo alloy. *Dent Med Probl*. 2018;55(4):395–398. doi:10.17219/dmp/96237
10. Zhou Y, Li N, Yan J, Zeng Q. Comparative analysis of the microstructures and mechanical properties of Co–Cr dental alloys fabricated by different methods. *J Prosthet Dent*. 2018;120(4):617–623. doi:10.1016/j.prosdent.2017.11.015
11. Geurtsen W. Biocompatibility of dental casting alloys. *Crit Rev Oral Biol Med*. 2002;13(1):71–84. doi:10.1177/154411130201300108
12. Urano S, Hotta Y, Miyazaki T, Baba K. Bending properties of Ce-TZP/A nanocomposite clasps for removable partial dentures. *Int J Prosthodont*. 2015;28(2):191–197. doi:10.11607/ijp.4113
13. Takano T, Sakurai K. Overview of zirconia: Application as denture base material substituting for metal. *Int J Prosthodont Restor Dent*. 2013;3(4):153–156. doi:10.5005/jp-journals-10019-1096
14. Tanaka K, Tamura J, Kawanabe K, et al. Ce-TZP/Al_2O_3 nanocomposite as a bearing material in total joint replacement. *J Biomed Mater Res*. 2002;63(3):262–270. doi:10.1002/jbm.10182
15. Hagiwara Y, Nakajima K. Application of Ce-TZP/Al_2O_3 nanocomposite to the framework of an implant-fixed complete dental prosthesis and a complete denture. *J Prosthodont Res*. 2016;60(4):337–343. doi:10.1016/j.jpor.2016.02.002
16. Akar T, Dündar A, Kırmalı Ö, et al. Evaluation of the shear bond strength of zirconia to a self-adhesive resin cement after different surface treatment. *Dent Med Probl*. 2021;58(4):463–472. doi:10.17219/dmp/135652
17. Ataol AS, Ergun G, Yayman M. Effects of the substructure thickness, the resin cement color and the finishing procedure on the color and translucency of zirconia-based ceramic restorations. *Dent Med Probl*. 2023;60(1):137–144. doi:10.17219/dmp/149356
18. Mohamed S, Emarah A. Metal versus poly ether-ether Ketone secondary copings for rigid telescopic retained implant supported mandibular over dentures. Evaluation of clinical retention forces. *Egypt Dent J*. 2020;66(3):1769–1778. doi:10.21608/edj.2020.31446.1134
19. AlHelal A, AlRumaih HS, Kattadiyil MT, Baba NZ, Goodacre CJ. Comparison of retention between maxillary milled and conventional denture bases: A clinical study. *J Prosthet Dent*. 2017;117(2):233–238. doi:10.1016/j.prosdent.2016.08.007
20. Nishiyama H, Tanaka S, Nemoto R, Miura H, Baba K. Zirconia-reinforced framework for maxillary complete dentures. *Int J Prosthodont*. 2018;31(2):114–116. doi:10.11607/ijp.4999

21. Im SM, Huh YH, Cho LR, Park CJ. Comparison of the fracture resistances of glass fiber mesh- and metal mesh-reinforced maxillary complete denture under dynamic fatigue loading. *J Adv Prosthodont*. 2017;9(1):22–30. doi:10.4047/jap.2017.9.1.22
22. Hada T, Suzuki T, Minakuchi S, Takahashi H. Reduction in maxillary complete denture deformation using framework material made by computer-aided design and manufacturing systems. *J Mech Behav Biomed Mater.* 2020;103:103514. doi:10.1016/j.jmbbm.2019.103514
23. Faty MA, Sabet ME, Thabet YG. A comparison of denture base retention and adaptation between CAD/CAM and conventional fabrication techniques. *Int J Prosthodont*. 2023;36(4):469–478. doi:10.11607/ijp.7193
24. Goodacre BJ, Goodacre CJ, Baba NZ, Kattadiyil MT. Comparison of denture base adaptation between CAD–CAM and conventional fabrication techniques. *J Prosthet Dent*. 2016;116(2):249–256. doi:10.1016/j.prosdent.2016.02.017
25. Abd El Galil EG, Mohamed SL, Rizk FN, Sabet ME. Evaluation of two computer-aided design software on the adaptation of digitally constructed maxillary complete denture. *J Indian Prosthodont Soc*. 2021;21(4):383–390. doi:10.41010.4103/jips.jips_137_21
26. El-Mekawy N, Gomaa A, Habib A. Comparison of three different forms of denture adhesives: Direct measurement of denture retention and study of patient satisfaction. *Egypt Dent J*. 2012;58(3):1–9. https://www.researchgate.net/profile/Nesreen-El-Mekawy/publication/268503832_Comparison_of_Three_Different_Forms_of_Denture_Adhesives_Direct_Measurement_of_Denture_Retention_and_Study_of_Patient_Satisfaction/links/5b8d027192851c1e124437ee/Comparison-of-Three-Different-Forms-of-Denture-Adhesives-Direct-Measurement-of-Denture-Retention-and-Study-of-Patient-Satisfaction.pdf. Accessed November 20, 2023.
27. Rizk FN, Elhadary AA. Study of retention and physical properties of conventional and microwave-cured acrylic resin complete dentures: An in-vivo and in-vitro investigation. *Egypt Dent J*. 2016;62(4):5005–5015. https://buescholar.bue.edu.eg/cgi/viewcontent.cgi?article=1104&context=dentistry. Accessed November 20, 2023.
28. Teraoka F, Nakagawa M, Takahashi J. Adaptation of acrylic dentures reinforced with metal wire. *J Oral Rehabil*. 2001;28(10):937–942. doi:10.1111/j.1365-2842.2001.00752.x
29. Barker E, AlQobaly L, Shaikh Z, Franklin K, Moharamzadeh K. Implant soft-tissue attachment using 3D oral mucosal models – a pilot study. *Dent J (Basel)*. 2020;8(3):72. doi:10.3390/DJ8030072
30. Bae SY, Park JY, Kim JH, Kim HY, Kim MB, Kim WC. The comparison of accuracy on three-unit fixed dental prosthesis made with CAD/CAM milling machines. *J Tech Dent*. 2015;37(1):9–15. doi:10.14347/kadt.2015.37.1.9
31. Lo Russo L, Guida L, Zhurakivska K, Troiano G, Chochlidakis K, Ercoli C. Intaglio surface trueness of milled and 3D-printed digital maxillary and mandibular dentures: A clinical study. *J Prosthet Dent*. 2023;129(1):131–139. doi:10.1016/j.prosdent.2021.05.003
32. Wang C, Shi YF, Xie PJ, Wu JH. Accuracy of digital complete dentures: A systematic review of in vitro studies. *J Prosthet Dent*. 2021;125(2):249–256. doi:10.1016/j.prosdent.2020.01.004
33. ElCharkawi HG, Goodkind RJ, DeLong R, Douglas WH. The effect of the resilient-layer distal-extension partial denture on movement of the abutment teeth: A new methodology. *J Prosthet Dent*. 1988;60(5):622–630. doi:10.1016/0022-3913(88)90226-0
34. Tehini G, Baba NZ, Berberi A, Majzoub Z, Bassal H, Rifai K. Effect of simulated mastication on the retention of locator attachments for implant-supported overdentures: An in vitro pilot study. *J Prosthodont*. 2020;29(1):74–79. doi:10.1111/jopr.12670
35. Yılmaz EC, Sadeler R. Investigation of three-body wear of dental materials under different chewing cycles. *Sci Eng Compos Mater*. 2018;25(4):781–787. doi:10.1515/secm-2016-0385
36. Sia PKS, Masri R, Driscoll CF, Romberg E. Effect of locator abutment height on the retentive values of pink locator attachments: An in vitro study. *J Prosthet Dent*. 2017;117(2):283–288. doi:10.1016/j.prosdent.2016.08.012
37. Zidan S, Silikas N, Haider J, Alhotan A, Jahantigh J, Yates J. Evaluation of equivalent flexural strength for complete removable dentures made of zirconia-impregnated PMMA nanocomposites. *Materials (Basel)*. 2020;13(11):2580. doi:10.3390/ma13112580
38. Stock V, Schmidlin PR, Merk S, et al. PEEK primary crowns with cobalt–chromium, zirconia and galvanic secondary crowns with different tapers – a comparison of retention forces. *Materials (Basel)*. 2016;9(3):187. doi:10.3390/ma9030187
39. Stock V, Wagner C, Merk S, et al. Retention force of differently fabricated telescopic PEEK crowns with different tapers. *Dent Mater J*. 2016;35(4):594–600. doi:10.4012/dmj.2015-249
40. Bayer S, Stark H, Mues S, Keilig L, Schrader A, Enkling N. Retention force measurement of telescopic crowns. *Clin Oral Investig*. 2010;14(5):607–611. doi:10.1007/s00784-009-0315-z
41. Hwang HJ, Lee SJ, Park EJ, Yoon HI. Assessment of the trueness and tissue surface adaptation of CAD–CAM maxillary denture bases manufactured using digital light processing. *J Prosthet Dent*. 2019;121(1):110–117. doi:10.1016/j.prosdent.2018.02.018
42. Kontonasaki E, Giasimakopoulos P, Rigos AE. Strength and aging resistance of monolithic zirconia: An update to current knowledge. *Jpn Dent Sci Rev*. 2020;56(1):1–23. doi:10.1016/j.jdsr.2019.09.002
43. Kim HR, Jang SH, Kim YK, et al. Microstructures and mechanical properties of Co–Cr dental alloys fabricated by three CAD/CAM-based processing techniques. *Materials (Basel)*. 2016;9(7):596. doi:10.3390/MA9070596
44. John J, Gangadhar SA, Shah I. Flexural strength of heat-polymerized polymethyl methacrylate denture resin reinforced with glass, aramid, or nylon fibers. *J Prosthet Dent*. 2001;86(4):424–427. doi:10.1067/mpr.2001.118564
45. Salim S, Sadamori S, Hamada T. The dimensional accuracy of rectangular acrylic resin specimens cured by three denture base processing method. *J Prosthet Dent*. 1992;67(6):879–881. doi:10.1016/0022-3913(92)90606-B
46. Wong DM, Cheng LY, Chow TW, Clark RK. Effect of processing method on the dimensional accuracy and water sorption of acrylic resin dentures. *J Prosthet Dent*. 1999;81(3):300–304. doi:10.1016/S0022-3913(99)70273-8
47. Amaral M, Cesar PF, Bottino MA, Lohbauer U, Valandro LF. Fatigue behavior of Y-TZP ceramic after surface treatments. *J Mech Behav Biomed Mater*. 2016;57:149–156. doi:10.1016/j.jmbbm.2015.11.042
48. Hong MH, Lee DH, Hanawa T, Kwon TY. Comparison of microstructures and mechanical properties of 3 cobalt–chromium alloys fabricated with soft metal milling technology. *J Prosthet Dent*. 2022;127(3):489–496. doi:10.1016/j.prosdent.2020.07.037
49. Lee CJ, Bok SB, Bae JY, Lee HH. Comparative adaptation accuracy of acrylic denture bases evaluated by two different methods. *Dent Mater J*. 2010;29(4):411–417. doi:10.4012/dmj.2009-105
50. Emera RMK, Shady M, Alnajih MA. Comparison of retention and denture base adaptation between conventional and 3D-printed complete dentures. *J Dent Res Dent Clin Dent Prospects*. 2022;16(3):179–185. doi:10.34172/joddd.2022.030

Review

Laser therapies for oral lichen planus: An overview of systematic reviews and meta-analyses with evidence-based recommendations from the Polish Society for Laser Dentistry

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Abstract

The objective of the study was to comprehensively synthesize and critically evaluate the effectiveness, safety and clinical applicability of various laser therapies in the management of oral lichen planus (OLP), including photodynamic therapy (PDT), photobiomodulation therapy (PBMT) and high-intensity laser therapy (HILT), and to provide guidelines for clinicians.

A structured literature review was conducted following the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines, and recommendations were formulated. A total of 23 relevant studies published between 2015 and 2025 were identified through searches of multiple biomedical databases. The risk of bias and quality of evidence were assessed, focusing on clinical outcomes such as reduction in lesion severity, pain relief, mucosal healing, and recurrence rates.

Laser therapies demonstrated effectiveness in reducing lesion size and pain, with safety profiles comparable to or superior to those of conventional corticosteroid treatments. Photobiomodulation therapy and HILT showed particularly favorable outcomes, including lower recurrence rates and fewer adverse effects. However, heterogeneity in treatment protocols and laser parameters limited direct comparisons, with corticosteroids remaining the first-line treatment.

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Laser-based therapies are valuable adjuncts or alternatives for patients with OLP who are intolerant to corticosteroids. Standardized treatment protocols and further large-scale randomized controlled trials with longer follow-up periods are needed to optimize clinical guidelines and enable personalized therapy. Photobiomodulation therapy is recommended as the safest and most effective modality for erosive OLP.

Keywords: photodynamic therapy, oral lichen planus, low-level laser therapy, high-intensity laser therapy, photobiomodulation

Highlights

- These recommendations and structured literature review synthesize evidence from 23 systematic reviews and meta-analyses evaluating laser-based therapies for oral lichen planus (OLP).
- Photobiomodulation and photodynamic therapy demonstrate clinical effectiveness comparable to topical corticosteroids in reducing pain and lesion severity.
- High-intensity laser therapy provides superior short-term analgesia but requires strict parameter control due to thermal risks.
- Laser-based therapies show favorable safety profiles and lower recurrence rates, particularly in patients with steroid-resistant or steroid-intolerant OLP.
- Significant heterogeneity in laser parameters and short follow-up periods limit direct comparison and long-term conclusions.

Introduction

Rationale

Although lichen planus may present as a systemic mucocutaneous disorder involving both oral and extraoral sites, the present review focuses specifically on its oral manifestation (oral lichen planus (OLP)), as the majority of available clinical studies do not stratify outcomes according to systemic involvement.[1–3] Oral lichen planus should be interpreted within the broader spectrum of lichen planus as a systemic, immune-mediated disorder that may involve multiple anatomical sites, including the oral mucosa, skin, genital mucosa, and nails. From a clinical and pathophysiological perspective, it is critical to distinguish isolated oral manifestations from multi-organ disease, as these phenotypes may differ in immune activity, chronicity and therapeutic response. These recommendations focus on the topical treatment of oral symptoms.[3] The condition is characterized by periods of remission and exacerbation, which lead to fluctuating clinical manifestations and require long-term management and monitoring due to its chronic nature and potential for malignant transformation in some forms.[3–5] The etiology of OLP is multifactorial and complex, involving a dysregulated immune response in genetically predisposed individuals. Oral lichen planus is considered a T-cell-mediated autoimmune disease where cytotoxic $CD8^+$ T lymphocytes trigger apoptosis of basal keratinocytes in the oral epithelium. Lichen planus occurs in cutaneous (skin) and mucosal (organ-specific, e.g., oral) forms. In its etiology, mast cells play a key role by releasing mediators like histamine and cytokines, contributing to the inflammatory infiltrate and basal keratinocyte damage characteristic of the disease. In cutaneous and mucosal lichen planus, laser therapy is effective mainly in the superficial type, where laser radiation targets lesions limited to the epidermis, minimizing penetration into deeper layers. Factors implicated in the pathogenesis of OLP include genetic predispositions, infections such as the hepatitis C virus, psychological stress, certain medications, and dental materials that may act as triggers or exacerbating agents.[6,7] Epigenetic modifications and alterations in the oral microbial environment may also contribute to disease onset and progression. Systemic associations such as autoimmune thyroid disease, diabetes mellitus and hypertension (Grinspan's syndrome) have also been described. Clinically, 6 types of OLP are distinguished: reticular; papular; plaque-like; atrophic; erosive (ulcerative); and bullous. The reticular form is the most common and is typically asymptomatic, characterized by bilateral white, lace-like Wickham's striae on the buccal mucosa, tongue and gingiva. The erosive and atrophic forms present with pain, erythema, ulceration, and mucosal atrophy and are associated with a higher risk of

malignant transformation and significant morbidity. The plaque-like form resembles leukoplakia, while the bullous type features vesicles or bullae that rupture easily. Each subtype may require different therapeutic approaches tailored to symptom severity and risk profile.[7–11] Laser-based therapies have emerged as an important treatment modality in the management of OLP, especially its erosive and ulcerative types that are refractory to conventional corticosteroid therapy. Various types of lasers, including photobiomodulation therapy (PBMT),[12–17] diode lasers,[18,19] CO_2 lasers,[19–23] and neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers,[17,19–21,24–26] provide targeted anti-inflammatory[12–14,17–30] and analgesic effects,[15,16,20–22,27,29] promote mucosal healing[13,14,29] and reduce lesion size with minimal adverse effects.[19,22,28,29] The advantages of laser treatments include precision application, reduced need for systemic immunosuppressants, decreased recurrence rates, and improved patient comfort and quality of life.[11,31,32] These features make laser therapy a valuable addition or alternative in the management of OLP.

Objectives

The aim of these recommendations and the structured literature review was to systematically synthesize and critically appraise high-quality evidence derived from systematic reviews and meta-analyses evaluating laser-based therapies in the management of OLP. Specifically, the review seeks to assess the clinical effectiveness, safety profile and practical applicability of different laser modalities, including PBMT, photodynamic therapy (PDT) and high-intensity laser therapy (HILT), with respect to pain reduction, improvement in lesion severity, mucosal healing, and recurrence control. In addition, the objective is to identify strengths and limitations within the existing evidence base, with particular emphasis on heterogeneity in laser parameters and treatment protocols. By consolidating current evidence, the review intends to support informed clinical decision-making, contribute to the optimization and standardization of laser treatment strategies, and provide evidence-based recommendations endorsed by the Polish Society for Laser Dentistry, especially for patients who are resistant or intolerant to conventional corticosteroid therapy.[32–36]

Material and methods

These recommendations and the structured literature review were conducted in accordance with the Joanna Briggs Institute (JBI) and PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 guidelines for umbrella reviews. The study was registered in the PROSPERO database (registration No. CRD420251172714).[37–41]

PICO question

The following PICO question was formulated: In patients diagnosed with OLP (Population), do laser-based therapies, including PBMT, PDT and HILT (Intervention), compared with conventional pharmacologic management (such as topical corticosteroids), placebo or sham laser treatment, or other non-laser therapeutic approaches (Comparison), lead to improved clinical outcomes, including reduction in pain, lesion severity, enhancement of mucosal healing, and decreased recurrence rates (Outcome)?[42]

Search strategy

A detailed and systematic search was performed across major electronic databases, including PubMed®/MEDLINE, Embase, Scopus, and the Cochrane Library, to identify published systematic reviews and meta-analyses examining laser therapy for the prevention or treatment of OLP. Three reviewers conducted the search using a predefined set of Medical Subject Headings (MeSH) terms and free-text keywords related to OLP and laser therapy. The initial search, limited to English-language studies with no publication year restrictions, included all records available up to October 1, 2025. The screening process followed a two-step procedure: first, titles and abstracts were screened, and then full-text articles were assessed by 3 reviewers against predefined inclusion and exclusion criteria. To capture any additional relevant publications, reference lists of all eligible reviews were manually checked. Overall, the search strategy was designed to identify systematic reviews and other high-quality evidence on the efficacy of laser-based interventions in the management of OLP. The final syntax covered literature published between 2015 and 2025 across the selected biomedical databases (Table 1).

Study selection process

To ensure methodological rigor and minimize potential bias, study selection followed a structured and transparent screening process conducted independently by 3 reviewers. The procedure began with an initial assessment of titles and abstracts based on predefined inclusion and exclusion criteria specifically designed for the objectives of these recommendations and the comprehensive literature review. The study focused on systematic reviews and meta-analyses that examined the effectiveness of laser-based therapies and related photobiomodulation approaches in the management of OLP. Eligible studies were required to report clinically relevant outcomes, such as reductions in lesion size, pain intensity, burning sensation, erythema, or improvement in mucosal healing. To ensure data quality, only peer-reviewed articles with a transparent methodology, including control or

Table 1. Search strategy used in the study

Source	Search syntax	Filters	Search results, *n*
PubMed®/MEDLINE	("Oral Lichen Planus"[MeSH Terms] OR "Oral Lichen Planus"[Title/Abstract] OR "OLP" [Title/Abstract]) AND ("Laser Therapy"[MeSH Terms] OR "Laser"[Title/Abstract] OR "Lasers"[Title/Abstract] OR "Laser Treatment"[Title/Abstract] OR "Low-Level Laser Therapy"[Title/Abstract] OR "Photobiomodulation"[Title/Abstract] OR "PBM"[Title/Abstract] OR "Photodynamic Therapy"[Title/Abstract] OR "PDT"[Title/Abstract] OR "CO_2 laser"[Title/Abstract] OR "Er:YAG laser"[Title/Abstract] OR "Er,Cr:YSGG laser"[Title/Abstract] OR "Nd:YAG laser"[Title/Abstract] OR "Diode laser"[Title/Abstract] OR "KTP laser"[Title/Abstract] OR "Phototherapy"[Title/Abstract] OR "Light therapy"[Title/Abstract] OR "Laser irradiation"[Title/Abstract])	systematic reviews 2015–2025	30
Embase	('oral lichen planus'/exp OR 'oral lichen planus':ti,ab OR 'olp':ti,ab) AND ('laser therapy'/exp OR 'laser':ti,ab OR 'lasers':ti,ab OR 'laser treatment':ti,ab OR 'low level laser therapy':ti,ab OR 'photobiomodulation':ti,ab OR 'pbm':ti,ab OR 'photodynamic therapy'/exp OR 'photodynamic therapy':ti,ab OR 'pdt':ti,ab OR 'co2 laser':ti,ab OR 'er:yag laser':ti,ab OR 'er,cr:ysgg laser':ti,ab OR 'nd:yag laser':ti,ab OR 'diode laser':ti,ab OR 'ktp laser':ti,ab OR 'phototherapy'/exp OR 'phototherapy':ti,ab OR 'light therapy':ti,ab OR 'laser irradiation':ti,ab)	reviews 2015–2025	60
Scopus	(TITLE-ABS-KEY("oral lichen planus") OR TITLE-ABS-KEY(OLP) AND TITLE-ABS-KEY("laser therapy") OR TITLE-ABS-KEY(laser) OR TITLE-ABS-KEY(lasers) OR TITLE-ABS-KEY("laser treatment") OR TITLE-ABS-KEY("low level laser therapy") OR TITLE-ABS-KEY(photobiomodulation) OR TITLE-ABS-KEY(PBM) OR TITLE-ABS-KEY("photodynamic therapy") OR TITLE-ABS-KEY(PDT) OR TITLE-ABS-KEY("CO_2 laser") OR TITLE-ABS-KEY("Er:YAG laser") OR TITLE-ABS-KEY("Er,Cr:YSGG laser") OR TITLE-ABS-KEY("Nd:YAG laser") OR TITLE-ABS-KEY("diode laser") OR TITLE-ABS-KEY("KTP laser") OR TITLE-ABS-KEY(phototherapy) OR TITLE-ABS-KEY("light therapy") OR TITLE-ABS-KEY("laser irradiation"))	reviews 2015–2025	94
Cochrane Library	("oral lichen planus" OR OLP) AND ("laser therapy" OR laser OR lasers OR "laser treatment" OR "low level laser therapy" OR photobiomodulation OR PBM OR "photodynamic therapy" OR PDT OR "CO_2 laser" OR "Er:YAG laser" OR "Er,Cr:YSGG laser" OR "Nd:YAG laser" OR "diode laser" OR "KTP laser" OR phototherapy OR "light therapy" OR "laser irradiation")	reviews 2015–2025	81

comparison groups and measurable outcomes, were included. Exclusion criteria comprised non-peer-reviewed publications, opinion pieces (such as narrative reviews or editorials), conference abstracts, and unpublished theses. Studies not written in English, lacking sufficient methodological details, or not directly evaluating laser interventions for OLP were also excluded. Duplicated reports and secondary analyses derived from the same dataset were omitted unless they provided novel insights or additional data.

To ensure objectivity and minimize the potential for selection bias, the initial screening of titles and abstracts was performed independently by 3 reviewers. The level of inter-reviewer agreement was quantified using Cohen's kappa coefficient, providing an objective measure of consistency across assessments. Any discrepancies in study selection were resolved through structured consensus discussions to maintain transparency and uniformity throughout the review process.[40–42]

Data extraction

Essential information collected from each included review comprised the first author's name, year of publication, review design (systematic review or meta-analysis), clinical context, and demographic characteristics of the patient populations. Extracted data included the type of laser or light source, wavelength, power output, energy density, application technique, duration of exposure, and the number of treatment sessions. When available, additional parameters such as treatment frequency, target tissue site, and whether laser therapy was used as a primary or adjunctive treatment were also documented. Outcomes of interest encompassed both primary and secondary clinical measures, including reduction in lesion size or severity, pain or burning sensation relief, improvement in mucosal healing, and enhancement of patients' quality of life, where applicable.

Assessment of the risk of bias

The methodological quality of each included study was evaluated independently by 3 reviewers using a structured assessment of the risk of bias. This tool encompassed 9 domains aimed at assessing both methodological transparency and internal validity. The evaluation criteria were as follows:

1. Comprehensive identification and description of the laser system and treatment parameters (e.g., wavelength, power output, energy density, and application technique);
2. Clear definition of treatment protocols, including frequency, duration, and whether the therapy was used as a primary or adjunctive intervention;
3. Specification of clinically relevant outcomes such as improvement in lesion severity, pain reduction or mucosal healing;
4. Inclusion of appropriate control or comparator groups;
5. Transparent inclusion criteria with detailed characterization of the patient population;
6. Consideration of bias control measures, including randomization and blinding where applicable;
7. Appropriateness, transparency and reproducibility of statistical analyses;

8. Clarity and completeness in outcome reporting, including adverse effects and follow-up data;
9. Disclosure of funding sources and potential conflicts of interest.

This risk-of-bias assessment was developed by the authors due to the lack of a standardized scoring system applicable to the synthesis of clinical recommendations.

Results

Study selection

The PRISMA flow diagram outlines the process of study selection (Fig. 1). A total of 265 records were initially identified through database searches, including PubMed®/MEDLINE (n = 30), Embase (n = 60), Scopus (n = 94), and the Cochrane Library (n = 81). After removing 97 duplicate records, 168 unique records were screened for eligibility. Of these, 143 were excluded based on title and abstract screening, leaving 25 reports for full-text assessment. Two of these were excluded as duplicates, resulting in 23 studies meeting the inclusion criteria and being included in the final review. Additionally, 7 records were identified through other sources, namely websites (n = 5) and citation search (n = 2). However, all 7 were excluded after full-text evaluation because they did not meet the inclusion criteria.

Risk of bias

As summarized in Table 2, all but one study were judged to be at low risk of bias. Importantly, no studies were excluded solely based on their risk-of-bias rating.

Characteristics of the included reviews

A total of 23 systematic reviews and meta-analyses published between 2017 and 2025 were included in this overview (Table 3). These studies collectively examined the efficacy of various phototherapeutic approaches, such as PDT, PBMT and HILT, in comparison with conventional corticosteroid therapy for the management of OLP and related oral potentially malignant disorders. Early evidence indicated that both PDT and PBMT were effective in alleviating OLP symptoms but not consistently superior to topical corticosteroids.[43–46] Subsequent reviews confirmed that phototherapy improved pain and lesion severity with minimal adverse effects.[47,49,53,54,62,63] More recent meta-analyses provided stronger evidence

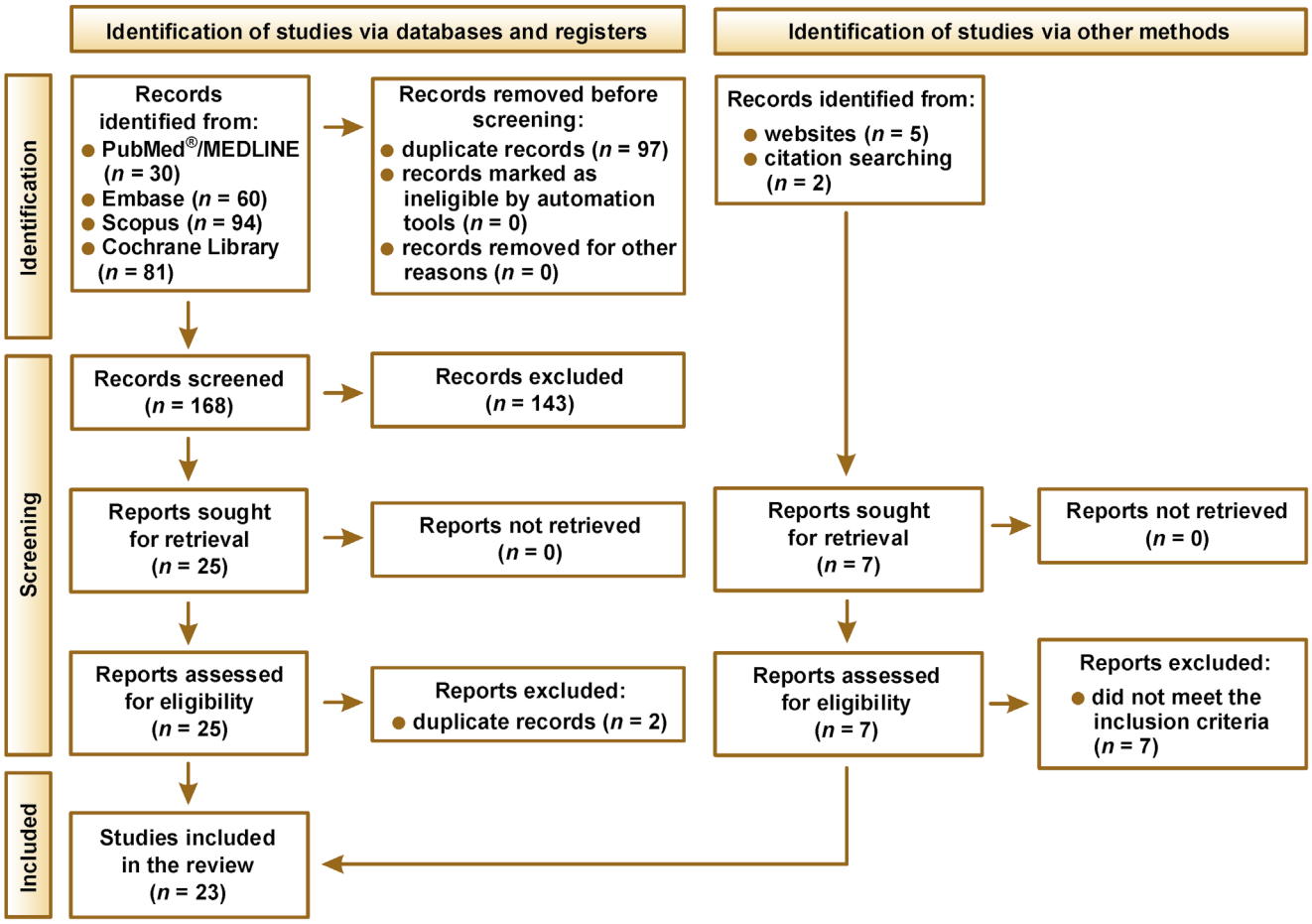

Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 flow diagram of the study selection process

Table 2. Results of the risk-of-bias assessment

Study	Score (0–9)	Risk
Akram et al. 2018[43]	8	low
Akram et al. 2018[44]	9	low
Al-Maweri et al. 2017[45]	8	low
Al-Maweri et al. 2018[46]	9	low
Binnal et al. 2022[47]	9	low
Bordoloi et al. 2024[48]	7	low
Choudhary et al. 2022[49]	7	low
de Carvalho et al. 2022[50]	7	low
Gulzar et al. 2023[51]	6	moderate
Hanna et al. 2023[52]	8	low
He et al. 2020[53]	9	low
Jajarm et al. 2018[54]	8	low
Leong et al. 2023[55]	9	low
Liu et al. 2025[56]	9	low
Lu et al. 2025[57]	8	low
Mahuli et al. 2024[58]	8	low
Nagi et al. 2023[59]	8	low
Ruiz Roca et al. 2022[60]	9	low
Sandhu et al. 2022[61]	7	low
Serafini et al. 2023[62]	7	low
Soh et al. 2024[63]	9	low
Sridharan and Sivaramakrishnan 2021[64]	8	low
Waingade et al. 2022[65]	8	low

that PBMT and HILT significantly reduced pain and produced clinical outcomes comparable or superior to corticosteroids.[55–59] Reviews focused on aminolevulinic acid (ALA)-mediated PDT and symptomatic OLP further supported the effectiveness, safety and tolerability of these light-based treatments.[43,48,51,60] Overall, the included reviews consistently demonstrated that phototherapies are effective, safe and minimally invasive alternatives or adjuncts to corticosteroid therapy for managing symptomatic and erosive forms of OLP.

Photodynamic therapy consistently improves clinical scores and symptoms in OLP, though it is generally not superior to corticosteroid treatment in efficacy.[43] Studies have shown that PDT is effective and safe. Some papers indicate similar or better outcomes after PDT compared to topical steroids, but heterogeneity in trials limits firm conclusions.[46] Photodynamic therapy yields high response rates and is minimally invasive, though protocols vary widely.[47] Aminolevulinic acid-mediated PDT specifically shows strong lesion reduction, symptom improvement and low recurrence without adverse effects.[48] Multiple studies have confirmed that PDT using various photosensitizers significantly improves OLP symptoms, with ALA-mediated PDT often yielding the best outcomes.[49] Meta-analyses reveal that the reductions in pain and lesion size with PDT are comparable to corticosteroid treatment, but limited RCTs and data heterogeneity prevent definitive conclusions.[51] Phototherapy combining PDT and PBMT improves symptoms, although many studies have high risk of bias and use diverse laser parameters.[52] Photodynamic therapy shows similar effectiveness to corticosteroids, and ALA-mediated PDT is a suitable alternative when steroids are contraindicated.[53] According to a network meta-analysis, PDT improves clinical and pain scores to a greater extent than placebo treatment but is inferior to corticosteroids, which remain the most effective modality.[62] Methylene blue (MB)-mediated PDT is a safe alternative comparable to corticosteroids, but its superiority is not established.[65] Photobiomodulation therapy is generally considered effective and safe, though evidence remains weaker and protocols vary.[44,45,61] This method has been shown to significantly reduce pain and lesion severity, comparable to the efficacy of corticosteroids, while maintaining a favorable safety profile.[50,58,60] Photobiomodulation therapy also helps to relieve long-term pain and reduce the likelihood of recurrence; however, larger trials are required to confirm these outcomes.[57] Some studies suggest that PBMT is a reasonable alternative to corticosteroids, while the effectiveness of PDT remains less conclusive.[54] High-intensity laser therapy shows superior pain relief compared to steroids, with PBMT demonstrating similar efficacy.[56] Photobiomodulation therapy is as effective as corticosteroids and is recommended for steroid-resistant cases, though standardization is needed.[63] Overall, laser-based therapies are useful adjuncts to corticosteroids, especially in resistant or steroid-contraindicated OLP, but corticosteroids remain the first-line treatment.[62]

In general, the included evidence indicates that PBMT and PDT provide clinical improvement comparable to topical corticosteroids, while HILT may offer stronger analgesic effects. CO_2 laser ablation shows promising long-term outcomes in refractory cases. However, heterogeneity in laser parameters and short follow-up periods limit the strength of the conclusions (Table 4).

Table 3. Summary of the studies included in the review

Study	Study type	Studies included, *n*	Laser intervention	Databases searched	Range of publication year	Study design	Outcomes
Akram et al. 2018[43]	SR	6	PDT	MEDLINE, Embase, CENTRAL	up to August 2017	clinical trials	PDT improved OLP symptoms but was not superior to corticosteroids.
Akram et al. 2018[44]	SR	5	PBMT	MEDLINE, PubMed®, Embase, ISI Web of Knowledge, Science Direct, Scopus, CENTRAL, Cochrane Oral Health Trials Register	up to April 2017	3 RCTs, 2 non-RCTs	Evidence is weak, with no clear superiority of PBMT: 3 studies favored corticosteroids; 1 study favored PBMT.
Al-Maweri et al. 2017[45]	SR	6	PBMT	PubMed®, Scopus, Web of Science	up to December 2016	clinical trials	PBMT is effective for symptomatic OLP, with no adverse effects, and may serve as an alternative to corticosteroids.
Al-Maweri et al. 2018[46]	SR	5	PDT	PubMed®/MEDLINE, Scopus, ISI Web of Knowledge	2014–July 2017	3 RCTs, 2 non-RCTs	All studies showed that PDT is effective for symptomatic OLP; 2 studies found it as effective as steroids, one found it superior and 2 found it inferior. Overall, the evidence is limited and heterogeneous.
Binnal et al. 2022[47]	SR and M	49	PDT	PubMed®/MEDLINE, Embase, Cochrane Library, CINAHL	up to September 2021	RCTs, non-RCTs, single-arm studies, case series, retrospective cohort studies	A 60.6% CR and 93.7% AR were reported. PDT was most effective for dysplasia/CIS (CR: 81%), followed by actinic cheilitis (CR: 73.9%); no significant differences as compared to other treatments were observed in RCTs. Overall, the evidence is moderate.
Bordoloi et al. 2024[48]	SR	17	ALA-mediated PDT	PubMed®/MEDLINE, Google Scholar	January 2008–February 2023	prospective clinical studies, comparative studies	ALA-mediated PDT is effective, minimally invasive, safe, and well tolerated. CR and PR are common, recurrence rates are low, and outcomes vary depending on ALA form, concentration and light source. No severe adverse events or photosensitivity were reported.
Choudhary et al. 2022[49]	SR	18	ALA-, MB- and TBO-mediated PDT	PubMed®/MEDLINE, Web of Science, Cochrane Library	2000–2020	clinical intervention studies, comparative studies, case series	ALA-mediated PDT is a promising first-line option for OEL lesions, with both LED and laser light providing effective clinical results.
de Carvalho et al. 2022[50]	SR and M	17	PBMT	PubMed®/MEDLINE, Embase, Scopus, Web of Science	up to July 2020	RCTs	PBMT reduced pain and lesion severity and was comparable to corticosteroids.
Gulzar et al. 2023[51]	SR	8	PDT vs. corticosteroids	PubMed®, Embase, Google Scholar, Semantic Scholar, X-MOL, Dimensions	up to May 2023	RCTs	PDT improved symptoms of OLP.
Hanna et al. 2023[52]	SR	9	PBMT and aPDT	PubMed®/MEDLINE, PubMed Central, Embase, CINAHL, Cochrane Library, ProQuest, Scopus, ClinicalTrials.gov	up to February 2023	RCTs	PBMT/aPDT improved pain and quality of life. There was high heterogeneity among the studies.
He et al. 2020[53]	SR and M	16	PDT vs. corticosteroids or pre/post PDT	PubMed®, Web of Science, Cochrane Library, Embase, EBSCO	up to December 2019	clinical studies, case series	PDT improved lesion response, reduced lesion size and was comparable to corticosteroids.

Study	Study type	Studies included, *n*	Laser intervention	Databases searched	Range of publication year	Study design	Outcomes
Jajarm et al. 2018[54]	SR and M	13	PBMT and PDT	ISI Web of Knowledge, Cochrane Library, MEDLINE, Embase	up to April 2018	RCTs, before-and-after trials	PBMT significantly reduced pain and sign scores, whereas the evidence for PDT was less conclusive.
Leong et al. 2023[55]	SR and M	37	PDT	MEDLINE, Embase, CENTRAL	up to 2022	RCTs	Topical corticosteroids remain the preferred treatment for OLP.
Liu et al. 2025[56]	SR and M	16	HILT and PBMT	PubMed®, Embase, Cochrane Library, Web of Science, CNKI, SinoMed	up to October 2024	RCTs	HILT provided greater pain relief than TCS, and PBMT showed similar effects, performing best below 120 J/cm^2. Both treatments lowered recurrence rates and increased cure rates. PBMT had no side effects, unlike HILT and TCS. HILT and PBMT appear to be useful alternatives to TCS, although larger studies are still needed.
Lu et al. 2025[57]	SR and M	12	PBMT	PubMed®, Embase, CINAHL, Cochrane Library, Web of Science	up to February 2023	RCTs	PBMT improved pain and lesion severity, reduced relapse risk, and showed no adverse events.
Mahuli et al. 2024[58]	SR and M	10	PBMT	Cochrane Library, MEDLINE, PubMed®, Scopus, LILACS, Google Scholar, ClinicalTrials.gov, CTRI	2011–2022	RCTs	The study favored PBMT for pain reduction (n = 274, *MD* = –0.48, 95% *CI*: –0.66 to –0.30), with no adverse effects reported for laser therapy.
Nagi et al. 2023[59]	SR	10	MB- or TBO-mediated PDT vs. corticosteroids (dexamethasone, triamcinolone, betamethasone)	PubMed®/MEDLINE, Embase, Scopus, Web of Science, Google Scholar, ScienceDirect	January 2015–July 2021	RCTs and controlled trials	PDT reduced pain and lesion size. Four studies favored PDT, 1 favored corticosteroids, and 2 showed equal outcomes. No major adverse effects were reported.
Ruiz Roca et al. 2022[60]	SR	7	PBMT	PubMed®, Google Scholar, Cochrane Library	May 2017–May 2022	clinical studies	PBMT improved symptoms in erosive OLP and showed no adverse effects.
Sandhu et al. 2022[61]	SR	70	PBMT and PDT	PubMed®, Scopus, Web of Science	up to March 2021	RCTs	Topical corticosteroids remain the most cost-effective and reliable choice.
Serafini et al. 2023[62]	SR	15	PBMT and PDT	PubMed®/MEDLINE	January 2005–September 2022	RCTs	Further research is necessary to establish the role of PDT in the treatment of OLP (first-line treatment vs. adjuvant therapy).
Soh et al. 2024[63]	SR and M	8	PBMT	PubMed®, Scopus, Cochrane Library	up to June 2023	RCTs, observational studies	PBMT is as effective as other therapies and is a suitable alternative when corticosteroids are contraindicated.
Sridharan and Sivaramakrishnan 2021[64]	SR and M	55	PDT	PubMed®, CENTRAL	up to October 2020	RCTs	Corticosteroids are the most effective treatment for OLP, while PDT is among the effective alternatives for symptom reduction.
Waingade et al. 2022[65]	SR and M	5	MB-mediated PDT	PubMed®/MEDLINE, PubMed Central, CENTRAL, Google Scholar, ScienceDirect, DOAJ	January 2010–February 2022	RCTs	Both MB-mediated PDT and corticosteroids are effective. MB-mediated PDT is a safe alternative with similar outcomes.

ALA – aminolevulinic acid; aPDT – antimicrobial photodynamic therapy; AR – any response; CENTRAL – Cochrane Central Register of Controlled Trials; *CI* – confidence interval; CINAHL – Cumulative Index to Nursing and Allied Health Literature; CIS – carcinoma in situ; CNKI – China National Knowledge Infrastructure; CR – complete response; CTRI – Clinical Trials Registry – India; DOAJ – Directory of Open Access Journals; HILT – high-intensity laser therapy; LED – light-emitting diode; LILACS – Latin American and Caribbean Health Sciences Literature; M – meta-analysis; MB – methylene blue; *MD* – mean difference; OEL – oral erythroleukoplakia; OLP – oral lichen planus; PBMT – photobiomodulation therapy; PDT – photodynamic therapy; PR – partial response; RCTs – randomized controlled trials; SR – systematic review; TBO – toluidine blue O; TCS – topical corticosteroids.

Table 4. Laser parameters and treatment protocols for oral lichen planus (OLP)

Laser type	Wavelength [nm]	Power	Energy density	Session duration [min]	Session frequency	Clinical effectiveness and additional notes	References
PBMT red diode	630	10 mW	1.5 J/cm^2	2.5	every 3 days, for approx. 10 sessions	effective in erosive/ulcerative OLP; reduces pain, lesion size and soreness; no significant side effects; may serve as an alternative to corticosteroids	9, 34, 35, 63, 71, 84, 85, 88
Diode laser (980 nm)	980	moderate range (varies by study)	variable (low to moderate)	2–10	twice per week for 4 weeks	provides symptom relief within 1 week; lesion disappearance after approx. 1 month; no recurrence; good safety profile	31, 34, 63, 85, 89
Nd:YAG laser	1,064	high	high	5–10	weekly to biweekly	superior to topical corticosteroids for pain relief; some side effects may occur; reduces recurrence	31, 52, 63, 85
CO_2 laser	10,600	moderate–high	adjusted to tissue type and lesion thickness	5–15	variable (typically a few sessions)	useful for ablative vaporization in severe or refractory cases; requires professional use; associated with a risk of mucosal damage	32, 81–83
Er:YAG laser	2,940	moderate–high	similar to CO_2 laser	5–10	variable	used for mucosal ablation; causes less thermal damage than CO_2 lasers; less commonly used for OLP	31, 52, 63, 85

Er:YAG – erbium-doped yttrium aluminium garnet; Nd:YAG – neodymium-doped yttrium aluminium garnet.

Discussion

Rationale behind laser therapy

Topical corticosteroids remain the first-line therapy for symptomatic OLP due to their well-established effectiveness.[66–68] However, recurrence rates after corticosteroid therapy often reach 30–60% within 3–6 months, and long-term remission is infrequent.[66–69] This has prompted interest in laser-based therapies as adjuncts or alternatives, particularly for patients with corticosteroid intolerance, mucosal atrophy, recurrent candidiasis, or refractory disease.[70–81] Across multiple systematic reviews, PBMT and HILT demonstrate analgesic and anti-inflammatory effects comparable to topical corticosteroids, while PBMT offers an excellent safety profile when energy density remains below 120 J/cm^2.[70–74,79,81] High-intensity laser therapy may provide stronger short-term pain relief but carries a higher risk of thermal injury, emphasizing the importance of operator experience and appropriate parameter selection.[70,79,81] Photodynamic therapy also achieves clinically meaningful symptom reduction and, in some studies, lower recurrence rates than corticosteroids, although heterogeneity across protocols limits direct comparison.[75–78,80] CO_2 laser vaporization appears beneficial in selected refractory lesions, with some studies reporting markedly reduced recurrence rates and durable symptom resolution.[82] Long-term data further supports the efficacy of the CO_2 laser. A study reported that 85% of patients remained pain-free after a mean follow-up of 8 years.[83] Recurrences were successfully managed by retreatment.[83] Nevertheless, ablative approaches require advanced clinical training and careful case selection. Photobiomodulation therapy and HILT have shown durable symptom control and sustained efficacy, with meta-analyses reporting reduced recurrence rates compared to corticosteroids (relative risk for recurrence: 0.43 (PBMT), 0.33 (HILT)) and higher cure rates at 3–6 months.[70–74,79,81] For refractory or steroid-resistant OLP, both PDT and PBMT have achieved lasting lesion healing and minimal side effects in case series and clinical trials.[80,84,85] Given these findings, laser-based therapies are most appropriate for patients who do not respond adequately to corticosteroids or for whom pharmacologic treatment is contraindicated.[70–81] Patient selection criteria for topical corticosteroids include symptomatic, erosive or ulcerative OLP, as these agents are considered first-line therapy for most patients due to their established efficacy and ease of use.[66–68] Treatment choice should consider lesion severity, subtype, comorbidities, prior response to therapy, and practical factors such as equipment availability and clinician expertise.[66–87] For laser-based modalities, contraindications include photosensitivity disorders (especially for PDT), pregnancy (relative) and inability to comply with treatment protocols.[70,71,75,79,86] Laser-based therapies, while non-invasive and generally safe, require specialized equipment (such as diode lasers or PDT kits), operator training and adherence to safety protocols, which can limit availability.[70,71,73,75,79,86,87] Photobiomodulation therapy and PDT are particularly advantageous owing to their minimal adverse effects and favorable safety profiles.[70,71,73,75,79,86,87] High-intensity laser therapy requires greater expertise due to the higher risk of tissue damage if misapplied.[79,87] Direct comparative and cost-effectiveness data remains limited, but accumulating

evidence demonstrates that laser-based therapies (PDT, PBMT, HILT, CO_2 laser ablation) offer at least equivalent, and in some studies superior, long-term efficacy and lower recurrence rates compared to topical corticosteroids, with fewer adverse effects and improved patient tolerance.[70–83] These modalities are particularly valuable for patients who cannot tolerate or do not respond to corticosteroids. In summary, topical corticosteroids remain the first-line treatment for symptomatic OLP due to established efficacy, while laser-based therapies represent effective, safe and durable alternatives for selected patients, particularly those with contraindications or refractory disease. Individualized therapy selection should be based on lesion characteristics, prior treatment response, comorbidities, and available resources.[66–87] While corticosteroids remain the standard of care, laser modalities provide safe, effective and adaptable options that complement or extend traditional management strategies (Fig. 2).

Secondary infection of oral lichen planus

Secondary infection can exacerbate the inflammatory burden of OLP, intensifying pain and delaying healing.[88,89] Although topical or intralesional corticosteroids remain the standard management, recurrent or refractory cases may benefit from laser-based interventions.[88–90] Photobiomodulation therapy and HILT have shown meaningful symptom reduction in infected or highly inflamed lesions, with PBMT providing predictable clinical improvement and an excellent safety profile at standard parameters. CO_2 laser vaporization may also achieve effective lesion removal and reduced recurrence in persistent cases, though its use should be restricted to trained clinicians due to the ablative nature and risk of mucosal injury. Photodynamic therapy likewise offers an alternative for steroid-intolerant patients, improving lesion control with minimal adverse effects when delivered according to established protocols. Overall, laser modalities represent well-tolerated options that may help restore symptom stability and promote healing in OLP complicated by secondary infection, particularly when conventional therapy is insufficient or contraindicated.

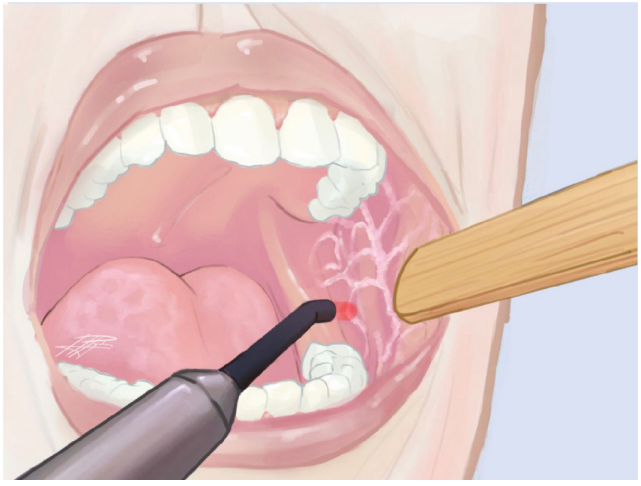

Fig. 2. Laser irradiation in oral lichen planus (OLP)

Parameters for each laser type

Table 4 reflects diverse laser modalities with typical parameters and clinical outcomes reported in the current literature for OLP management. Photobiomodulation therapy (red diode ~630 nm) is widely supported for safe, effective pain reduction in erosive OLP. Diode lasers with a wavelength of approx. 980 nm show similar positive results. High-intensity laser therapy and CO_2 laser ablation provide more aggressive treatment options, often reserved for refractory or severe lesions. Treatment frequency generally spans 3–4 weeks and encompasses multiple sessions tailored to lesion response and patient tolerance. These laser therapies offer promising adjunct or alternative options to conventional corticosteroid treatment with fewer side effects.[88]

From evidence limitations to clinical decision-making

Although the available evidence supports the clinical effectiveness and safety of laser-based therapies in OLP, several limitations must be considered when translating these findings into routine practice. Substantial heterogeneity in laser parameters, treatment protocols and outcome measures limits direct comparison between modalities and restricts the strength of long-term conclusions. Reporting of clinical outcomes was also inconsistent: while pain reduction was commonly assessed with the visual analogue scale (VAS), lesion size, clinical severity indices, healing dynamics, and recurrence rates were not uniformly measured or standardized. Follow-up periods were short in most studies (4–12 weeks), and the sample sizes were frequently small (often <50 participants), reducing generalizability. This limitation raises uncertainty as to whether reported outcomes reflect sustained disease control or only short-term symptomatic improvement. In this context, laser therapies should be interpreted predominantly as symptom-oriented interventions, unless long-term remission data is demonstrated. In addition, inconsistent reporting of adverse effects and malignant transformation further limits the interpretability and clinical applicability of the available evidence. Most included studies did not stratify patients according to systemic manifestations of lichen planus or associated autoimmune conditions, which may influence treatment response. Another major limitation is the substantial heterogeneity in laser parameters (wavelength, fluence, power density), treatment protocols (session frequency, duration) and outcome measures across studies. This precludes quantitative comparison between modalities and limits the validity of any hierarchy of effectiveness.

Despite these limitations, the consistency of findings across systematic reviews allows for the formulation of cautious, evidence-informed clinical recommendations. Rather than defining a single universal protocol, current evidence supports a stepwise, indication-driven approach, in which laser modalities are selected based on the response to corticosteroids, disease severity, patient tolerance, and safety considerations. This approach enables clinicians to integrate laser therapies into OLP management while acknowledging existing evidence gaps and the need for individualized treatment planning.

These findings indicate that laser-based therapies should be integrated within a structured, stepwise treatment framework rather than used as universal replacements for pharmacologic management. In this context, corticosteroids remain the reference first-line therapy, while laser modalities may be integrated as adjunctive or alternative options in selected clinical scenarios.

Future research should prioritize well-designed randomized controlled trials that directly compare laser modalities with topical corticosteroids using standardized dosimetry and harmonized outcome measures, including pain scores, lesion severity, recurrence at 6–12 months, and patient-reported quality of life. Longer follow-up and cost-effectiveness analyses are essential to refine clinical pathways and support personalized treatment strategies.

Conclusions

This structured review and recommendations demonstrate that laser-based therapies, including PBMT, PDT and HILT, are effective and safe adjuncts or alternatives to conventional corticosteroid treatment for OLP. Across systematic reviews and meta-analyses, these modalities consistently achieved clinically meaningful reductions in pain and lesion severity, with safety profiles comparable to or better than topical corticosteroids. Photobiomodulation therapy showed the most favorable balance of efficacy and safety and was associated with lower recurrence rates, particularly in erosive or steroid-resistant disease. Despite these promising outcomes, substantial heterogeneity in laser parameters, treatment protocols and outcome measures, together with short follow-up periods, limits definitive conclusions regarding long-term efficacy and direct modality comparison. As a result, topical corticosteroids remain the first-line therapy for symptomatic OLP. However, laser-based approaches represent valuable complementary or alternative options for patients with contraindications, intolerance or inadequate response to pharmacologic treatment. Future research should focus on standardized laser protocols, longer follow-up and well-designed randomized controlled trials directly comparing laser modalities with corticosteroids. Such efforts are essential to refine clinical guidelines, support personalized treatment strategies and optimize long-term outcomes for patients with OLP.

Consensus-based clinical guidance from the Polish Society for Laser Dentistry

The Polish Society for Laser Dentistry presents the following evidence-based clinical guidance regarding laser therapy in the management of symptomatic OLP. These recommendations integrate findings from systematic reviews, meta-analyses and clinical trials published between 2015 and 2025 and reflect current best practices in oral medicine and laser dentistry.

General therapeutic principles

Subtype-guided selection of PBMT, PDT or ablative lasers is recommended, as different clinical subtypes of OLP respond differently to laser therapy:

- reticular and papular forms typically do not require laser intervention;
- atrophic and erosive lesions benefit most from PBMT due to its analgesic and anti-inflammatory effects, whereas ulcerative lesions may require extended PBMT or PDT protocols;
- bullous OLP is generally less suitable for laser treatment due to fragile, blistering epithelium and the higher risk of tissue disruption.

Topical corticosteroids remain the first-line treatment for symptomatic, erosive or ulcerative OLP due to their established effectiveness, accessibility and predictable short-term outcomes.

Laser-assisted therapies may be used as adjunctive or alternative treatments in the following clinical scenarios:

- inadequate response to topical corticosteroids (steroid-refractory OLP);
- contraindications or intolerance to corticosteroids (e.g., mucosal atrophy, recurrent candidiasis, hypersensitivity);
- patient preference for non-pharmacologic or minimally invasive interventions.

Additionally, treatment selection should be individualized based on:

- clinical presentation (atrophic, erosive, ulcerative lesions);
- lesion extent and localization;
- comorbidities and contraindications;
- previous therapeutic response;
- availability of equipment and operator experience.

Preferred laser modalities

Photobiomodulation therapy

Photobiomodulation therapy is recommended among laser-based approaches as a first-choice alternative or adjunct. The method is most effective when applied with energy densities below 120 J/cm^2 and delivered in multiple sessions (2–3 treatments per week over 2–4 weeks), as supported by recent meta-analyses.

Treatment parameters

The following parameters of PBMT are recommended:

- wavelength: red (630–660 nm) for superficial mucosal lesions or near-infrared (808–980 nm) for deeper inflammatory components or symptomatic erosive lesions;
- power output: 50–120 mW (for contact or near-contact delivery);
- energy density (fluence): 4–8 J/cm^2 for mild erythematous lesions or 8–12 J/cm^2 for erosive or symptomatic lesions (delivered per point);
- application technique: contact or near-contact; perpendicular orientation to the mucosal surface; point-by-point technique, spacing points by ~1 cm;
- exposure time: typically 20–40 s per point, adjusted to reach the target dose;
- treatment frequency: 2–3 sessions per week for 4–6 weeks or until clinical improvement stabilizes;
- total number of sessions: usually 8–12 sessions for initial therapy, with additional sessions scheduled as needed for symptom recurrence.

Photobiomodulation therapy has demonstrated consistent pain reduction and improvement in lesion severity, particularly in erosive or ulcerative forms of OLP, while maintaining an excellent safety profile with no documented adverse events when energy densities remained below 120 J/cm^2. These characteristics support its preferential use as the first-choice laser-based modality in appropriate clinical scenarios.

Photobiomodulation parameters in OLP must be carefully individualized. The selection of dose, wavelength and application technique depends on several factors, including tissue characteristics (such as thickness, sensitivity and vascularity), anatomical location (e.g., lateral tongue vs. palate), severity and morphology of lesions (erythematous, erosive or ulcerative), and the patient's response during the course of therapy. The anatomical site plays a significant role in protocol adjustment. Lesions of the buccal mucosa and inner lips are typically flat or mildly erythematous, located in well-tolerated tissue where standard PBMT parameters can be applied. In contrast, lesions on the lateral borders or dorsal surface of the tongue require reduced energy densities due to the thin, highly innervated, and more reactive nature of the lingual mucosa. Similarly, lesions on the floor of the mouth and sublingual area – regions characterized by delicate, highly vascular tissues – necessitate conservative dosing and shorter exposure times. Conversely, the palatal mucosa, being thicker and less sensitive, can tolerate higher energy densities. Gingival and marginal periodontal lesions often require moderate parameters because of their sensitivity and variable inflammatory burden.

The severity of the disease further influences PBMT adjustment. Erythematous lesions, representing mild inflammatory activity, generally respond well to lower energy densities aimed at modulating surface inflammation. Erosive lesions of moderate severity are typically symptomatic and demand more robust anti-inflammatory and analgesic dosing. Chronic, ulcerative or extensive lesions, which are often fragile, long-standing and highly reactive, require the most cautious approach, beginning with conservative energy densities and progressively increasing them based on tissue tolerance and clinical response.

Malignant transformation considerations

Oral lichen planus is recognized as an oral potentially malignant disorder, with an estimated malignant transformation rate of 0.5–1.4%. Current evidence does not demonstrate an increased risk of dysplasia or malignant transformation associated with PBMT, PDT, HILT, or CO_2 laser ablation. Photobiomodulation therapy operates at non-thermal energy levels that do not induce DNA damage, while PDT selectively targets dysplastic epithelium and may theoretically reduce malignant risk by eliminating atypical keratinocytes.

However, long-term surveillance remains essential, and any OLP lesion, whether treated with corticosteroids or lasers, should undergo periodic clinical follow-up and biopsy when changes in appearance occur.

Current evidence does not indicate an increased risk of malignant transformation related to laser therapy. Photobiomodulation therapy operates at low energy levels that modulate cellular activity without causing DNA-damaging effects, and clinical studies using PBMT for OLP have reported symptomatic improvement without signs of increased oncogenic potential. However, due to limited long-term data, the possibility cannot be completely excluded.

Photodynamic therapy

Photodynamic therapy is recommended as a strong alternative for symptomatic, steroid-intolerant, or steroid-resistant OLP. The method offers favorable safety, low recurrence and minimal systemic effects. ALA- and MB-mediated PDT demonstrate efficacy comparable to topical corticosteroids in many studies. However, patient photosensitivity and adherence to light-avoidance protocols must be considered.

Treatment parameters

Pre-treatment procedures include clinical assessment (documentation of lesion morphology (reticular, erythematous, erosive), pain scoring and photographic evaluation), mucosal preparation, removal of surface debris, and gentle drying of the lesion.

A typical research-grade protocol of the photosensitizer phase using ALA-mediated PDT includes the following elements:

- photosensitizer form: 5-ALA gel or cream (commonly 5% formulations);

- application: a thin, uniform layer over the affected mucosa;
- incubation period: tens of minutes to a few hours, depending on the formulation, allowing conversion of ALA to protoporphyrin IX (PpIX) within epithelial cells;
- occlusion: in some studies, lesions are covered with a light-shielding dressing to prevent premature activation.

A protocol for MB-mediated PDT is as follows:

- photosensitizer form: MB aqueous solution or gargle (commonly 1–5%, often 5%);
- application: direct application via a microbrush;
- pre-irradiation time: a short-defined interval to allow tissue binding (3–10 min);
- excess removal: gentle blotting to prevent pooling.

Additional parameters encompass the following factors:

- light source: approx. 630–660 nm for MB and for activation of PpIX (ALA → PpIX);
- illumination technique: direct, perpendicular exposure to the lesion, fixed distance from the mucosal surface based on device specifications, using uniform scanning or a point-by-point technique;
- energy density (fluence): typical clinical PDT studies report the use of high light doses (many MB studies report ~100–120 J/cm^2 per session). Aminolevulinic acid protocols in recent series have used session-based dosing tailored to deliver the desired effect;
- treatment schedule: multiple sessions (weekly or several sessions over 1–3 weeks for MB, or longer series such as 8–10 weekly sessions for ALA).

High-intensity laser therapy

High-intensity laser therapy is recommended for selective use in steroid-resistant cases requiring rapid pain relief. Due to the thermal nature of HILT, inappropriate parameter settings may cause mucosal overheating or tissue damage, making operator experience essential for safe use. This method provides significant analgesic effects and may reduce recurrence risk. However, its higher thermal potential requires experienced operators, careful parameter selection and strict safety monitoring.

CO_2 laser ablation

CO_2 laser ablation is recommended for localized, severe or refractory lesions. It should only be performed by clinicians with advanced training in ablative laser procedures. The method is effective for long-term symptom control and for reducing recurrence. However, CO_2 laser ablation is not intended as a first-line therapy due to the risk of mucosal damage and the need for specialized equipment.

Key conceptual aspects (principles) for HILT or CO_2 laser ablation include:

- superficial removal of affected epithelium only (minimal thermal penetration);
- controlled ablation while avoiding deep stromal injury;
- use of a non-contact or near-contact technique, depending on system design;
- use of local anesthesia due to the ablative nature of the procedures;
- protection of adjacent mucosa with shields or barriers;
- the aim to produce a clean, uniform superficial wound bed.

Safety, documentation and procedural standards

The following procedural standards were formulated:

- all laser procedures require appropriate ocular and tissue protection according to device manufacturer guidelines;
- clinicians must document all treatment parameters, including wavelength, power, energy density (fluence), exposure time, spot size, and the number of sessions[89];
- PDT requires additional precautions for photosensitive individuals;
- HILT necessitates heightened attention to avoid overheating and collateral tissue injury.

Research and standardization needs

The Polish Society for Laser Dentistry emphasizes the need for:

1. Large, multicenter, long-term randomized controlled trials directly comparing PBMT, PDT, HILT, CO_2 laser ablation, and corticosteroids using standardized settings;
2. Uniform outcome measures, including:
   - VAS/numeric rating scale (NRS) pain score,
   - validated clinical severity indices (e.g., Thongprasom score),
   - lesion dimensions,
   - recurrence rates at 6 and 12 months,
   - patient-reported quality of life (Oral Health Impact Profile-14 (OHIP-14) or equivalent);
3. Cost-effectiveness analyses of laser modalities compared to corticosteroid regimens;
4. Assessment of biological response markers (cytokine modulation, oxidative stress markers, microbiome composition) to aid personalized treatment selection.

Summary of key recommendations

- Corticosteroids remain the first-line therapy for symptomatic OLP.
- PBMT is recommended as the safest and most broadly applicable laser modality.
- PDT is an effective alternative for patients unable or unwilling to use steroids.
- HILT is reserved for experienced operators managing steroid-resistant disease.

- CO_2 laser ablation is appropriate for localized, recalcitrant lesions in specialist settings.
- Laser therapies should be used with clear documentation, appropriate safety measures and clinical justification.

Practical clinical protocol for laser use in OLP

Laser therapy may be initiated in symptomatic erosive or ulcerative OLP when (1) symptoms persist despite optimized topical corticosteroid therapy, (2) corticosteroids are contraindicated or (3) the patient prefers a non-pharmacologic alternative. The following approach is recommended:

- PBMT should be the first-choice modality, applied with red (630–660 nm) or near-infrared (808–980, 1,064 nm) wavelengths, using energy densities of 4–12 J/cm^2 delivered 2–3 times per week for 2–4 weeks;
- ALA- or MB-mediated PDT may be applied weekly in steroid-intolerant or steroid-refractory cases, with standard illumination at 630–660 nm;
- HILT and CO_2 laser ablation should be restricted to refractory lesions and performed only by experienced operators due to the high thermal risk.

All procedures require documentation of wavelength, power, energy density (fluence), exposure time, spot size, and the number of sessions, according to the standards of the Polish Society for Laser Dentistry.

Stepwise practice-oriented clinical recommendations

Based on the synthesized evidence from this structured literature review, laser-based therapies for OLP should be implemented using a structured, stepwise clinical strategy rather than as standalone or empirical interventions.

Step 1 – First-line management

In patients with symptomatic erosive or ulcerative OLP, topical corticosteroids remain the first-line therapy due to their established efficacy and accessibility.

Step 2 – Second-line therapy

The effectiveness and optimal selection of laser modalities may vary depending on disease phenotype. In localized oral disease, PBMT and PDT may provide meaningful symptomatic control. In contrast, in patients with multi-organ involvement or strong autoimmune background, these modalities should be considered adjunctive, as they do not address the underlying systemic immune dysregulation.

Step 3 – Alternative or adjunctive therapy

In patients with steroid intolerance, recurrent disease or partial response to PBMT, PDT, particularly ALA- or MB-mediated protocols, may be used as an alternative or adjunctive modality, provided that appropriate photosensitivity precautions are observed.

Step 4 – Refractory disease management

High-intensity laser therapy may be considered in selected steroid-resistant cases requiring rapid analgesia but should be restricted to experienced operators due to its higher thermal risk profile.

Step 5 – Localized refractory lesions

CO_2 laser ablation should be reserved for localized, severe or treatment-refractory lesions and should not be used as a first-line approach.

Step 6 – Surveillance

Regardless of the therapeutic modality used, all patients with OLP require regular clinical follow-up, with vigilance for lesion progression and potential malignant transformation.

Trial registration

The study was registered in PROSPERO (registration No. CRD420251172714).

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

AI-assisted tools were used exclusively for language editing and improvement of readability. No AI tools were used for data analysis, interpretation or generation of scientific conclusions. All content was reviewed and approved by the authors.

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References

1. Krupaa RJ, Sankari SL, Masthan KMK, Rajesh E. Oral lichen planus: An overview. *J Pharm Bioallied Sci*. 2015;7(Suppl 1):S158–S161. doi:10.4103/0975-7406.155873
2. Louisy A, Humbert E, Samimi M. Oral lichen planus: An update on diagnosis and management. *Am J Clin Dermatol*. 2024;25(1):35–53. doi:10.1007/s40257-023-00814-3
3. Li C, Tang X, Zheng X, et al. Global prevalence and incidence estimates of oral lichen planus: A systematic review and meta-analysis. *JAMA Dermatol*. 2020;156(2):172–181. doi:10.1001/jamadermatol.2019.3797
4. de Carvalho MFMS, Cavalieri D, Do Nascimento S, et al. Cytokines levels and salivary microbiome play a potential role in oral lichen planus diagnosis. *Sci Rep*. 2019;9(1):18137. doi:10.1038/s41598-019-54615-y
5. Ślebioda Z, Drożdżyńska J, Karpińska A, et al. Oral lichen planus: Clinical presentation, demographic characteristics, and risk factors in a retrospective study of 186 Polish patients. *J Clin Med*. 2024;13(23):7363. doi:10.3390/jcm13237363
6. Le Cleach L, Chosidow O. Lichen planus. *N Engl J Med*. 2012;366(8):723–732. doi:10.1056/NEJMcp1103641
7. Budimir V, Richter I, Andabak-Rogulj A, Vučićević-Boras V, Budimir J, Brailo V. Oral lichen planus – retrospective study of 563 Croatian patients. *Med Oral Patol Oral Cir Bucal*. 2014;19(3):e255–e260. doi:10.4317/medoral.18940
8. Elenbaas A, Enciso R, Al-Eryani K. Oral lichen planus: A review of clinical features, etiologies, and treatments. *Dent Rev*. 2022;2(1):100007. doi:10.1016/j.dentre.2021.100007
9. Mahdavi O, Boostani N, Jajarm HH, Falaki F, Tabesh A. Use of low level laser therapy for oral lichen planus: Report of two cases. *J Dent (Shiraz)*. 2013;14(4):201–204. PMID:24724146.
10. Silva Prado RG, Passos LAB, Silva Filho WJE, Prado PGS, Ribeiro MAG, Sá MC. Use of low-power laser in the treatment of oral lichen planus: A narrative review. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2022;134(3):e242. doi:10.1016/j.oooo.2022.01.784
11. Wang K, Miao T, Lu W, et al. Analysis of oral microbial community and Th17-associated cytokines in saliva of patients with oral lichen planus. *Microbiol Immunol*. 2015;59(3):105–113. doi:10.1111/1348-0421.12232
12. Grzech-Leśniak K. Making use of lasers in periodontal treatment: A new gold standard? *Photomed Laser Surg*. 2017;35(10):513–514. doi:10.1089/pho.2017.4323
13. Michalak F, Dominiak M, Grzech-Leśniak Z, Kiryk J, Grzech-Leśniak K. Photobiomodulation in medication-related osteonecrosis of the jaw: Outcomes in stage I and its adjunctive role in advanced cases. *Biomedicines*. 2025;13(5):1042. doi:10.3390/biomedicines13051042
14. Kocherova I, Bryja A, Błochowiak K, et al. Photobiomodulation with red and near-infrared light improves viability and modulates expression of mesenchymal and apoptotic-related markers in human gingival fibroblasts. *Materials (Basel)*. 2021;14(12):3427. doi:10.3390/ma14123427
15. Matys J, Flieger R, Gedrange T, et al. Effect of 808 nm semiconductor laser on the stability of orthodontic micro-implants: A split-mouth study. *Materials (Basel)*. 2020;13(10):2265. doi:10.3390/ma13102265
16. Matys J, Flieger R, Świder K, et al. A clinical trial of photobiomodulation effect on orthodontic microscrews stability using a 635 nm red laser light. *Photobiomodul Photomed Laser Surg*. 2020;38(10):607–613. doi:10.1089/photob.2020.4863
17. Grzech-Leśniak Z, Szwach J, Lelonkiewicz M, et al. Effect of Nd:YAG laser irradiation on the growth of oral biofilm. *Microorganisms*. 2024;12(11):2231. doi:10.3390/microorganisms12112231
18. El Mobadder M, Nammour S, Namour M, Namour A, Grzech-Leśniak K. Disinfection potential of 980 nm diode laser and hydrogen peroxide (3%) in "critical probing depths" periodontal pockets: Retrospective study. *Life (Basel)*. 2022;12(3):370. doi:10.3390/life12030370
19. Nammour S, El Mobadder M, Namour M, et al. Aesthetic treatment outcomes of capillary hemangioma, venous lake, and venous malformation of the lip using different surgical procedures and laser wavelengths (Nd:YAG, Er,Cr:YSGG, CO_2, and diode 980 nm). *Int J Environ Res Public Health*. 2020;17(22):8665. doi:10.3390/ijerph17228665
20. Nammour S, Brugnera Junior A, Zeinoun T, et al. Global consensus report of the World Federation for Laser Dentistry (WFLD) on laser-assisted caries treatment and prevention. *Dent Med Probl*. 2025;62(5):783–800. doi:10.17219/dmp/211449
21. Arnabat-Dominguez J, Del Vecchio A, Todea C, et al. Laser dentistry in daily practice during the COVID-19 pandemic: Benefits, risks and recommendations for safe treatments. *Adv Clin Exp Med*. 2021;30(2):119–125. doi:10.17219/acem/130598
22. Murias I, Grzech-Leśniak K, Murias A, et al. Efficacy of various laser wavelengths in the surgical treatment of ankyloglossia: A systematic review. *Life (Basel)*. 2022;12(4):558. doi:10.3390/life12040558
23. Reddy N, Golob Deeb J, Kitten T, Carrico CK, Grzech-Leśniak K. The in vitro effect of laser irradiation (Er:YAG and CO_2) and chemical reagents (hydrogen peroxide, sodium hypochlorite, chlorhexidine, or sodium fluoride) alone or in combination on reducing root caries bacteria. *Int J Mol Sci*. 2022;23(24):15732. doi:10.3390/ijms232415732
24. Golob Deeb J, Reddy N, Kitten T, Carrico CK, Grzech-Leśniak K. Viability of bacteria associated with root caries after Nd:YAG laser application in combination with various antimicrobial agents: An in vitro study. *Dent Med Probl*. 2023;60(4):649–655. doi:10.17219/dmp/171690
25. Grzech-Leśniak Z, Pyrkosz J, Szwach J, et al. Antibacterial effects of Er:YAG laser irradiation on *Candida–Streptococcal* biofilms. *Life (Basel)*. 2025;15(3):474. doi:10.3390/life15030474
26. Grzech-Leśniak K, Belvin BR, Lewis JP, Golob Deeb J. Treatment with Nd:YAG laser irradiation combined with sodium hypochlorite or hydrogen peroxide irrigation on periodontal pathogens: An in vitro study. *Photobiomodul Photomed Laser Surg*. 2021;39(1):46–52. doi:10.1089/photob.2019.4775
27. Milc A, Kotuła J, Kiryk J, et al. Treatment of deciduous teeth in children using the Er:YAG laser compared to the traditional method: A randomized clinical trial. *Dent Med Probl*. 2025;62(4):579–589. doi:10.17219/dmp/194009
28. Kolberg-Babrzyńska I, Grzech-Leśniak K, Kiryk J, Dominiak M, Matys J. Effects of endodontic retreatment by conventional therapy compared to combined therapy with an Er:YAG laser and photobiomodulation: A randomized clinical trial. *Dent Med Probl*. 2025;62(6):1079–1087. doi:10.17219/dmp/188864
29. Bahrami R, Eftekharian S, Jafarieh R, Pourhajibagher M, Bahador A. The potential effect of photobiomodulation on oral mucositis induced by head and neck radio-chemotherapy: A literature review. *J Dent Sci*. 2025;20(4):2039–2045. doi:10.1016/j.jds.2025.06.002
30. Grzech-Leśniak K, Matys J. The effect of Er:YAG lasers on the reduction of aerosol formation for dental workers. *Materials (Basel)*. 2021;14(11):2857. doi:10.3390/ma14112857
31. Sivakumar S, R SN, N A, et al. Efficacy of intralesional platelet-rich plasma versus intralesional corticosteroids in the management oral lichen planus: A systematic review and meta-analysis of randomized controlled trials using random effects and weighted least square methods. *Evid Based Dent*. 2025;26(3):152. doi:10.1038/s41432-025-01145-4
32. Ibrahim R, Abdul-Hak M, Kujan O, Hamadah O. CO_2 laser vaporisation in treating oral lichen planus: A split-mouth randomised clinical trial. *Oral Dis*. 2024;30(4):2306–2313. doi:10.1111/odi.14669
33. Cheng S, Kirtschig G, Cooper S, Thornhill M, Leonardi-Bee J, Murphy R. Interventions for erosive lichen planus affecting mucosal sites. *Cochrane Database Syst Rev*. 2012;2012(2):CD008092. doi:10.1002/14651858.CD008092.pub2
34. Lax SJ, Harvey J, Axon E, et al. Strategies for using topical corticosteroids in children and adults with eczema. *Cochrane Database Syst Rev*. 2022;3(3):CD013356. doi:10.1002/14651858.CD013356.pub2

35. Cafaro A, Arduino PG, Massolini G, Romagnoli E, Broccoletti R. Clinical evaluation of the efficiency of low-level laser therapy for oral lichen planus: A prospective case series. *Lasers Med Sci*. 2014;29(1):185–190. doi:10.1007/s10103-013-1313-6
36. Salinas-Gilabert C, Gómez García F, Galera Molero F, Pons-Fuster E, Vander Beken S, Lopez Jornet P. Photodynamic therapy, photobiomodulation and acetonide triamcinolone 0.1% in the treatment of oral lichen planus: A randomized clinical trial. *Pharmaceutics*. 2022;15(1):30. doi:10.3390/pharmaceutics15010030
37. Aromataris E, Fernandez R, Godfrey CM, Holly C, Khalil H, Tungpunkom P. Summarizing systematic reviews: Methodological development, conduct and reporting of an umbrella review approach. *Int J Evid Based Healthc*. 2015;13(3):132–140. doi:10.1097/XEB.0000000000000055
38. Schiavo JH. PROSPERO: An International Register of Systematic Review Protocols. *Med Ref Serv Q*. 2019;38(2):171–180. doi:10.1080/02763869.2019.1588072
39. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
40. Higgins JPT, Thomas J, Chandler J, et al., eds. *Cochrane Handbook for Systematic Reviews of Interventions* version 6.5 (updated August 2024). Cochrane; 2024. https://www.cochrane.org/authors/handbooks-and-manuals/handbook/current. Accessed October 20, 2025.
41. Watson PF, Petrie A. Method agreement analysis: A review of correct methodology. *Theriogenology*. 2010;73(9):1167–1179. doi:10.1016/j.theriogenology.2010.01.003
42. Eriksen MB, Frandsen TF. The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: A systematic review. *J Med Libr Assoc*. 2018;106(4):420–431. doi:10.5195/jmla.2018.345
43. Akram Z, Javed F, Hosein M, et al. Photodynamic therapy in the treatment of symptomatic oral lichen planus: A systematic review. *Photodermatol Photoimmunol Photomed*. 2018;34(3):167–174. doi:10.1111/phpp.12371
44. Akram Z, Abduljabbar T, Vohra F, Javed F. Efficacy of low-level laser therapy compared to steroid therapy in the treatment of oral lichen planus: A systematic review. *J Oral Pathol Med*. 2018;47(1):11–17. doi:10.1111/jop.12619
45. Al-Maweri SA, Kalakonda B, Al-Soneidar WA, Al-Shamiri HM, Alakhali MS, Alaizari N. Efficacy of low-level laser therapy in management of symptomatic oral lichen planus: A systematic review. *Lasers Med Sci*. 2017;32(6):1429–1437. doi:10.1007/s10103-017-2233-7
46. Al-Maweri SA, Ashraf S, Kalakonda B, Halboub E, Petro W, AlAizari NA. Efficacy of photodynamic therapy in the treatment of symptomatic oral lichen planus: A systematic review. *J Oral Pathol Med*. 2018;47(4):326–332. doi:10.1111/jop.12684
47. Binnal A, Tadakamadla J, Rajesh G, Tadakamadla SK. Photodynamic therapy for oral potentially malignant disorders: A systematic review and meta-analysis. *Photodiagnosis Photodyn Ther*. 2022;37:102713. doi:10.1016/j.pdpdt.2022.102713
48. Bordoloi B, Goswami A, Roy D, Goswami P, Das I. Efficacy of aminolevulinic acid mediated photodynamic therapy in the treatment of oral premalignant lesions: A systematic review. *Asian Pac J Cancer Prev*. 2024;25(9):2971–2978. doi:10.31557/APJCP.2024.25.9.2971
49. Choudhary R, Reddy SS, Nagi R, Nagaraju R, Kunjumon SP, Sen R. The effect of photodynamic therapy on oral-premalignant lesions: A systematic review. *J Clin Exp Dent*. 2022;14(3):e285–e292. doi:10.4317/jced.59348
50. de Carvalho MM, Hidalgo MAR, Scarel-Caminaga RM, et al. Photobiomodulation of gingival lesions resulting from autoimmune diseases: Systematic review and meta-analysis. *Clin Oral Investig*. 2022;26(5):3949–3964. doi:10.1007/s00784-021-04362-0
51. Gulzar MA, Gul N, Alvi FD, et al. Comparison of photodynamic therapy and corticosteroid therapy in management of oral lichen planus: A systematic review of randomized controlled trials. *Photodiagnosis Photodyn Ther*. 2023;44:103747. doi:10.1016/j.pdpdt.2023.103747
52. Hanna R, Dalvi S, Tomov G, et al. Emerging potential of phototherapy in management of symptomatic oral lichen planus: A systematic review of randomised controlled clinical trials. *J Biophotonics*. 2023;16(7):e202300046. doi:10.1002/jbio.202300046
53. He Y, Deng J, Zhao Y, et al. Efficacy evaluation of photodynamic therapy for oral lichen planus: A systematic review and meta-analysis. *BMC Oral Health*. 2020;20(1):302. doi:10.1186/s12903-020-01260-x
54. Jajarm HH, Asadi R, Bardideh E, Shafaee H, Khazaei Y, Emadzadeh M. The effects of photodynamic and low-level laser therapy for treatment of oral lichen planus – a systematic review and meta-analysis. *Photodiagnosis Photodyn Ther*. 2018;23:254–260. doi:10.1016/j.pdpdt.2018.07.001
55. Leong XY, Gopinath D, Syeed SM, Veettil SK, Shetty NY, Menon RK. Comparative efficacy and safety of interventions for the treatment of oral lichen planus: A systematic review and network meta-analysis. *J Clin Med*. 2023;12(8):2763. doi:10.3390/jcm12082763
56. Liu P, Zhou Q, Bao J, et al. Effect of high-intensity laser therapy and photobiomodulation therapy on oral lichen planus – a systematic review and meta-analysis. *Lasers Med Sci*. 2025;40(1):151. doi:10.1007/s10103-025-04398-8
57. Lu C, Mo L, Li X, et al. Effects of low-level light therapy on pain and related lesions in patients with oral lichen planus: A systematic review and meta-analysis. *J Evid Based Dent Pract*. 2025;25(2):102126. doi:10.1016/j.jebdp.2025.102126
58. Mahuli SA, Rai A, Shree P, Haque ZU, Mahuli AV. Efficacy of photobiomodulation in the management of oral lichen planus in comparison to topical corticosteroids: Systematic review, meta-analysis, and GRADE-based assessment of certainty of evidence. *J Stomatol Oral Maxillofac Surg*. 2024;125(12 Suppl 2):101798. doi:10.1016/j.jormas.2024.101798
59. Nagi R, Muthukrishnan A, Rakesh N. Effectiveness of photodynamic therapy (PDT) in the management of symptomatic oral lichen planus – A systematic review. *J Oral Biol Craniofac Res*. 2023;13(2):353–359. doi:10.1016/j.jobcr.2023.03.003
60. Ruiz Roca JA, López Jornet P, Gómez García FJ, Marcos Aroca P. Effect of photobiomodulation on atrophic–erosive clinical forms of oral lichen planus: A systematic review. *Dent J (Basel)*. 2022;10(12):221. doi:10.3390/dj10120221
61. Sandhu S, Klein BA, Al-Hadlaq M, et al. Oral lichen planus: Comparative efficacy and treatment costs – a systematic review. *BMC Oral Health*. 2022;22(1):161. doi:10.1186/s12903-022-02168-4
62. Serafini G, De Biase A, Lamazza L, Mazzucchi G, Lollobrigida M. Efficacy of topical treatments for the management of symptomatic oral lichen planus: A systematic review. *Int J Environ Res Public Health*. 2023;20(2):1202. doi:10.3390/ijerph20021202
63. Soh WK, Cheah KF, Veettil SK, Pandiar D, Nimbalkar S, Gopinath D. Photobiomodulation therapy in the management of oral lichen planus: A systematic review and meta-analysis. *Eur J Dent*. 2024;18(4):976–986. doi:10.1055/s-0044-1782213
64. Sridharan K, Sivaramakrishnan G. Interventions for oral lichen planus: A systematic review and network meta-analysis of randomized clinical trials. *Aust Dent J*. 2021;66(3):295–303. doi:10.1111/adj.12835
65. Waingade M, Medikeri RS, Rathod P. Effectiveness of methylene blue photosensitizers compared to corticosteroids in the management of oral lichen planus: A systematic review and meta-analysis. *J Dent Anesth Pain Med*. 2022;22(3):175–186. doi:10.17245/jdapm.2022.22.3.175
66. Lodi G, Manfredi M, Mercadante V, Murphy R, Carrozzo M. Interventions for treating oral lichen planus: Corticosteroid therapies. *Cochrane Database Syst Rev*. 2020;2(2):CD001168. doi:10.1002/14651858.CD001168.pub3
67. Bachmeyer C, Benchaa B, Rohaut B, Petitjean B, Danne O, Blum L. Lichen planus: An unusual cause of oesophageal stricture. *J Eur Acad Dermatol Venereol*. 2007;21(5):719–721. doi:10.1111/j.1468-3083.2006.02028.x
68. Lodi G, Carrozzo M, Furness S, Thongprasom K. Interventions for treating oral lichen planus: A systematic review. *Br J Dermatol*. 2012;166(5):938–947. doi:10.1111/j.1365-2133.2012.10821.x
69. Gordon M, Sinopoulou V, Akobeng AK, Pana M, Gasiea R, Moran GW. Tacrolimus (FK506) for induction of remission in corticosteroid-refractory ulcerative colitis. *Cochrane Database Syst Rev*. 2022;4(4):CD007216. doi:10.1002/14651858.CD007216.pub2
70. Al-Maweri SA, Al-Qadhi G, Alqutaibi AY, et al. Curcumin versus corticosteroids for symptomatic oral lichen planus: A systematic review and meta-analysis. *Clin Exp Dent Res*. 2025;11(5):e70227. doi:10.1002/cre2.70227

71. Ferri EP, Cunha KRL, Abboud CS, et al. Photobiomodulation is effective in oral lichen planus: A randomized, controlled, double-blind study. *Oral Dis*. 2021;27(5):1205–1216. doi:10.1111/odi.13662
72. Ferri EP, Gallo CB, Abboud CS, et al. Efficacy of photobiomodulation on oral lichen planus: A protocol study for a double-blind, randomised controlled clinical trial. *BMJ Open*. 2018;8(10):e024083. doi:10.1136/bmjopen-2018-024083
73. Zborowski J, Kida D, Szarwaryn A, et al. A comparison of clinical efficiency of photodynamic therapy and topical corticosteroid in treatment of oral lichen planus: A split-mouth randomised controlled study. *J Clin Med*. 2021;10(16):3673. doi:10.3390/jcm10163673
74. Zborowski J, Kida D, Karolewicz B, Jurczyszyn K, Konopka T. A comparison of photodynamic therapy and topical clobetasol in treatment of oral lichen planus: A split-mouth randomized controlled study. *J Clin Med*. 2025;14(3):681. doi:10.3390/jcm14030681
75. Wiśniewski P, Sulewska M, Tomaszuk J, et al. Effects of photodynamic therapy and glucocorticosteroids on salivary oxidative stress in oral lichen planus: A randomized clinical trial. *Antioxidants (Basel)*. 2025;14(8):1017. doi:10.3390/antiox14081017
76. Sulewska M, Pietruska A, Tomaszuk J, et al. Efficacy of photodynamic therapy with 5-aminolevulinic acid-loaded oromucosal emulgel in patients with oral lichen planus: A randomized controlled clinical study. *Int Dent J*. 2025;75(6):103925. doi:10.1016/j.identj.2025.103925
77. Cosgarea R, Pollmann R, Sharif J, et al. Photodynamic therapy in oral lichen planus: A prospective case–controlled pilot study. *Sci Rep*. 2020;10(1):1667. doi:10.1038/s41598-020-58548-9
78. Pelet Del Toro N, Strunk A, Garg A, Han G. Validity of lichen planus and lichen planopilaris case identification using diagnostic codes from a clinical database. *Dermatology*. 2023;239(6):906–911. doi:10.1159/000533247
79. Ming J, Yang Z, Wang T, Wang J, Zeng X. Photodynamic therapy for refractory erosive oral lichen planus: A case series study. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2024;137(3):e41–e44. doi:10.1016/j.oooo.2023.11.013
80. Trakarnboonkij J, Tanya S, Sarideechaigul W, Subarnbhesaj A, Tabbon P, Sattayut S. Recalcitrant oral lichen planus involving bilaterally buccal mucosae treated with a combination of photodynamic and photobiomodulation therapies. *F1000Res*. 2024;13:152. doi:10.12688/f1000research.146733.1
81. Fekrazad R, Gholami GA, Sadr Eshkevari P, Nokhbatolfoghahaei H, Mohaghegh S. Management of recurrent aphthous ulcers with therapeutic Nd:YAG laser, using two different methods. *Dent Med Probl*. 2023;60(3):467–472. doi:10.17219/dmp/147048
82. van der Hem PS, Egges M, van der Wal JE, Roodenburg JLN. CO_2 laser evaporation of oral lichen planus. *Int J Oral Maxillofac Surg*. 2008;37(7):630–633. doi:10.1016/j.ijom.2008.04.011
83. Mahon-Smith A, Skingley G, Ayala-Nunes L, et al. Evaluating the appropriateness of existing health-related quality of life measures in lichen planus. *Dermatol Ther (Heidelb)*. 2023;13(11):2817–2837. doi:10.1007/s13555-023-00990-4
84. Duś-Ilnicka I, Rybińska A, Rusiecka A, et al. Prevalence of human papillomavirus DNA in the saliva of patients with oral lichen planus. *Dent Med Probl*. 2025;62(6):1089–1097. doi:10.17219/dmp/212617
85. Hanna R, Dalvi S, Benedicenti S, et al. Photobiomodulation therapy in oral mucositis and potentially malignant oral lesions: A therapy towards the future. *Cancers (Basel)*. 2020;12(7):1949. doi:10.3390/cancers12071949
86. Derikvand N, Ghasemi SS, Moharami M, Shafiei E, Chiniforush N. Management of oral lichen planus by 980 nm diode laser. *J Lasers Med Sci*. 2017;8(3):150–154. doi:10.15171/jlms.2017.27
87. Grzech-Leśniak K, Fiegler-Rudol J, Grzech-Leśniak Z, et al. Laser therapy for the management of oral mucositis: An umbrella review – official recommendations of the Polish Society for Laser Dentistry. *Dent Med Probl*. 2025;62(6):1157–1175. doi:10.17219/dmp/213958
88. Dillenburg CS, Martins MAT, Munerato MC, et al. Efficacy of laser phototherapy in comparison to topical clobetasol for the treatment of oral lichen planus: A randomized controlled trial. *J Biomed Opt*. 2014;19(6):068002. doi:10.1117/1.JBO.19.6.068002
89. Parker S, Grzech-Leśniak K, Cronshaw M, Matys J, Brugnera A Jr, Nammour S. Full operating parameter recording as an essential component of the reproducibility of laser–tissue interaction and treatments. *Adv Clin Exp Med*. 2024;33(6):653–656. doi:10.17219/acem/189795

Review

Association between periodontal disease and rheumatoid arthritis: A systematic review and meta-analysis

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Abstract

This systematic review and meta-analysis aimed to evaluate the association between periodontitis and rheumatoid arthritis (RA) in middle-aged populations.

The study followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 guidelines. A comprehensive search of major electronic databases was conducted for studies published between 2013 and 2023. In addition, a manual search was performed to identify relevant studies examining an association between these 2 conditions. The Newcastle–Ottawa scale was used to assess the risk of bias for the included cross-sectional and case–control studies. The certainty of evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) analysis, and a meta-analysis of eligible studies was conducted.

A total of 11 articles were included in the systematic review, with 9 incorporated into the meta-analysis. A statistically significant positive association was observed between periodontitis and RA for clinical attachment loss (CAL). The GRADE assessment indicated low to moderate level of certainty across studies, indicating strong evidence regarding clinical parameters. The meta-analysis showed a high overall odds ratio (*OR*) (*OR* = 11.37; 95% confidence interval (95% *CI*): 4.22–30.61; $p < 0.01$), suggesting that patients with RA have a significantly higher risk of developing periodontitis.

In conclusion, this systematic review and meta-analysis identified a positive association between periodontitis and RA. However, the GRADE analysis highlights the need for clinical and longitudinal studies with extended follow-up periods to further clarify this relationship and account for potential confounding factors.

Keywords: periodontitis, meta-analysis, rheumatoid arthritis, rheumatoid factors, GRADE analysis

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Highlights

- This systematic review and meta-analysis demonstrate a strong positive association between periodontitis and rheumatoid arthritis (RA), with RA patients showing an approx. 11-fold higher risk of periodontitis (*OR* = 11.37; 95% *CI*: 4.22–30.61; $p < 0.01$).
- Clinical attachment loss (CAL) was the most consistently affected periodontal parameter, and the majority of the included studies reported an association between RA and moderate-to-severe periodontitis.
- Shared risk factors, including smoking, vitamin D deficiency, female sex, advancing age, and elevated anti-*Porphyromonas gingivalis* antibody titers, may contribute to the relationship between both conditions.
- The certainty of evidence was rated as low to moderate (GRADE), highlighting the need for well-designed longitudinal and population-based studies using standardized diagnostic criteria.
- The findings support integrated dental–rheumatologic care, with routine periodontal screening in patients with RA potentially enabling earlier identification of at-risk individuals and improved disease management.

Introduction

Periodontitis is a chronic inflammatory disease resulting from a bacterial infection that can potentially initiate and increase systemic levels of various cytokines.[1] Recent advancements in periodontology have discovered an association between periodontitis and systemic diseases such as diabetes, myocardial infarction, stroke, arteriosclerosis, Alzheimer's disease, osteoporosis, and rheumatoid arthritis (RA) in adults.

Rheumatoid arthritis and periodontitis are chronic inflammatory conditions that share similar inflammatory mediators, including interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α).[2] Moreover, both diseases are known to impact connective tissue degradation. Rheumatoid arthritis is a systemic autoimmune condition characterized by persistent joint inflammation, resulting in joint destruction and functional loss. Studies have reported that globally, around 1.0% of adults suffer from this condition, with a higher predisposition observed in females (male:female ratio of 1:3). In the United States, around 2% of adults suffer from RA.[3–5] Studies have suggested 3 types of disease manifestation among RA patients: self-limited – patients with self-diagnosed RA who do not have symptoms 2–3 years after diagnosis[6]; easily controlled – conditions managed with non-steroidal anti-inflammatory drugs (NSAIDs)[7]; and progressive – conditions that typically require second-line therapy and may not fully resolve despite treatment.[8]

Numerous hypotheses have been developed regarding the correlation between periodontitis and systemic diseases. An intriguing one centers around paraoxonase-1 (PON1), an enzyme with established antiatherogenic and antioxidant activities. Current research investigates the potential role of PON1 in mitigating both dental biofilm formation and periodontitis.[9] Many studies have investigated the association between periodontitis and RA.[10–12] While some studies have indicated that periodontitis could be a potential risk factor for RA, others have demonstrated a notable increase in tooth and alveolar bone loss in individuals with longstanding RA.[1,13–15] Patients diagnosed with periodontitis exhibit a four-fold increase in the likelihood of self-reported RA, which may be related to the beneficial outcomes of non-surgical periodontal therapy (NSPT) in reducing the severity of RA.[2,16,17] In RA, the synovial membrane produces pro-inflammatory mediators due to systemic immunological failure, leading to synovitis, joint pain and bone damage.[18,19] The correlation between RA and periodontitis may be attributed to the association of both conditions with environmental and genetic risk factors, as well as comparable pathobiology.

Periodontitis and RA are characterized by chronic inflammation that leads to increased infiltration of lymphocytes of the T and B cell types, neutrophils, and monocytes.[8,12] The elevated production of proinflammatory cytokines leads to the destruction of joint and alveolar bone tissues in RA and periodontitis, respectively.[1,14] Fibroblasts and lymphocytes increase the expression of receptor activator of nuclear factor-κB ligand (RANKL) during osteoclast formation in both conditions. Autoimmunity to citrullinated proteins has been suggested to contribute to the relationship between RA and inflammation.[20] *Porphyromonas gingivalis* is a significant periodontopathogen found in the subgingival plaque. It demonstrates the distinctive capability of citrullinating proteins, thereby accelerating the autoimmune response in RA.[21,22]

Both conditions share a common etiology, pathology, immunogenetic background, and pathways of bone cartilage destruction. Additionally, RA and severe periodontal disease are associated with lower oral health-related quality of life (OHRQoL) scores.[23] Various studies have also proposed NSPT for these conditions.[16,18,19] This may be evidenced by the ability of NSPT to reduce disease activity score-28 with C-reactive protein (DAS28-CRP), erythrocyte sedimentation rate (ESR) and anticitrullinated protein antibody (ACPA) levels in serum.[24] Nonetheless, very limited literature shows this bidirectional

association, especially in middle-aged adults. The National Health and Nutrition Examination Survey-I (NHANES-I) showed that individuals with periodontitis or missing teeth presented increased odds of prevalent or incident RA.[25] However, most of the results were not statistically significant and lacked dose–response relationships. The observed variations may stem from inconsistent criteria for defining the 2 illnesses. The association between these 2 conditions can be explained in various forms: 1) the role of periodontal pathogens (*P. gingivalis*, anticyclic citrullinated peptide (anti-CCP)); 2) common immunogenetics; 3) common risk factors (smoking, age-related factors, psychological issues).[1,12,13,26]

Several studies have reported discrepancies regarding the correlation between periodontitis and RA. However, recent reports indicate a significant link (biological and immunological) between these prevalent chronic inflammatory conditions.[8,11,15] Nonetheless, a potential correlation between the severity of RA and periodontitis cannot be established due to inconclusive evidence. Hence, this systematic review aims to evaluate an association between periodontitis and RA among middle-aged populations.

Material and methods

Focus question

Based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines 2020, a specific question was structured according to the PECOS (Participants, Exposure, Control, Outcomes, and Study design) framework. The focus question for this systematic review was as follows: Among middle-aged populations (P), is there an association between periodontitis (E) and rheumatoid arthritis (O), compared to healthy individuals (C), in observational studies (S)?

Protocol and registration

The research problem, focus question and criteria for inclusion and exclusion were identified after a preliminary literature review. A protocol was prepared and registered prospectively with PROSPERO (registration No. CRD42023490679).

Eligibility criteria

Studies fulfilling the following criteria were included: participants aged ≥30 years; observational studies; studies reporting risk estimates as odds ratios (*OR*s) or hazard ratios (*HR*s) with corresponding 95% confidence intervals (95% *CI*s) for the association between RA and periodontitis; inclusion of a control group (healthy individuals); diagnosis of periodontitis and RA according to accepted diagnostic criteria; adjustment for potential confounding factors; studies with ≥15 patients per group; mean RA duration of ≥5 years; articles published between 2013 and 2023.

The exclusion criteria were as follows: letters to the editor; commentaries; reviews; case reports; case series; animal studies; in vitro studies; and articles published in languages other than English. If more than 1 paper from the same study was retrieved, both were included when different outcomes were reported. However, if the same result was reported in different studies, the one with the larger sample size and/or non-overlapping cohort analyses was selected for the meta-analysis.

Literature search protocol

The search strategy included published and unpublished articles, and this review employed a two-step procedure for selecting studies. An initial search of PubMed®/MEDLINE was conducted, followed by screening of titles, abstracts and keywords.

The second search utilized keywords and index terms across different databases. PubMed®/MEDLINE, Embase, Scopus, Web of Science, and ProQuest were searched from 2013 to 2023 using various combinations of keywords and Medical Subject Headings (MeSH) terms related to periodontitis and RA. Unpublished articles were searched in the OpenGrey database. Moreover, a manual search and citation chasing were performed in various dental and medical journals. The PubMed® search strategy is available from the corresponding author upon reasonable request.

Selection of articles

The retrieved articles were imported into EndNote 20 (Clarivate, Philadelphia, USA), and duplicates were removed. The relevant articles were entered into a Microsoft Excel (Microsoft Corp., Redmond, USA) sheet, and the final articles were selected by 2 independent reviewers (SeA, BJ), who examined the titles, abstracts and full texts according to the eligibility criteria. Disagreements between the reviewers were resolved through discussion with a third reviewer (SuA). Intraobserver reliability was assessed, and the value of K-alpha between the reviewers was 0.9.

Data extraction

The data was extracted using tools predesigned for research questions. The following details were extracted: 1) authors/year/country; 2) number of males and females included in the test and control groups; 3) mean age of participants; 4) exposure assessment of periodontitis; 5) outcome assessment of RA; 6) RA clinical parameters; 7) periodontal clinical measures; 8) risk estimates (crude and adjusted): *OR*s, relative risks and *HR*s with 95% *CI*s

and *p*-values, if available; 9) number of RA patients with a history of periodontitis; 10) factors adjusted for in the analysis, e.g., age, smoking status and other inflammatory conditions; and 11) conclusions. The reviewers resolved discrepancies through discussion, and the authors were contacted if any data from the primary study was missing.

Assessment of methodological quality

The methodological quality of the included articles was assessed using the Newcastle–Ottawa quality assessment scale for case–control[27] and cross-sectional studies.[28] A scoring system was used to evaluate the quality of articles, with 17–18 points indicating high, 12–16 indicating moderate, and <12 suggesting low quality. Eligible studies were assessed for quality by 2 reviewers, and any disagreements were resolved through discussion or by the third reviewer.

Data synthesis and assessment of the quality of evidence

A meta-analysis of the included case–control and cross-sectional articles was conducted, as the included studies possessed similar designs, and the pooling of statistical data yielded a concise outcome. Moreover, the quality of evidence was assessed using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) system.[29]

Results

Study selection

The primary search identified 15,982 relevant articles, out of which 15,778 papers were found to be duplicates and were removed. The titles and abstracts of the remaining 204 articles were screened based on the eligibility criteria, and 166 were excluded after careful evaluation. The full texts of the remaining 38 articles were examined, and 27 articles were removed based on their objectives or eligibility criteria. Finally, 11 articles were considered eligible for this systematic review. Figure 1 illustrates the study selection process.

Characteristics of the included studies

This review included 11 primary studies, consisting of 5 case–control and 6 cross-sectional studies comprising 4,055 participants (1,396 males and 2,659 females) (Table 1). The included papers were published between 2013 and 2023 and, originating from different countries (India, USA, China, South Korea, Sweden, Spain, Brazil, and Lithuania), represented various ethnic backgrounds. In most of the included studies, the majority of the participants were females aged ≥40 years. Out of the 11 studies, only 2 studies excluded smokers,[11,30] and 1 study did not mention this criterion.[17] Only 5 included studies reported medications used by the patients.[17,31–34] Most of the studies evaluated periodontal health using validated indices, and RA was diagnosed based on the 1987 (revised) American Rheumatism Association (ARA) criteria and 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA.

Clinical parameters defining periodontitis

Ten of the 11 included studies evaluated CAL,[11,12,17,30,31,33–37] either alone or in combination with other periodontal indices; 1 study did not assess CAL and recorded only PPD and BOP.[32] Five studies evaluated PPD and BOP in combination with CAL.[11,12,30,34,35] The odds of developing periodontitis were higher in patients with RA. However, no significant differences were reported regarding the severity of periodontitis in relation to RA. Secondary parameters recorded included the plaque index, the gingival index, tooth loss, and tooth mobility. Only 5 studies evaluated interexaminer calibration reports.[12,30,32,34,35]

Clinical parameters defining rheumatoid arthritis

All the included articles diagnosed RA clinically based on the DAS28 criteria as low, moderate or high. Eight papers reported RA treatment, while others did not

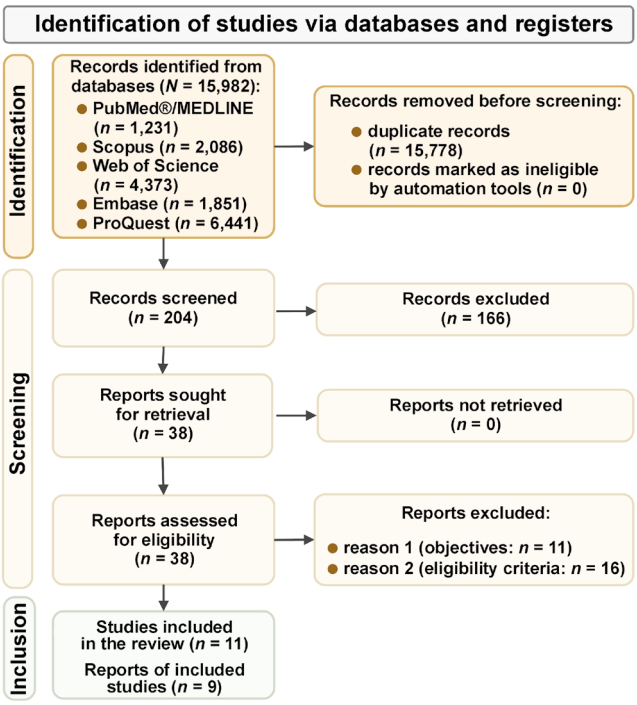

Fig. 1. Study selection process

Table 1. Characteristics of the included studies

Study	Country	Study design	Participants, *n*	Sex (M/F), *n*	Age [years]	Results	Conclusions
Posada-López et al. 2023[37]	USA	cross-sectional	75 (PD without RA: 21; PD+RA: 33; reduced periodontium with RA: 21)	20/55	52.8 (50.3–55.3)	In RA patients, milder periodontal conditions were observed. Patients without PD displayed the highest ACPA levels. Factors like age, *P. gingivalis*, diabetes, smoking status, osteoporosis, and medication were not significantly correlated with RA ($p < 0.05$).	PD was not associated with RA. Furthermore, no correlation emerged between periodontal clinical parameters and RA biochemical markers.
Moura et al. 2021[36]	Brazil	case–control	471 (RA: 157; controls: 314)	145/326	49.13 ±7.88	The study demonstrated a significantly higher frequency ($p < 0.001$), severity ($p = 0.006$) and extent ($p = 0.018$) of periodontitis in RA patients compared to controls. RA demonstrated an *OR* of 2.53 (95% *CI*: 1.24–3.86, $p < 0.001$) for PD, while PD showed an *OR* of 3.12 (95% *CI*: 1.47–4.26, $p < 0.001$) for RA.	Individuals with RA have a high frequency of PD. There is a significant correlation between the occurrence, severity and extent of PD, RA and smoking habits.
Punceviciene et al. 2021[31]	Lithuania	cross-sectional	93 (RA+PD: 63; RA only: 30)	14/79	54.01 ±11.01	29% of RA patients had severe PD. Those with moderate–severe PD showed higher DAS28-CRP scores (4.49 ±1.22 vs. 3.86 ±1.58, $p = 0.033$). Lower vitamin D levels were found in RA+PD patients with severe PD (39.61 ±17.12 vs. 52.07 ±18.23 nmol/L, $p = 0.031$).	RA patients exhibited a notable prevalence of severe PD, with a significant association between severe PD and elevated RA disease activity. Additionally, lower vitamin D levels were noted in the RA+PD group.
Renvert et al. 2020[32]	Sweden	case–control	375 (RA: 126; controls: 249)	147/228	70 ±6.6 (RA); 70 ±7.1 (controls)	The RA group had a significantly higher prevalence of PD (61.1%) than the control group (33.7%) ($p = 0.001$). Logistic regression showed associations between RA and BMI > 25 (*OR*: 6.2), PD (*OR*: 2.5) and female sex (*OR*: 2.3).	The RA group exhibited a higher prevalence of PD than controls. Patients with RA had higher odds of developing PD (*OR*: 2.5, 95% *CI*: 1.5–4.2, $p < 0.001$), especially among females or obese individuals.
Rodríguez-Lozano et al. 2019[33]	Spain	case–control	344 (RA: 187; controls: 157)	96/248	54.4 ±10.8 (RA); 55.5 ±23.7 (controls)	A significant association was found between PD and RA (*aOR*: 20.57, 95% *CI*: 6.02–70.27, $p < 0.001$). RA patients showed worse periodontal parameters than controls ($p < 0.001$). The severity of PD was correlated with RA disease activity (*aOR*: 2.66, 95% *CI*: 1.24–5.74, $p = 0.012$).	A strong correlation persists between PD and RA, remaining significant despite controlling for other variables. This association is notably stronger among individuals with severe PD and higher RA activity.
Eriksson et al. 2019[12]	Sweden	cross-sectional	40 (RA without PD: 10; RA+PD: 30)	5/35	50 ±14 (RA without PD); 64 ±7.8 (RA+PD)	Most patients (75%) had moderate or severe PD, while others were diagnosed with no or mild PD. ACPA positivity was more prevalent in moderate/severe PD (86%) when compared to no/mild cases (50%). There were significant differences in plaque microbial composition.	RA patients with moderate/severe PD exhibited significant associations with ACPA positivity, altered subgingival microbial profiles and elevated inflammatory mediator levels.
Zhao et al. 2019[17]	China	case–control	237 (RA: 128; controls: 109)	33/204	44.36 ±8.16	RA patients exhibited significantly worse periodontal health, with a 10.26-fold increased risk of periodontitis after adjusting for factors. Independent associations included DAS28, ESR, educational level, and anti-CCP positivity.	RA patients demonstrate higher odds of developing PD.
Kim et al. 2018[35]	South Korea	cross-sectional	346 (RA: 260; controls: 86)	45/301	58.13 ±11.65 (RA); 58.08 ±12.17 (controls)	The RA group showed significantly higher periodontal indices ($p < 0.05$), except for teeth count. BOP correlated with DAS28, RA duration, ESR, anti-Pg, and anti-CCP titers. CAL and the severity of PD were associated with anti-Pg titers.	GI, BOP and PPD correlated positively with RA indices, but CAL did not. The anti-Pg titer correlated positively with PPD, BOP, CAL, and PD severity.
Choi et al. 2016[34]	South Korea	cross-sectional	352 (RA: 264; controls: 88)	44/308	58.2 ±12.0 (RA); 58.2 ±11.6 (controls)	RA patients had a higher prevalence of moderate/severe PD than controls (63.6% vs. 34.1%, $p < 0.001$). BOP correlated positively with DAS28 ($r = 0.128$, $p = 0.041$) and RA disease duration ($r = 0.211$, $p = 0.001$).	The prevalence of moderate/severe PD was higher in RA patients compared to controls.
Joseph et al. 2013[30]	India	case–control	212 (RA: 100; controls: 112)	50/162	46.54 ±8.5 (RA); 45.91 ±9.76 (controls)	Significant differences in GI, OHI-S, PPD, CAL, ESR, and CRP levels were observed between RA and non-RA groups ($p < 0.05$). However, there was no association between disease activity and PD severity in patients with RA.	The occurrence and severity of PD were higher in RA subjects compared to controls.
Dev et al. 2013[11]	India	cross-sectional	1,520 (RA+PD: 852; controls: 668)	802/718	–	Among patients referred for periodontal treatment, the prevalence of RA was 4.4%. Females (3.2%) and those aged >50 years (3.5%) had a significantly higher prevalence of RA ($p < 0.001$). Odds of RA in females were nearly 3 times higher compared to males (*OR* = 2.81, $p < 0.05$).	Individuals with moderate to severe periodontal disease are at higher risk of developing RA, and vice versa.

ACPA – anticitrullinated protein antibody; anti-CCP – anticyclic citrullinated peptide antibody; anti-Pg – antibody against *P. gingivalis*; *aOR* – adjusted odds ratio; BMI – body mass index; BOP – bleeding on probing; CAL – clinical attachment level; CRP – C-reactive protein; DAS28 – disease activity score-28; ESR – erythrocyte sedimentation rate; GI – gingival index; OHI-S – oral hygiene index-simplified; *OR* – odds ratio; PD – periodontitis; *P. gingivalis* – *Porphyromonas gingivalis*; PPD – probing pocket depth; RA – rheumatoid arthritis; 95% *CI* – 95% confidence interval.

Table 2. Risk estimates of the included studies

Study	Inclusion of smokers	Exposure (definition and assessment of periodontitis)	Outcome (definition and assessment of RA)	RA clinical parameters
Posada-López et al.[37]	yes	2 or more non-adjacent interdental sites with loss of CAL, PPD ≥ 4 mm and BOP	ACR; DAS28-CRP ≥ 3.2	ACPA [U/mL]: PD (*Me* (*IQR*)): 0.5 (0.5–0.65) PD+RA: 98.6 (10.9–200.0) non-PD+RA: 45.8 (8.2–507.4) ($p < 0.001$) RF [U/mL]: PD: 15.0 (0.0–0.0) PD+RA: 72.6 (9.0–144.2) non-PD+RA: 157.9 (16.0–268.0) ($p < 0.001$)
Moura et al.[36]	yes	stage II: ≥2 interproximal sites with CAL of 3–4 mm and PPD ≤5 mm	ACR; DAS28	N/A
Punceviciene et al.[31]	yes	2018 AAP/EFP classification	ACR/EULAR; DAS28-CRP	DAS28: total: 4.19 ±1.43 RA+PD: 4.37 ±1.28 RA only: 3.82 ±1.65 ($p = 0.08$) CRP [mg/L]: total: 12.08 ±21.5 RA+PD: 13.82 ±24.44 RA only: 8.44 ±12.99 ($p = 0.98$)
Renvert et al.[32]	yes	PPD and BOP; CAL not assessed	ACR; DAS28-ESR	N/A
Rodríguez-Lozano et al.[33]	yes	CAL and PPD (based on Tonetti and Claffey criteria, 2005)	DAS28-ESR or DAS28-CRP	N/A
Eriksson et al.[12]	yes (no current smokers but former smokers)	PPD, CAL and BOP at 6 sites per tooth	DAS28-CRP	DAS28: RA without PD: 2.7 ±1.5 RA+PD: 3.3 ±1.4 ACPA-positive: RA without PD: 5 ±50 RA+PD: 25 ±86
Zhao et al.[17]	not mentioned	AAP (Armitage et al., 2003 classification)	DAS28-ESR	DAS28-ESR: RA+PD: 4.03 ±1.38 RA without PD: 3.64 ±1.46 ($p = 0.148$) DAS28-CRP: RA+PD: 12.95 ±15.13 RA without PD: 9.29 ±8.13 ($p = 0.149$)
Kim et al.[35]	yes	CAL (AAP, 1999)	DAS28	DAS28: RA: 3.62 ±1.35 ACPA [U/mL]: RA: 67.5
Choi et al.[34]	yes	CAL (AAP, 1999)	DAS28-ESR	DAS28: RA: 3.3 ±1.4 ACPA [U/mL]: RA: 69.1
Joseph et al.[30]	no	CAL (AAP, 1999)	DAS28-ESR	not mentioned
Dev et al.[11]	no	CPI with CAL based on the WHO guidelines (1997)	ARA, 1988	not mentioned

AAP – American Academy of Periodontology; ACR – American College of Rheumatology; ARA – American Rheumatism Association; EFP – European Federation of Periodontology; EULAR – European League Against Rheumatism; *IQR* – interquartile range; *Me* – median; NSAIDs – non-steroidal anti-inflammatory drugs; RF – rheumatoid factor; *rOR* – reporting odds ratio; WHO – World Health Organization.

Periodontal clinical measures	Risk estimates and adjusted risk estimates	RA patients with a history of periodontitis, *n*	Factors adjusted for in the analysis
PD (stage III): PD: 5 (23.8%) PD+RA: 19 (57.6%) PD (stage IV): PD: 16 (76.2%) PD+RA: 12 (36.4%)	crude *OR*: 0.078 (0.1–0.3)	31	sex, age, *P. gingivalis*, diabetes, smoking, osteoporosis, NSAIDs, corticosteroids, antihypertensive medication
CAL [mm]: test: 4.8 ±1.8 control: 4.0 ±1.5	crude *OR*: 2.64 (1.78–3.94); $p < 0.0001$ *aOR*: 3.12 (1.47–4.26)	84	older age, female sex, smoking, protective effect of alcohol use
severe PD (stages III–IV): 27 (29.0%)	not mentioned	27	not mentioned
RA ($n = 126$): 61 patients with PD (48%) controls ($n = 249$): 96 patients with PD (38.6%); $p = 0.001$	crude *OR* (PD: composite index): 2.5 (1.5–4.2); $p < 0.001$	61	not mentioned
CAL [mm]: RA: 3.99 ±1.27 controls: 3.34 ±1.03	crude *OR* (periodontitis): 14.75 (5.66–34.4); $p < 0.001$ *aOR*: 20.57 (6.02–70.27); $p < 0.001$	182	sex, smoking, socioeconomic status, annual dental prophylaxis, osteoporosis
CAL ≥ 4 mm: RA without PD: 0.5 ±0.5 RA+PD: 10 ±6.7	not mentioned	30	sex, age, smoking habits
CAL [mm]: RA: 3.57 ±1.59 controls: 2.11 ±1.13; $p < 0.001$	crude *OR*: 4.68 (2.70–8.11) *aOR*: 10.26 (4.67–22.58)	87	age, education, BMI
CAL [mm]: RA: 3.25 ±0.74 controls: 2.91 ±0.52	not mentioned	not mentioned	not mentioned
CAL [mm]: RA: 3.25 ±0.73 controls: 2.90 ±0.51	crude *OR*: 3.38 (2.50–4.57)	not mentioned	not mentioned
CAL [mm]: RA: 2.88 ±1.10 controls: 0.98 ±1.04	*rOR*: 3.05	58	not mentioned
not mentioned	not mentioned	572	not mentioned

mention the therapy. Disease duration was evaluated as a factor associated with the outcome in 6 publications, whereas 5 did not assess disease incidence. Eight studies examined ESR and posterior-anterior hand and wrist radiographs,[17,30–33,35–37] 4 reports assessed visual analog scores and tender joint counts to measure the occurrence of RA,[12,30,34,35] and 5 studies recorded the CRP levels.[12,30,34–36] All the included papers utilized validated measures to record RA clinical parameters.

Association between rheumatoid arthritis and periodontitis

Case–control studies

Moura et al. reported a high frequency of periodontitis among individuals with high DAS28 scores (adjusted odds ratio (*aOR*): 3.12; 95% *CI*: 1.47–4.26).[36] Additionally, a significant correlation between periodontitis, RA and smoking was noted ($p < 0.01$).[36] Adjusted risk estimates considered older age, female sex, smoking, and the protective effect of alcohol use. Renvert et al. found that high DAS28-ESR was linked to a composite index for periodontitis (*OR*: 2.5; 95% *CI*: 1.5–4.2; $p < 0.001$).[32] Rodríguez-Lozano et al. demonstrated that the DAS28-ESR and DAS28-CRP scores were significantly associated with periodontal clinical measures (*aOR*: 20.57; 95% *CI*: 6.02–70.27; $p < 0.001$) when adjusted for sex, smoking status, socioeconomic status, annual dental prophylaxis, and osteoporosis.[33] Zhao et al. also found DAS28-ESR outcomes to be associated with periodontal clinical measures, revealing significant results (*aOR*: 10.26; 95% *CI*: 4.67–22.58).[17] Similarly, Joseph et al. found that the DAS28-ESR scores were correlated with CAL, with a relatively significant *OR* when smokers were excluded.[30]

Cross-sectional studies

Posada-López et al. reported that periodontitis was not associated with RA.[37] This study adjusted for confounders such as sex, age, *P. gingivalis*, diabetes, smoking, osteoporosis, NSAIDs, corticosteroids, and antihypertensive medication. All periodontal variables and *P. gingivalis* were negatively correlated with RA biochemical markers ($p < 0.05$). However, Punceviciene et al. reported a high prevalence of severe periodontitis in RA patients.[31]

Table 3. Assessment of the risk of bias for case–control studies (Newcastle–Ottawa scale)[27]

Domain	Item	Study				
		Joseph et al.[30]	Zhao et al.[17]	Rodríguez-Lozano et al.[33]	Renvert et al.[32]	Moura et al.[36]
Selection	adequacy of case definition	**	**	**	*	**
	representativeness of cases	**	**	**	*	**
	selection of controls	**	**	**	*	**
	definition of controls	**	**	**	*	**
Comparability	comparability of cases and controls based on design or analysis	**	**	**	**	**
Exposure	ascertainment of exposure	**	**	**	*	**
	same method of ascertainment for case and control groups	–	–	–	–	–
	non-response rate	*	–	–	*	*
Quality		good	good	good	poor	good

Table 4. Assessment of the risk of bias for cross-sectional studies (Newcastle–Ottawa scale)[27]

Domain	Item	Study					
		Dev et al.[11]	Choi et al.[34]	Kim et al.[35]	Eriksson et al.[12]	Punceviciene et al.[31]	Posada-López et al.[37]
Selection	representativeness of cases	**	**	**	**	**	**
	sample size	*	*	*	*	*	*
	non-response rate	–	–	–	*	*	–
	ascertainment of the screening/surveillance tool	–	–	–	–	–	–
Comparability	assessment of potential confounders (subgroup or multivariable analysis)	–	**	**	**	**	**
Outcome	assessment of the outcome	*	**	**	**	**	**
	statistical test	**	**	**	**	**	**
Quality		poor	moderate	good	good	good	moderate

This relationship was significantly associated with higher RA disease activity and lower vitamin D levels in the RA–periodontitis group.[31] Similarly, Eriksson et al. found that RA parameters correlated with periodontal clinical measures in a study that included non-smokers.[12] Kim et al. reported that DAS28 outcomes were associated with CAL in a cohort that included individuals who smoked.[35] Furthermore, Choi et al. noted that DAS28-ESR outcomes were correlated with CAL and significantly elevated *OR*s.[34] Dev et al. also documented that the American Rheumatism Association (ARA) 1988 criteria were associated with periodontal clinical measures in a study in non-smokers, where periodontitis was evaluated using the community periodontal index (CPI) and CAL was assessed based on the World Health Organization (WHO) guidelines.[11]

To summarize, the increased risk of periodontitis was observed in RA patients in 10 studies, and 1 study showed no correlation between the 2 conditions.[37] Additionally, 10 studies demonstrated an association between moderate to severe forms of periodontitis in RA patients. Six papers mentioned smoking, vitamin D deficiency and alcohol consumption as risk factors associated with periodontal health and RA.[17,32,33,35–37] Three studies demonstrated anti-*P. gingivalis* antibody titers and reported a positive association with CAL, PPD and BOP in patients with RA.[12,30,35] One study noted a significantly higher risk of periodontitis (p = 0.01) among obese females with RA.[36] However, 1 report showed no association between the severity of periodontitis in RA patients.[37] Tables 1 and 2 illustrate the relevant findings of the included studies.

Risk of bias analysis

The Newcastle–Ottawa scale was employed to evaluate the risk of bias in the included case–control (Table 3) and cross-sectional studies (Table 4), analyzing case selection, comparison and outcome criteria. Out of the 5 case–control studies, the case and control selection domains scored low, while the comparison and outcome domains achieved higher scores. Accordingly, 1 study was identified as having a high risk of bias,[32] and 4 studies had a low risk of bias.[17,30,33,36] Similarly, in cross-sectional studies, one study was of poor quality,[11] 2 were of moderate quality,[34,37] and 3 studies were classified as having a low risk of bias.[12,31,35]

GRADE evaluation

The GRADE analysis reported low certainty of evidence in the analyzed case–control studies on the association between periodontitis and RA (Table 5). Meanwhile, cross-sectional studies demonstrated moderate certainty of evidence. The findings of the GRADE analysis indicate that more clinical and longitudinal studies are required to establish an association between both conditions.

Table 5. Grading of Recommendations Assessment, Development and Evaluation (GRADE) analysis

Certainty assessment							Patients with RA/healthy controls, *n*	Effect			Certainty (GRADE)	Importance
category	studies, *n* (design)	risk of bias	inconsistency	indirectness	imprecision	other considerations		relative effect	assumed baseline risk	anticipated absolute effect (95% *CI*)		
periodontal health of patients with and without RA (case–control studies)	5 (observational)	serious[a]	serious[b]	not serious	not serious	There was a strong association between both conditions. Plausible residual confounding would reduce the demonstrated effect.	5,060/5,759	*OR*: 22.76 (95% *CI*: 6.26–82.70)	10% 33%	5,060 more per 10,000 (159–383 more) 3,868 more per 10,000 (2,773–4,755 more)	⨁⨁◯◯ low	important
periodontal health of patients with and without RA (cross-sectional studies)	6 (observational)	serious[a]	serious[b]	not serious	not serious	Plausible residual confounding would suggest a spurious effect, while no effect was observed.	6,027/12,741	*OR*: 5.34 (95% *CI*: 2.28–12.51)	10% 33%	1,281 more per 10,000 (124–1,574 more) 2,327 more per 10,000 (282–2,721 more)	⨁⨁⨁◯ moderate	important

[a] risk of bias rated as serious owing to the predominantly observational study design, the use of convenience sampling and incomplete reporting of confounders in several included studies; [b] inconsistency rated as serious because of substantial statistical heterogeneity (I^2 = 87–95%) across the included studies.

Meta-analysis results

The results of the meta-analysis revealed high heterogeneity among the included studies (I^2 = 95%), and a high overall *OR* between the groups (*OR* = 22.76, 95% *CI*: 6.26–82.70; $p < 0.001$) (Fig. 2A). Sensitivity analysis of the included studies also showed a high *OR* for the development of periodontitis in RA patients (*OR* = 11.37, 95% *CI*: 4.22–30.61) (Fig. 2B). Figure 3A represents the overall analysis of cross-sectional studies, which revealed high heterogeneity (I^2 = 91%). The overall *OR* between the groups was 5.34 (95% *CI*: 2.28–12.51), which was statistically significant ($p < 0.001$). Overall, sensitivity analysis of the included cross-sectional studies yielded an *OR* of 7.52 (95% *CI*: 2.95–19.14), demonstrating a 3 times higher risk of developing periodontitis in patients with RA (Fig. 3B). In the context of the 5 studies that were considered in this analysis, only 1 study[37] concluded that the effect in question was not significant. The subgroup analysis was not performed, as the authors' primary focus was on assessing the overall association.

Discussion

To the best of our knowledge, this is the first systematic review to critically analyze and synthesize the available literature on the association between periodontal disease and RA. The results of this study indicate a statistically significant positive association between periodontal disease and RA. According to the results of the meta-analysis, there is an 11.37-fold higher risk of developing periodontitis in patients with RA. However, the GRADE analysis reported low to moderate certainty of evidence, suggesting that more research is needed on this association.

Previous studies have systematically evaluated the association between periodontitis and RA.[1,10,11,38,39] Based on the selection criteria, 11 articles (5 case–control and 6 cross-sectional studies) were included in this systematic review, with 9 articles being incorporated into the meta-analysis. The studies showed a positive association between periodontitis and RA, as 10 out of 11 papers demonstrated a strong correlation. The meta-analysis of cross-sectional studies revealed similar findings. A systematic review and meta-analysis by Kaur et al. showed a significant association between the conditions in 17 out of 19 studies (examining various RA-related

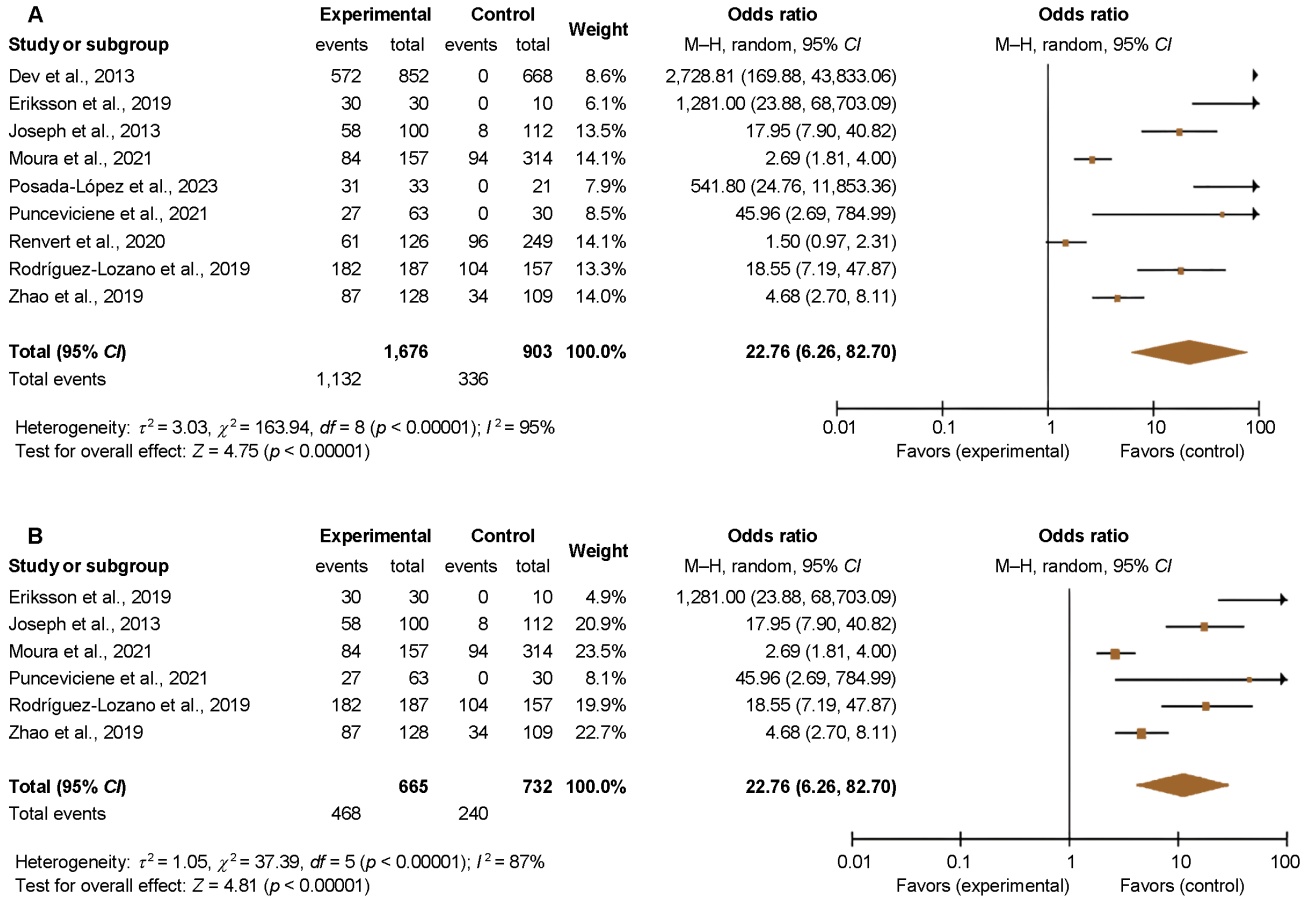

Fig. 2. Forest plot of the effect size for the association between periodontitis and rheumatoid arthritis (RA) in the included studies

A. Overall analysis; B. Sensitivity analysis.

95% *CI* – 95% confidence interval; *df* – degrees of freedom; M–H – Mantel–Haenszel.

factors and periodontitis).[40] Another systematic review conducted by Kriauciunas et al. found a link between oral bacterial growth and RA.[41] Agnihotri and Gaur reported significantly more severe CAL in their systematic review of elderly patients with RA.[42] In a recent scoping review, Nik-Azis et al. found that seropositive RA patients had higher odds of developing chronic periodontitis than the control group.[43]

The meta-analysis of cross-sectional studies indicated that there is a 5-fold increased risk for RA in patients with moderate to severe periodontitis (95% *CI*: 2.28–12.35; $p = 0.01$). The results also indicate that an inverse relationship may exist between the risk ratio of RA and periodontitis. The results of this review support the relationship between these 2 variables. However, given the small number of point estimates available in this analysis, there was a lack of statistical significance rather than an absence of an association. Similarly, in their meta-analysis, Del Rei Daltro Rosa et al. reported a reduction in the RA score index (mean difference = −1.81) in patients treated for periodontitis.[44] The risk estimate was lower than that reported in the current study. In the present review, we considered the studies included in the paper of Del Rei Daltro Rosa et al., although some of them could not be quantitatively synthesized. Moreover, the mentioned review included prospective and cross-sectional studies, whereas the current review focused primarily on cross-sectional evidence.

Rheumatoid arthritis is a risk factor for periodontal disease.[40] This relationship can be attributed to a possible link between systemic and concurrent alveolar bone resorption in RA and periodontitis. As systemic bone mineral density declines, for instance in RA, a simultaneous decrease occurs in the density of the maxillary and mandibular arches.[43] This increases bone fragility, inflammation and fracture risk. The majority of studies conducted on middle-aged adults have shown a positive association between RA and periodontal disease, which could be attributed to estrogen-related bone remodeling that increases with age.[42] Additionally, estrogen deficiency plays a mediating role in this association, contributing to inflammation and increasing the risk of both periodontitis and RA.[12,44]

The clinical presentation of periodontitis may not accurately indicate the presence of *P. gingivalis*, as not all cases are associated with this bacterium. In some instances, the administration of antibiotics in conjunction with mechanical debridement does not result in substantial improvement for the treatment of chronic periodontitis.[45] The presence of various periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, highlights the

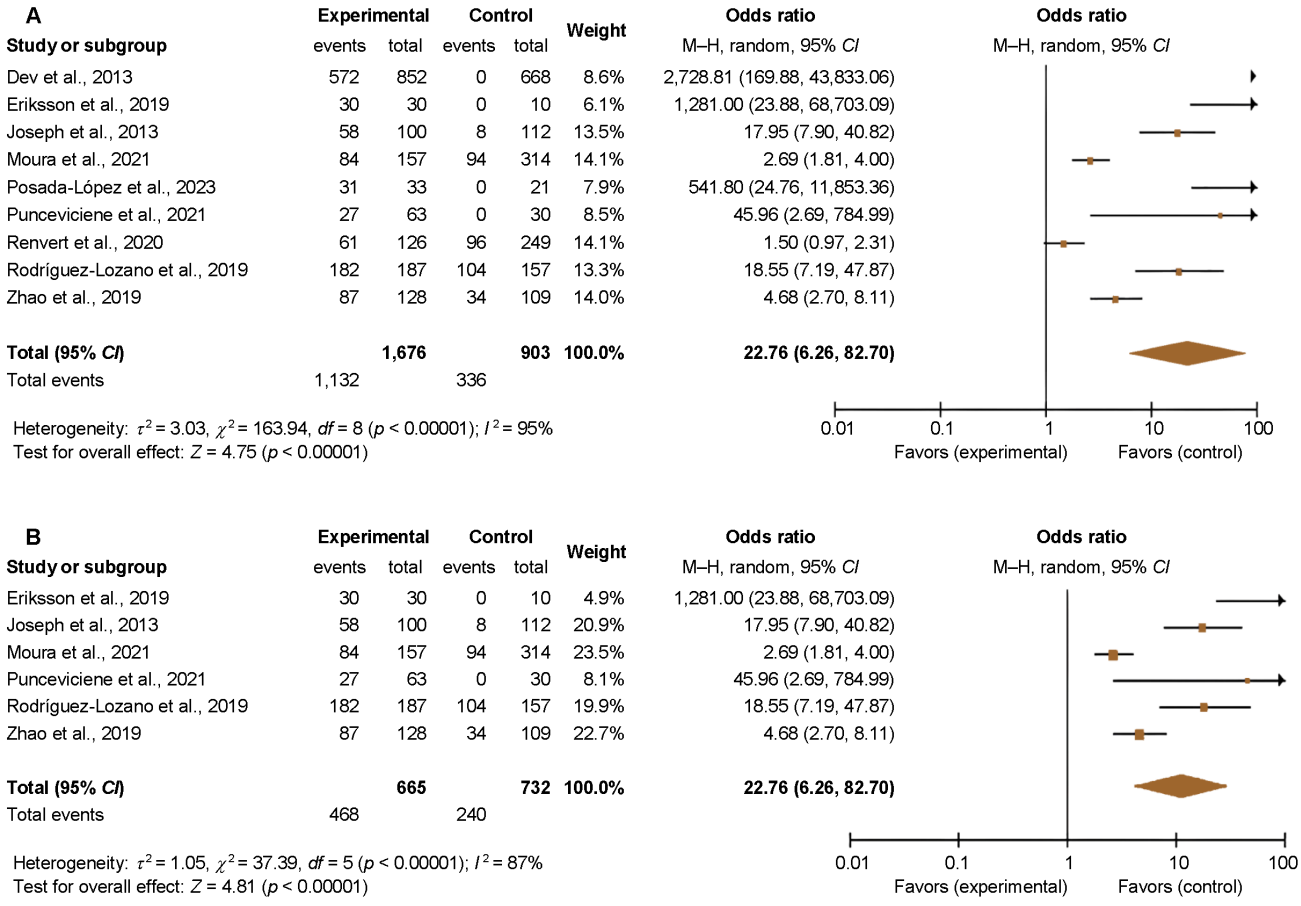

Fig. 3. Forest plot of the effect size for the association between periodontitis and rheumatoid arthritis (RA) in the included cross-sectional studies
A. Overall analysis; B. Sensitivity analysis.

need for improved study designs that consider the specific bacteria present in the subgingival microbiota.[20,26,39,46] Using an objective measurement of periodontal disease, such as quantifying the percentage of inflamed or ulcerated surface area, would address the issue of inconsistent definitions of periodontitis.[15,47,48]

Despite promising results, heterogeneity was observed among the included studies regarding the prevalence and severity of periodontal disease in RA-positive patients. In a cohort study of US veterans, periodontitis patients with RA were associated with the presence of ACPAs.[49] However, no significant association was found between periodontal disease, ACPA-positive RA patients and healthy controls.[50] In a Korean study, the authors indicated a positive association between periodontal inflammation and ACPA levels.[35] In a Japanese population cohort study designed to investigate this association, increased periodontal inflammation was reported in patients with seropositive RA as the level of ACPA rose above 2.34.[51,52] Choi et al. reported increased ACPA levels in patients with severe periodontitis.[34] By contrast, 2 studies on cohorts from Sweden and Malaysia found no association between ACPA levels and periodontal health in RA patients.[39,50]

The fundamental involvement of periodontal disease with the presence of salivary biomarkers is the basis for the hypothesized relationship between periodontitis and RA. Similar relationships have been studied using novel salivary adipokine levels (e.g., omentin-1). These molecules have the potential to serve as diagnostic tools to assess the progression of periodontal disease and treatment effectiveness.[53] However, the epidemiological evidence supporting this association is currently equivocal. These conflicts could be attributed to differences in study design and the selection of comparison groups (healthy controls, osteoporosis, osteoarthritis patients). Most of the included studies investigating this relationship did not specify the type and subsets of RA, even though these are important criteria for establishing an association between both conditions. The need for more specific and standardized criteria for defining periodontal disease and RA, inadequate information on confounding factors, and differences in eligibility criteria can also contribute to the conflicting evidence. Three included studies used data from hospital-based registries, while 2 studies employed national registries as the sample population. Such data provides concrete results due to larger sample sizes but may lack detailed information on confounding factors and associated diseases.

Limitations

The validity of the meta-analysis reported in this review is contingent upon the strength of the association between the 2 conditions – periodontitis and RA. Some studies have noted statistically significant associations between these 2 conditions, while others showed no significant correlations. Including such studies in our meta-analysis increases the validity of the findings and provides a more robust estimate of the association. However, the results of the meta-analysis should be interpreted carefully, as the inclusion of observational studies increases the risk of bias. The studies included in our review varied in methodological quality, resulting in differences in the strength of evidence. Well-controlled, population-based and clinical (molecular epidemiology) studies are needed to confirm the immunological and biological links between RA and periodontal disease. Future studies on this topic should follow the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines to facilitate the assessment of study strengths and limitations, as well as the generalizability of findings. Secondly, the review did not specifically consider severe periodontitis, as it is challenging to recruit patients with this condition. The majority of the included studies used convenience sampling methods, which pose a high risk of bias. Furthermore, although sex, age, smoking status, and RA treatment were considered in many studies, these parameters were not reported in detail. Hence, it was challenging to draw definitive conclusions on the role of potential confounding factors.

Conclusions

The current systematic review and meta-analysis found a positive association between periodontitis and RA. Moreover, some studies reported that patients diagnosed with RA present higher levels of salivary biomarkers and microbial load than healthy controls. The results of the GRADE analysis indicate the need for longitudinal clinical studies with longer follow-up durations to comprehensively evaluate this association and elucidate the potential role of confounding factors. Overall, the findings indicate that individuals diagnosed with RA demonstrate an increased risk of developing periodontal disease when compared to the general population. However, significant heterogeneity was observed across the included studies with respect to study populations, definitions of periodontal disease and RA assessments. Further research using standardized disease criteria and measurements is warranted. Understanding the interrelationship between periodontitis and RA may facilitate the identification of shared risk factors and disease mechanisms. Ultimately, clarifying this association has implications for the early identification of at-risk patients, preventive strategies and integrated treatment approaches that bridge oral health and chronic inflammatory disease management. The results of this review must be interpreted with caution, given the high heterogeneity among the included studies. Further research is necessary to clarify the extent and underlying nature of the periodontitis–RA relationship, as suggested by this initial evidence synthesis.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Georgiou TO, Marshall RI, Bartold PM. Prevalence of systemic diseases in Brisbane general and periodontal practice patients. *Aust Dent J*. 2004;49(4):177–184. doi:10.1111/j.1834-7819.2004.tb00070.x
2. Yang NY, Wang CY, Chyuan IT, et al. Significant association of rheumatoid arthritis-related inflammatory markers with non-surgical periodontal therapy. *J Formos Med Assoc*. 2018;117(11):1003–1010. doi:10.1016/j.jfma.2017.11.006
3. Pitones-Rubio V, Chávez-Cortez EG, Hurtado-Camarena A, González-Rascón A, Serafín-Higuera N. Is periodontal disease a risk factor for severe COVID-19 illness? *Med Hypotheses*. 2020;144:109969. doi:10.1016/j.mehy.2020.109969
4. Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. *Nat Rev Rheumatol*. 2017;13(10):606–620. doi:10.1038/nrrheum.2017.132
5. Ribeiro J, Leão A, Novaes AB. Periodontal infection as a possible severity factor for rheumatoid arthritis. *J Clin Periodontol*. 2005;32(4):412–416. doi:10.1111/j.1600-051X.2005.00689.x
6. O'Sullivan JB, Cathcart ES. The prevalence of rheumatoid arthritis. Follow-up evaluation of the effect of criteria on rates in Sudbury, Massachusetts. *Ann Intern Med*. 1972;76(4):573–577. doi:10.7326/0003-4819-76-4-573
7. Pincus T, Marcum SB, Callahan LF. Longterm drug therapy for rheumatoid arthritis in seven rheumatology private practices: II. Second line drugs and prednisone. *J Rheumatol*. 1992;19(12):1885–1894. PMID:1294735.
8. Cotti E, Schirru E, Acquas E, Usai P. An overview on biologic medications and their possible role in apical periodontitis. *J Endod*. 2014;40(12):1902–1911. doi:10.1016/j.joen.2014.08.013
9. Ghorbani P, Rezaei Esfahrood ZR, Foroughi M. Paraoxonase-1, a novel link between periodontitis and ischemic heart disease: A case–control study. *Dent Med Probl*. 2023;60(1):55–59. doi:10.17219/dmp/152181
10. de Molon RS, Rossa C Jr., Thurlings RM, Cirelli JA, Koenders MI. Linkage of periodontitis and rheumatoid arthritis: Current evidence and potential biological interactions. *Int J Mol Sci*. 2019;20(18):4541. doi:10.3390/ijms20184541
11. Dev YP, Khuller N, Basavaraj P, G S. Rheumatoid arthritis among periodontitis patients in Baddi industrial estate of Himachal Pradesh, India: A cross sectional study. *J Clin Diagn Res*. 2013;7(10):2334–2337. doi:10.7860/JCDR/2013/6237.3518
12. Eriksson K, Fei G, Lundmark A, et al. Periodontal health and oral microbiota in patients with rheumatoid arthritis. *J Clin Med*. 2019;8(5):630. doi:10.3390/jcm8050630
13. Eriksson K, Nise L, Kats A, et al. Prevalence of periodontitis in patients with established rheumatoid arthritis: A Swedish population based case–control study. *PLoS One*. 2016;11(5):e0155956. doi:10.1371/journal.pone.0155956
14. Gleissner C, Willershausen B, Kaesser U, Bolten WW. The role of risk factors for periodontal disease in patients with rheumatoid arthritis. *Eur J Med Res*. 1998;3(8):387–392. PMID:9707521.
15. Gonzalez SM, Payne JB, Yu F, et al. Alveolar bone loss is associated with circulating anti-citrullinated protein antibody (ACPA) in patients with rheumatoid arthritis. *J Periodontol*. 2015;86(2):222–231. doi:10.1902/jop.2014.140425
16. Takeuchi-Hatanaka K, Koyama Y, Okamoto K, Sakaida K, Yamamoto T, Takashiba S. Treatment resistance of rheumatoid arthritis relates to infection of periodontal pathogenic bacteria: A case–control cross-sectional study. *Sci Rep*. 2022;12(1):12353. doi:10.1038/s41598-022-16279-z
17. Zhao R, Gu C, Zhang Q, et al. Periodontal disease in Chinese patients with rheumatoid arthritis: A case–control study. *Oral Dis*. 2019;25(8):2003–2009. doi:10.1111/odi.13176
18. Rutger Persson G. Rheumatoid arthritis and periodontitis – inflammatory and infectious connections. Review of the literature. *J Oral Microbiol*. 2012;4. doi:10.3402/jom.v4i0.11829
19. Schulz S, Pütz N, Jurianz E, Schaller HG, Reichert S. Are there any common genetic risk markers for rheumatoid arthritis and periodontal diseases? A case–control study. *Mediators Inflamm*. 2019;2019:2907062. doi:10.1155/2019/2907062
20. Kobayashi T, Kido JI, Ishihara Y, et al. The *KCNQ1* gene polymorphism as a shared genetic risk for rheumatoid arthritis and chronic periodontitis in Japanese adults: A pilot case–control study. *J Periodontol*. 2018;89(3):315–324. doi:10.1002/JPER.17-0412
21. Jung ES, Choi YY, Lee KH. Relationship between rheumatoid arthritis and periodontal disease in Korean adults: Data from the Sixth Korea National Health and Nutrition Examination Survey, 2013 to 2015. *J Periodontol*. 2019;90(4):350–357. doi:10.1002/JPER.18-0290
22. Kässer UR, Gleissner C, Dehne F, Michel A, Willershausen-Zönnchen B, Bolten WW. Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis Rheum*. 1997;40(12):2248–2251. doi:10.1002/art.1780401221
23. Posada-López A, Botero JE, Duque-Castaño JD, Ronda-Pérez E, Agudelo-Suárez AA. Oral health-related quality of life in a group of patients with rheumatoid arthritis. *Dent Med Probl*. 2023;60(1):109–119. doi:10.17219/dmp/149455
24. Nguyen VB, Nguyen TT, Huynh NCN, Nguyen KD, Le TA, Hoang HT. Effects of non-surgical periodontal treatment in rheumatoid arthritis patients: A randomized clinical trial. *Dent Med Probl*. 2021;58(1):97–105. doi:10.17219/dmp/131266
25. Eklund SA, Burt BA. Risk factors for total tooth loss in the United States; longitudinal analysis of national data. *J Public Health Dent*. 1994;54(1):5–14. doi:10.1111/j.1752-7325.1994.tb01173.x
26. Gamel EB, Hashim NT, Satti A, Gismalla BG. Salivary TNFα levels in groups of subjects with rheumatoid arthritis and chronic periodontitis. *BMC Res Notes*. 2017;10(1):34. doi:10.1186/s13104-016-2341-7
27. Stang A. Critical evaluation of the Newcastle–Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. 2010;25(9):603–605. doi:10.1007/s10654-010-9491-z
28. Owens DK, Lohr KN, Atkins D, et al. AHRQ series paper 5: Grading the strength of a body of evidence when comparing medical interventions – agency for healthcare research and quality and the effective health-care program. *J Clin Epidemiol*. 2010;63(5):513–523. doi:10.1016/j.jclinepi.2009.03.009
29. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction–GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011;64(4):383–394. doi:10.1016/j.jclinepi.2010.04.026
30. Joseph R, Rajappan S, Nath SG, Paul BJ. Association between chronic periodontitis and rheumatoid arthritis: A hospital-based case–control study. *Rheumatol Int*. 2013;33(1):103–109. doi:10.1007/s00296-011-2284-1

31. Punceviciene E, Rovas A, Puriene A, et al. Investigating the relationship between the severity of periodontitis and rheumatoid arthritis: A cross-sectional study. *Clin Rheumatol*. 2021;40(8):3153–3160. doi:10.1007/s10067-021-05661-3
32. Renvert S, Berglund JS, Persson GR, Söderlin MK. The association between rheumatoid arthritis and periodontal disease in a population-based cross-sectional case–control study. *BMC Rheumatol*. 2020;4:31. doi:10.1186/s41927-020-00129-4
33. Rodríguez-Lozano B, González-Febles J, Garnier-Rodríguez JL, et al. Association between severity of periodontitis and clinical activity in rheumatoid arthritis patients: A case–control study. *Arthritis Res Ther*. 2019;21(1):27. doi:10.1186/s13075-019-1808-z
34. Choi IA, Kim JH, Kim YM, et al. Periodontitis is associated with rheumatoid arthritis: A study with longstanding rheumatoid arthritis patients in Korea. *Korean J Intern Med*. 2016;31(5):977–986. doi:10.3904/kjim.2015.202
35. Kim JH, Choi IA, Lee JY, et al. Periodontal pathogens and the association between periodontitis and rheumatoid arthritis in Korean adults. *J Periodontal Implant Sci*. 2018;48(6):347–359. doi:10.5051/jpis.2018.48.6.347
36. Moura MF, Cota LOM, Costa AM, Silva TA, Costa FO. Rheumatoid arthritis associated with the occurrence, severity and extension of periodontitis: A case–control study. *J Clin Exp Dent*. 2021;13(4):e389–e396. doi:10.4317/jced.57540
37. Posada-López A, Duque JD, Pineda-Tamayo RA, Bedoya-Giraldo E, Botero JE. Lack of association between periodontitis and rheumatoid arthritis. *Reumatol Clin (Engl Ed)*. 2023;19(3):123–129. doi:10.1016/j.reumae.2022.03.006
38. Fuggle NR, Smith TO, Kaul A, Sofat N. Hand to mouth: A systematic review and meta-analysis of the association between rheumatoid arthritis and periodontitis. *Front Immunol*. 2016;7:80. doi:10.3389/fimmu.2016.00080
39. Eriksson K, Lundmark A, Delgado LF, et al. Salivary microbiota and host-inflammatory responses in periodontitis affected individuals with and without rheumatoid arthritis. *Front Cell Infect Microbiol*. 2022;12:841139. doi:10.3389/fcimb.2022.841139
40. Kaur S, White S, Bartold PM. Periodontal disease and rheumatoid arthritis: A systematic review. *J Dent Res*. 2013;92(5):399–408. doi:10.1177/0022034513483142
41. Kriauciunas A, Gleiznys A, Gleiznys D, Janužis G. The influence of *Porphyromonas gingivalis* bacterium causing periodontal disease on the pathogenesis of rheumatoid arthritis: Systematic review of literature. *Cureus*. 2019;11(5):e4775. doi:10.7759/cureus.4775
42. Agnihotri R, Gaur S. Rheumatoid arthritis in the elderly and its relationship with periodontitis: A review. *Geriatr Gerontol Int*. 2014;14(1):8–22. doi:10.1111/ggi.12062
43. Nik-Azis NM, Mohd N, Mohd Fadzilah F, Mohamed Haflah NH, Mohamed Said MS, Baharin B. Rheumatoid arthritis serotype and synthetic disease-modifying anti-rheumatic drugs in patients with periodontitis: A case–control study. *PLoS One*. 2021;16(6):e0252859. doi:10.1371/journal.pone.0252859
44. Del Rei Daltro Rosa CD, de Luna Gomes JM, Dantas de Moraes SL, et al. Does non-surgical periodontal treatment influence on rheumatoid arthritis? A systematic review and meta-analysis. *Saudi Dent J*. 2021;33(8):795–804. doi:10.1016/j.sdentj.2021.09.007
45. Zúñiga-Loor D, Parise-Vasco JM, Montesinos-Guevara C. Mechanical debridement combined with amoxicillin and metronidazole compared with mechanical debridement alone for the treatment of chronic periodontitis: An overview of systematic reviews. *Dent Med Probl*. 2024;61(3):439–446. doi:10.17219/dmp/158925
46. Beyer K, Zaura E, Brandt BW, et al. Subgingival microbiome of rheumatoid arthritis patients in relation to their disease status and periodontal health. *PLoS One*. 2018;13(9):e0202278. doi:10.1371/journal.pone.0202278
47. Rahajoe PS, de Smit M, Schuurmans G, et al. Increased IgA anti-citrullinated protein antibodies in the periodontal inflammatory exudate of healthy individuals compared to rheumatoid arthritis patients. *J Clin Periodontol*. 2020;47(5):552–560. doi:10.1111/jcpe.13277
48. Rasch EK, Hirsch R, Paulose-Ram R, Hochberg MC. Prevalence of rheumatoid arthritis in persons 60 years of age and older in the United States: Effect of different methods of case classification. *Arthritis Rheum*. 2003;48(4):917–926. doi:10.1002/art.10897
49. Mikuls TR, Payne JB, Yu F, et al. Periodontitis and *Porphyromonas gingivalis* in patients with rheumatoid arthritis. *Arthritis Rheumatol*. 2014;66(5):1090–1100. doi:10.1002/art.38348
50. Mohamad WMW, Jia SK, Ghazali WSW, Taib H. Anti-cyclic citrullinated peptide antibody and periodontal status in rheumatoid arthritis patients. *Pak J Med Sci*. 2018;34(4):907–912. doi:10.12669/pjms.344.15007
51. Hashimoto H, Hashimoto S, Muto A, Dewake N, Shimazaki Y. Influence of plaque control on the relationship between rheumatoid arthritis and periodontal health status among Japanese rheumatoid arthritis patients. *J Periodontol*. 2018;89(9):1033–1042. doi:10.1002/JPER.17-0575
52. Hashimoto M, Yamazaki T, Hamaguchi M, et al. Periodontitis and *Porphyromonas gingivalis* in preclinical stage of arthritis patients. *PLoS One*. 2015;10(4):e0122121. doi:10.1371/journal.pone.0122121
53. Patil RS, Kalburgi NB, Koregol AC, Vinayaka AM, Puttarevanna T, Hannahson P. Effect of non-surgical periodontal therapy on the salivary levels of omentin-1, a novel adipokine biomarker in periodontitis: A clinico-biochemical study. *Dent Med Probl*. 2022;59(4):565–571. doi:10.17219/dmp/145593

Review

Orthodontically induced inflammatory root resorption in anterior teeth after treatment with clear aligners and fixed appliances, measured with cone-beam computed tomography: A systematic review and meta-analysis

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Abstract

Orthodontically induced inflammatory root resorption (OIIRR) is a common complication of orthodontic treatment. Clear aligners (CA) are thought to deliver lighter, intermittent forces than fixed appliances (FA), potentially reducing the extent of root resorption. However, comparative evidence remains limited, particularly from studies using cone-beam computed tomography (CBCT).

This meta-analysis aimed to compare anterior tooth root resorption following treatment with CA vs. FA in patients with similar malocclusions, using CBCT-based linear measurements.

A systematic search of MEDLINE/PubMed, Embase and the Cochrane Library was conducted in accordance with the PRISMA guidelines. Studies were eligible if they included patients older than 14 years with fully developed anterior roots and comparable malocclusion severity, and if they used CBCT to assess changes in the root length before and after treatment with either CA or FA. The random-effects meta-regression model was used to estimate the weighted mean difference (*WMD*) in root resorption. Subgroup analyses were performed according to tooth type and extraction status.

Four high-quality cohort studies, comprising a total of 1,204 anterior teeth, met the inclusion criteria. Root resorption was significantly lower in the CA group than in the FA group (*WMD*: –0.47 mm; 95% *CI*: –0.55 to –0.40 vs. *WMD*: –0.54 mm; 95% *CI*: –0.75 to –0.33; $p < 0.01$). The difference was most pronounced in non-extraction cases involving the maxillary central incisors.

Clear aligners are associated with significantly less anterior root resorption than FA in patients with comparable malocclusions. Although the absolute difference is modest, CA may represent a favorable treatment option for patients at increased risk of OIIRR.

Keywords: orthodontic treatment, root resorption, fixed appliances, clear aligners, orthodontically induced inflammatory root resorption

Highlights

- In patients with completed root apex development, undergoing treatment for comparable malocclusions over a similar treatment duration, the root resorption of anterior teeth was slightly but statistically significantly lower with clear aligner therapy (CAT) than with fixed appliance therapy (FAT).
- The risk of root resorption may be minimized through the application of light forces, precise tooth movements and the avoidance of jiggling movements, all of which are facilitated by the use of clear aligners.
- Regardless of the treatment modality used, the application of light forces and longer intervals between appliance activations is recommended.
- The choice of treatment modality should not be based solely on the potential extent of root apex resorption.

Introduction

Orthodontically induced inflammatory root resorption (OIIRR) is one of the most common complications of orthodontic treatment, posing risks to tooth vitality and long-term periodontal health. As the global demand for orthodontic treatment continues to rise – particularly among adults – understanding and minimizing OIIRR has become increasingly important. Mild to moderate root resorption affects approx. 40–60% of patients, whereas severe resorption (>5 mm) occurs in 1–5% of cases and may compromise tooth stability and function.[1–3] Orthodontically induced inflammatory root resorption most commonly affects the maxillary and mandibular incisors, as well as the maxillary canines, due to the extensive tooth movement often required during treatment.[4,5]

Orthodontically induced inflammatory root resorption results from the breakdown of cementum and dentin, typically initiated when excessive orthodontic forces exceed capillary pressure within the periodontal ligament, leading to localized ischemia and necrosis.[5,6] When reparative processes are insufficient to counteract tissue damage, irreversible root resorption may occur. Several factors have been associated with increased susceptibility to OIIRR, including force magnitude and duration, root morphology, occlusal trauma, pre-existing conditions, and genetic predisposition, particularly polymorphisms in the *IL-1β* gene.[3–7] Teeth with incomplete root formation or a history of endodontic treatment are generally considered more resistant to resorption.[3–7] Importantly, the loss of 3 mm of the apical root structure is estimated to correspond to 1 mm of marginal bone loss in terms of periodontal support, while root loss exceeding 6 mm is regarded as a sign of severe structural compromise.[6–8]

Contemporary orthodontic treatment modalities include both fixed appliances (FA), which remain the standard for managing complex malocclusions, and clear aligners (CA), which are increasingly favored for their esthetics and patient comfort. Although CA therapy has been associated with reduced root resorption, likely due to lighter forces and shorter periods of force application,[9] direct comparisons with FA must account for malocclusion severity to avoid biased conclusions.[10]

The radiographic assessment of root resorption remains challenging. Traditional two-dimensional (2D) imaging modalities, including panoramic and periapical radiographs, are limited by image distortion, magnification errors, and an inability to adequately detect buccal or lingual resorption.[11,12] In contrast, cone-beam computed tomography (CBCT), with its three-dimensional (3D) imaging capabilities, provides greater accuracy, repeatability and sensitivity for detecting subtle changes in root morphology.[4,5,13,14] Nevertheless, CBCT is associated with a substantially higher radiation dose, reported to be up to 33 times greater than that of panoramic radiography, depending on the field of view used.[10]

Previous meta-analyses comparing CA and FA have often lacked standardized imaging protocols and did not adequately control for differences in malocclusion severity.[4,9,15–17] The present study aimed to directly compare the extent of root resorption associated with CA and FA using CBCT in cohorts matched for baseline malocclusion severity, thereby providing more robust and clinically relevant evidence.

Objectives

This meta-analysis aimed to determine whether the root resorption of the maxillary and mandibular anterior teeth in patients treated with CA is comparable to that observed in patients treated with FA, as assessed by CBCT in cases with similar malocclusions. The analysis was based on the root length measurements obtained from CBCT performed before and after active orthodontic treatment.

The null hypothesis was that no statistically significant difference exists in root resorption between the maxillary and mandibular anterior teeth treated with CA and those treated with FA, as measured by CBCT.

Methodology

The study was registered in The International Prospective Register of Systematic Reviews – PROSPERO (CRD42023471622), and conducted in accordance with

the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. The preparation of the systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework, and included a systematic literature search, removal of duplicates, screening of titles and abstracts, and full-text assessment of selected studies.

Eligibility criteria (PICO framework)

To address the question of which treatment modality is associated with greater root resorption of anterior teeth, a search strategy based on the PICO framework was applied:

– **P**opulation: patients undergoing orthodontic treatment with fully developed anterior tooth roots;
– **I**ntervention: treatment with CA;
– **C**omparison: treatment with FA;
– **O**utcome: root resorption measured in millimetres on CBCT images as the difference in the root length before and after the completion of orthodontic treatment.

Studies were considered eligible if they included patients older than 14 years with fully developed anterior tooth roots and comparable malocclusion severity. Eligible studies were required to use CBCT to assess changes in the root length before and after orthodontic treatment with either CA or FA.

The exclusion criteria were as follows: animal studies; study designs other than randomized controlled trials (RCTs), prospective or retrospective clinical studies, or single-group studies; the assessment of the root length using 2D radiographic methods (panoramic or periapical imaging); measurements performed before the completion of orthodontic treatment; studies involving patients with incomplete development of anterior tooth roots; the lack of comparability between groups in terms of malocclusion type or severity; patients with a history of dental trauma; and patients with endodontically treated anterior teeth.

Literature search and study selection

The MEDLINE/PubMed, Embase and Cochrane Library databases were searched (Table 1). The search was further expanded to include gray literature and reference lists. All publications were independently screened, selected and assessed by two authors (RS and MM). Following title and abstract screening, full-text articles were retrieved and evaluated for eligibility based on the inclusion criteria. Studies meeting the criteria were included in the final analysis. Any disagreement was resolved through consultation with a third author (BG).

Assessment of study quality and risk of bias

The quality of non-randomized studies was assessed using the Newcastle–Ottawa Scale (NOS), which evaluates 3 domains: the selection of study groups (case and control representativeness); the comparability of groups at baseline; and outcome assessment with adequate follow-up and independent or blinded evaluation. One point was awarded for each criterion indicating high methodological quality.[18] The overall score was interpreted as follows: 7–9 points indicated grade A (good quality); 4–6 points indicated grade B (satisfactory quality); and 3 points or fewer indicated grade C (poor quality).[19]

The risk of bias was additionally evaluated using the ROBINS-I (Risk Of Bias In Non-randomized Studies – of Interventions) tool, which provides a structured framework for assessing bias across 7 domains that may occur before, during or after intervention implementation. These include: confounding (baseline differences between groups); the selection of participants; the classification of interventions; deviations from the intended interventions; missing outcome data; outcome measurement; and selective reporting. The ROBINS-I tool classifies studies into 4 levels of overall risk of bias: low; moderate; serious; and critical. A low risk of bias indicates that all or nearly all domains are at low risk, with only minor concerns regarding confounding. A moderate risk of bias is assigned when at least one domain raises moderate concerns, but none are serious or critical. A serious risk of bias indicates at least one domain with serious concerns or multiple domains with moderate concerns. A critical risk of bias reflects at least one domain with critical concerns, or multiple serious issues, and such studies are generally not recommended for inclusion in evidence syntheses.[20]

Table 1. Search strategy

Database	Search strategy
MEDLINE/PubMed	("root resorption"[MeSH Terms] OR ("root"[All Fields] AND "resorption"[All Fields]) OR "root resorption"[All Fields]) AND (("clear"[All Fields] OR "cleared"[All Fields] OR "clearing"[All Fields] OR "clearings"[All Fields] OR "clears"[All Fields]) AND ("align"[All Fields] OR "alignability"[All Fields] OR "alignable"[All Fields] OR "aligned"[All Fields] OR "alignement"[All Fields] OR "aligner"[All Fields] OR "aligners"[All Fields] OR "aligning"[All Fields] OR "alignment"[All Fields] OR "alignments"[All Fields] OR "aligns"[All Fields])) AND ("orthodontic appliances, fixed"[MeSH Terms] OR ("orthodontic"[All Fields] AND "appliances"[All Fields] AND "fixed"[All Fields]) OR "fixed orthodontic appliances"[All Fields] OR ("fixed"[All Fields] AND "appliances"[All Fields]) OR "fixed appliances"[All Fields])
Embase	('orthodontic root resorption' OR (('orthodontic'/exp OR orthodontic) AND ('root'/exp OR root) AND ('resorption'/exp OR resorption))) AND ('clear aligner'/exp OR 'clear aligner' OR (clear AND aligner)) AND ('fixed appliances' OR (fixed AND appliances))
Cochrane Library	("root resorption"):ti,ab,kw AND (clear aligner):ti,ab,kw AND ("fixed appliance"):ti,ab,kw

The risk of bias and methodological quality of all included studies were independently assessed by two authors (RS and MM). Any disagreement was resolved through discussion, and unresolved cases were adjudicated by a third author (BG).

Statistical analysis

Statistical analysis was performed using the R software, v. 3.6.1 (R Core Team, 2021; https://www.r-project.org). To evaluate differences in anterior tooth root resorption between the patients treated with FA and CA, the random-effects meta-regression model was applied. This approach combines meta-analysis and linear regression to assess the potential influence of study-level covariates on the effect size.

The treatment effect was expressed as a weighted mean difference (*WMD*) in millimetres, with corresponding 95% confidence intervals (*CI*s). Study weights were assigned using the inverse-variance method, thereby giving greater influence to studies with more precise estimates. The random-effects model was chosen a priori due to the anticipated clinical and methodological heterogeneity across the included studies.

Heterogeneity was assessed using the I^2 statistic, with values above 50% considered indicative of substantial heterogeneity and prompting additional subgroup analyses. Subgroup analyses were performed to compare extraction vs. non-extraction cases, as well as according to tooth type and location (incisors, canines, the maxilla, and the mandible), which were considered potential effect modifiers.

In contrast, malocclusion severity and treatment duration were not included in the meta-regression model, as these variables were comparable across the included studies, and therefore unlikely to account for between-study differences in the effect size.

Given the limited number of included studies, sensitivity analyses and the formal assessment of publication bias (e.g., funnel plots or Egger's test) were not performed, in accordance with methodological recommendations for low-powered datasets. A p-value <0.05 was considered statistically significant for all analyses.

Results

PRISMA flow of study selection

A total of 41 records were initially identified. After the removal of duplicates and the registered ongoing studies, 31 publications remained. Following title and abstract screening, 17 studies were excluded as review articles, or due to irrelevance to the topic, case report design, animal studies, or short observation periods. This resulted in 14 publications eligible for full-text assessment. Of these, 8 studies were included in the systematic review. Studies were excluded at this stage due to the absence of CBCT analysis,[21–24] the lack of specification of malocclusion type,[25] or exclusive focus on molar root resorption.[26] Ultimately, 4 studies qualified for the meta-analysis.[10,27–29] Additional exclusions were made for studies lacking a control group,[8,13] those involving teeth with incomplete root development,[30] and to ensure a more homogeneous study population. Specifically, only cases of crowding in Class I malocclusion with comparable discrepancy index (DI) scores according to the American Board of Orthodontics (ABO) were retained, leading to the exclusion of a further 3 studies (Fig. 1).[8,13,31]

Included studies

Eight studies were included in the qualitative synthesis (Table 2),[8,10,13,27–31] all of which were classified as high quality according to NOS (Table 3). However, the risk of bias varied among the included studies (Table 4). The majority were rated as having a moderate risk of bias,[8,10,28–31] while 2 studies were judged to have a low risk of bias.[13,27] In contrast, the study by Wang et al. was assessed as having a serious risk of bias, mainly due to the concerns related to confounding and outcome measurement.[30] Importantly, only studies with a low[27] or moderate risk of bias[10,28,29] were included in the meta-analysis to ensure the validity and reliability of the quantitative synthesis.

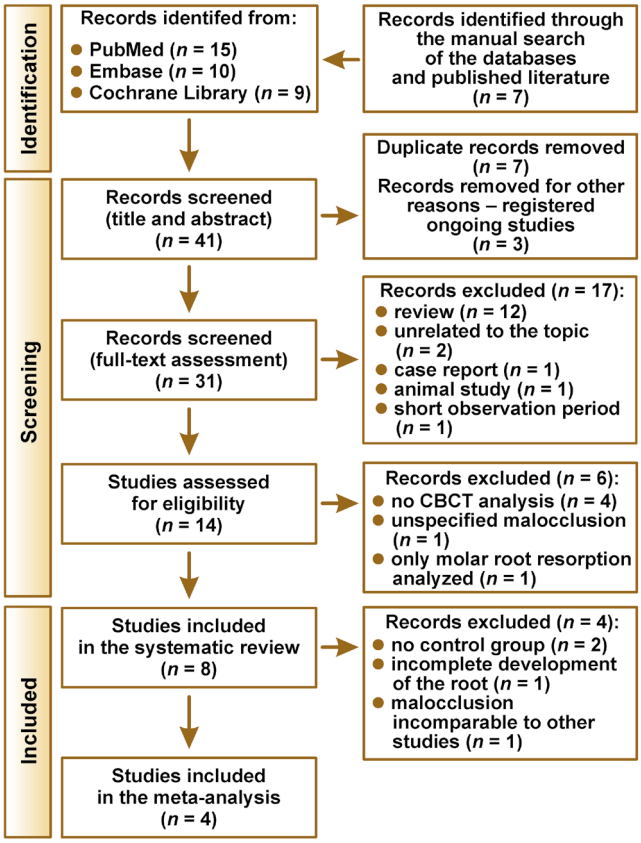

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the study

Table 2. Characteristics of the included studies

Study	Type of study	Number of participants (age [years])	Test group (CAT)	Control group (FAT)	Analyzed teeth	Type of malocclusion and DI (acc. to ABO)	Treatment duration [months]	Outcomes (root resorption in all teeth, measured on CBCT scans [mm])
Aman et al.[8] 2018 USA	cohort study	CAT:160 (34 ±16)	Invisalign	no FAT group	maxillary incisors	Class I, Class II, Class III, crowding, spacing, open bite, cross bite, extraction cases, previous trauma, previous orthodontic treatment	CAT: 2.19 ±0.81 years	CAT: central incisors: 0.53 ±0.59 lateral incisors: 0.49 ±0.57 $p = 0.550$
Li et al.[10] 2020 China	cohort study	CAT:35 (24.71 ±7.48) FAT: 35 (22.51 ±7.48)	Invisalign	Victory Series	maxillary and mandibular incisors and canines	malocclusion not specified, yet DI calculated: CAT: 18.80 ±7.74 FAT: 17.14 ±10.14 $p = 0.445$	CAT: 21.54 ±5.55 FAT: 23.31 ±6.25	CAT: 0.13 ±0.47 FAT: 1.12 ±1.34 $p < 0.001$
Liu et al.[13] 2021 China	cohort study	CAT: 40 (24.1 ±5.8)	Invisalign	no FAT group	maxillary and mandibular incisors	Class II, crowding	CAT: 21.45 ±7.24	CAT: 11.48 ±6.70 mm^3 $p < 0.010$
Almagrami et al.[27] 2023 China	cohort study	CAT: 20 (25.15 ±6.67) FAT: 20 (22.33 ±4.33)	Invisalign	Victory Series	maxillary incisors	mild–moderate crowding CAT: 12.69 ±0.52 FAT: 14.38 ±0.89 $p = 0.610$	CAT: 25.85 ±8.00 FAT: 29.67 ±7.71	CAT: 0.31 ±0.42 FAT: 0.62 ±0.78 $p < 0.005$
Eissa et al.[28] 2018 Canada	cohort study	CAT: 11 (18.35 ±2.83) FAT-DQ: 11 (17.71 ±2.22) FAT-VS: 11 (17.44 ±2.39)	SmartTrack	Damon Q Victory Series	maxillary incisors	Class I malocclusion with 4–6 mm crowding no DI, yet crowding severity measured: CAT: 5.13 ±0.59 FAT-DQ: 5.10 ±0.68 FAT-VS: 5.30 ±0.66 $p = 0.730$	CAT: 15.14 ±1.94 FAT-DQ: 15.75 ±1.94 FAT-VS: 16.27 ±2.74	CAT: 0.44 ±0.35 $p < 0.050$ FAT-DQ: 0.55 ±0.38 $p < 0.050$ FAT-VS: 1.04 ±0.67 $p < 0.050$
Jyotirmay et al.[29] 2021 India	cohort study	CAT: 55 (21.62 ±3.58) FAT: 55 (23.71 ±6.37)	In-line aligners	Victory Series	maxillary and mandibular incisors and canines	malocclusion not specified, yet DI calculated: CAT: 17.10 ±12.34 FAT: 16.13 ±11.23 $p = 0.561$	CAT: 22.23 ±7.34 FAT: 22.45 ±6.54	CAT: 1.12 ±1.34 $p = 0.002$ FAT: 1.51 ±1.34 $p < 0.001$
Wang et al.[30] 2017 China	cohort study	CAT: 28 (16 ±3.5) FAT: 28 (15 ±2.3)	Invisalign	Victory Series	maxillary and mandibular incisors	Class I, mild–moderate crowding CAT: 11.72 ±0.41 FAT: 12.19 ±3.26 $p > 0.050$	CAT: 1.5 ±0.3 years FAT: 1.7 ±0.4 years	prevalence of root resorption: CAT: 68.3% FAT: 85.3% $p < 0.05$ percentage of teeth with >3 mm resorption: CAT: 0% FAT: 23.21%
Chen et al.[31] 2023 China	cohort study	CAT: 22.71 ±3.44 FAT: 23.60 ±3.19 SL: 23.71 ±3.44	Invisalign	FAT: Victory Series SL: Damon Q	maxillary central incisors	Class II Division 2 DI: CAT: 12.00 ±3.38 FAT: 13.70 ±2.99 SL: 13.48 ±3.12 $p = 0.211$	CAT: 30.94 ±4.32 FAT: 28.30 ±5.08 SL: 27.67 ±4.72	CAT: 23.68 ±4.82 mm^3 FAT: 28.24 ±6.44 mm^3 SL: 28.17 ±6.07 mm^3 $p < 0.050$

CAT –clean aligner therapy; FAT – fixed appliance therapy; DQ – Damon Q; VS – Victory Series; SL – self-ligating bracket group; DI – discrepancy index; ABO – American Board of Orthodontics.

Regarding outcome assessment, most studies measured root resorption using linear CBCT measurements comparing the root length before and after orthodontic treatment. However, Liu et al.[13] and Chen et al.[31] employed volumetric CBCT analysis, reporting changes in the root volume [mm^3] rather than the linear root length.

Table 3. Quality evaluation of the included non-randomized studies according to the Newcastle–Ottawa Scale (NOS)

Study	Selection	Comparability	Outcomes	Total	Level of quality
Aman et al.[8] 2018	3	1	3	7	A
Li et al.[10] 2020	4	1	3	8	A
Liu et al.[13] 2021	3	1	3	7	A
Almagrami et al.[27] 2023	4	1	3	8	A
Eissa et al.[28] 2018	4	1	3	8	A
Jyotirmay et al.[29] 2021	4	1	3	8	A
Wang et al.[30] 2017	4	1	3	8	A
Chen et al.[31] 2023	4	1	3	8	A

Table 4. Risk of bias in the included non-randomized studies according to the ROBINS-I tool

Study	Confounding	Selection of participants	Classification of interventions	Deviations from the intended interventions	Missing outcome data	Outcome measurement	Selective reporting	Total
Aman et al.[8] 2018	low	low	low	moderate	low	low	low	moderate
Li et al.[10] 2020	moderate	moderate	moderate	low	low	low	low	moderate
Liu et al.[13] 2021	low	low	low	low	low	low	low	low
Almagrami et al.[27] 2023	low	low	low	low	low	low	low	low
Eissa et al.[28] 2018	low	low	low	moderate	low	low	low	moderate
Jyotirmay et al.[29] 2021	moderate	low	low	low	low	low	low	moderate
Wang et al.[30] 2017	serious	low	low	low	low	serious	low	serious
Chen et al.[31] 2023	moderate	low	low	moderate	low	low	low	moderate

Although volumetric measurements may provide a more comprehensive representation of root resorption, only studies using linear CBCT measurements were included in the meta-analysis. This was not defined as a standalone inclusion criterion; rather, the studies applying volumetric analysis were excluded, as they did not meet other essential eligibility requirements, including the need for comparable malocclusion severity,[13,31] and the presence of both treatment and control groups.[13]

The study groups consisted of patients treated with CA, including Invisalign®, SmartTrack™ and Inman/In-line aligners, while the control groups comprised patients treated with FA, such as Victory Series™ and Damon Q™ systems. Notably, 2 studies did not include a FA control group.[8,13]

With regard to malocclusion characteristics, the study by Aman et al. included a heterogeneous range of malocclusion types,[8] whereas Liu et al.[13] and Chen et al.[31] focused specifically on Class II malocclusion treatment. In contrast, Almagrami et al.,[27] Eissa et al.[28] and Wang et al.[30] and investigated patients with Class I malocclusion presenting anterior crowding. In the studies by Li et al.[10] and Jyotirmay et al.,[29] the specific malocclusion type was not explicitly stated; however, DI was reported to characterize baseline severity.

Most studies assessed the root length changes of the maxillary incisors[8,10,13,27,28,30,31] and the mandibular incisors.[10,13,29,30] Additionally, Li et al.[10] and Jyotirmay et al.[29] included canines in their analyses.

Meta-analysis

Four cohort studies were included in the meta-analysis.[10,27–29] These studies evaluated patients older than 14 years with fully developed anterior tooth roots.

In total, 1,204 teeth were assessed, including 602 in the CA group and 602 in the FA group.

The studies included in the meta-analysis used matched groups based on the type of treated malocclusion. In 2 studies, patients presented with moderate anterior crowding,[27,28] whereas in the remaining studies, malocclusion was not explicitly defined[10,29]; however, the DI, assessed according to ABO, was reported. No significant differences in the DI values were observed between groups. Neither DI nor treatment duration differentiated the study groups (Fig. 2–4).

All included studies performed blinded CBCT-based assessments of root resorption, ensuring that evaluators were unaware of treatment allocation. In the study by Eissa et al., radiographic measurements were performed by a single blinded investigator,[28] whereas in the remaining studies, assessments were conducted independently by two blinded examiners.[10,27,29] To evaluate measure-

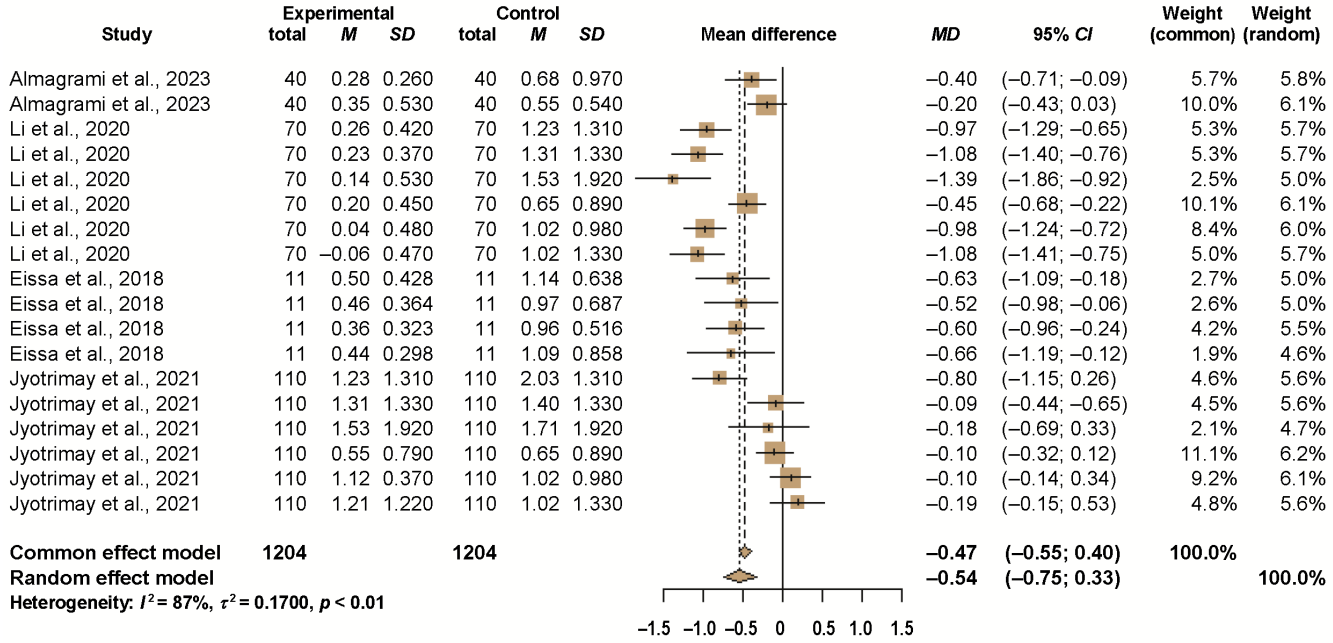

Fig. 2. Primary results for tooth root resorption in the clear aligner (CA) and fixed appliance (FA) groups

M – mean; *SD* – standard deviation; *MD* – mean difference; *CI* – confidence interval.

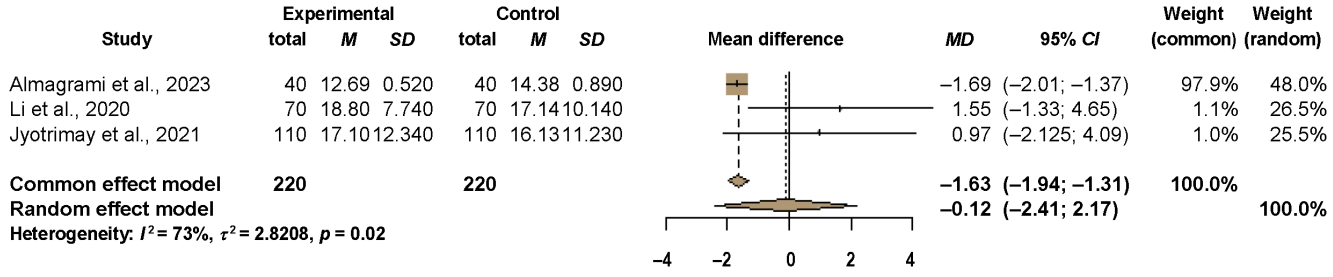

Fig. 3. Differences in the American Board of Orthodontics (ABO) discrepancy index (DI) in the included studies

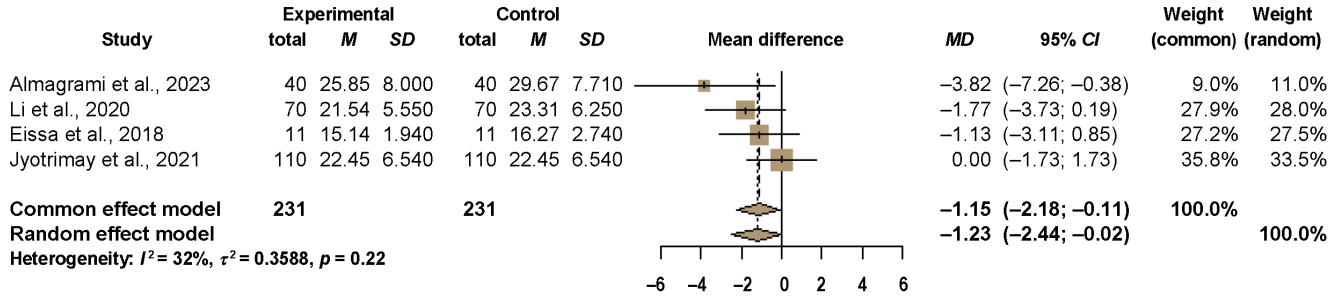

Fig. 4. Differences in treatment duration in the included studies

ment reproducibility and reliability, Eissa et al. repeated assessments after a 4-week interval and analyzed agreement using the κ statistic for the systematic error and the Dahlberg formula for the random error, both confirming acceptable measurement accuracy.[28] Li et al.[10] and Almagrami et al.[27] assessed inter- and intra-operator reliability using intra-class correlation coefficients (*ICCs*) based on repeated measurements performed at 2–4-week intervals. Both studies demonstrated excellent agreement, with the *ICC* values exceeding 0.97.[10,27] Among the 4 studies, the report by Jyotirmay et al. provided the least detailed methodology regarding reliability assessment.[29] Although concordance between the two investigators was reported, the study did not describe the statistical approach or provide a quantitative analysis to support this claim.[29]

Root resorption in meta-analysis

Root resorption was statistically significantly lower in the CA group as compared to the FA group (*WMD*: –0.47 mm; 95% *CI*: –0.55 to –0.40 vs. *WMD*: –0.54 mm; 95% *CI*: –0.75 to –0.33; $p < 0.01$).

Subgroup analyses demonstrated that, in non-extraction cases,[27,28] the root resorption of the maxillary central incisors was significantly lower in patients treated with CA. In contrast, no statistically significant differences between the treatment modalities were observed for the maxillary lateral incisors. Similarly, in extraction cases,[10,29] significantly lower root resorption of the maxillary central incisors was observed in the CA group. No statistically significant differences between the treatment modalities were identified for the lateral incisors or canines (Table 5).

The WMD values further confirmed a statistically significant reduction in root apex resorption in the CA group as compared to the FA group. The greatest reduction was observed for the maxillary central incisors in non-extraction cases ($p < 0.01$).

Discussion

The present meta-analysis evaluated differences in the extent of anterior tooth root apex resorption between clear aligner therapy (CAT) and fixed appliance therapy (FAT) in comparable malocclusions, using CBCT examinations performed before and after treatment. Both CAT and FAT were associated with apical root resorption. However, the mean root loss was slightly lower in the CAT group (–0.47 mm) than in the FAT group (–0.54 mm). The confidence intervals for CAT (95% *CI*: –0.55 to –0.40) were narrower than those for FAT (95% *CI*: –0.75 to –0.33), which may indicate greater homogeneity of results within the CA group. The limited overlap between *CI*s suggests a potentially meaningful difference between the 2 treatment modalities. Although the inter-group difference reached statistical significance ($p < 0.01$), its absolute magnitude (0.07 mm) was small. Consequently, the clinical relevance of this difference may be limited, particularly if it remains within the range considered biologically harmless to tooth vitality. Nevertheless, these findings suggest that CAT may constitute a more conservative alternative with respect to the risk of root resorption, which could be particularly relevant in patients predisposed to OIIRR.

Both extraction and non-extraction cases were included in the analysis. In all studies included in the meta-analysis,[10,27–29] root resorption in the CA group was statistically significantly lower than in the FA group. The greatest difference in root apex resorption observed in this meta-analysis concerned the central incisors, which was consistent with the findings reported in 3 of the included studies.[27–29] In contrast, Li et al. reported the greatest degree of resorption in the maxillary canines and lateral incisors.[10] Previous literature has identified the maxillary lateral incisors as the teeth most susceptible to OIIRR, with an average root loss ranging from 1.2 to 1.6 mm. This increased vulnerability may be associated with the damage caused by the erupting canines. Both the maxillary lateral and central incisors appear particu-

Table 5. Subgroup analysis – root resorption in individual tooth groups in non-extraction and extraction treatment with clear aligners (CA) vs. fixed appliances (FA)

Studies	Tooth group	*n*	*MD*	*SE*	95% *CI*		*Z* score	*p*-value
					lower bound	upper bound		
Non-extraction cases included[27,28]	UI1 + UI1r	102	–0.47	0.13	–0.73	–0.22	–3.60	0.0003*
	UI1 + UI1l	102	–0.44	0.13	–0.69	–0.18	–3.32	0.0009*
	UI2 + UI2r	102	–0.37	0.20	–0.76	0.01	–1.89	0.060
	UI2 + UI2l	102	–0.36	0.22	–0.79	0.07	–1.66	0.100
Extraction cases included[10,29]	UI1	360	–0.89	0.12	–1.13	–0.65	–7.40	<0.00001*
	UI2	360	–0.59	0.50	–1.56	0.38	–1.19	0.235
	UC	360	–0.79	0.60	–1.97	0.40	–1.30	0.192
	LI1	360	–0.27	0.17	–0.07	0.07	–1.56	0.119
	LI2	360	–0.44	0.54	–1.50	0.62	–0.81	0.415
	LC	360	–0.39	0.57	–1.51	0.74	–0.67	0.502

SE – standard error; U – upper; L – lower; I – incisor; C – canine; 1 – central; 2 – lateral; r – right; l – left;* statistically significant.

larly predisposed to resorption, likely because of their root morphology. Teeth with short, pointed, pipette-shaped, dilacerated, distorted, or curved roots are considered to be at greater risk.[32,33] Root resorption is generally more pronounced in the maxillary incisors than in the mandibular incisors, primarily due to the greater extent of tooth movement and overjet reduction during space closure, particularly in extraction cases. Therefore, carefully controlled orthodontic tooth movement remains essential for minimizing the risk of resorption.[13]

The type of malocclusion influences the extent of tooth movement, treatment mechanics, and, consequently, the risk of root resorption. Class II malocclusions and open bites are associated with a higher risk, as they often require greater ranges of tooth movement.[3,5] Accordingly, extraction cases appear to be particularly susceptible to this complication.[5] In contrast, the influence of crowding correction on root resorption remains unclear.[3,8] Root resorption has been most strongly associated with intrusive movements, retraction,[5] translation, lingual root torque,[6] round tipping, and the use of interarch elastics.[3] Increased resorption has also been reported when the root apex comes into contact with the palatal cortical plate, highlighting the importance of sagittal root positioning and careful radiographic monitoring throughout treatment. Proper torque control and carefully managed retraction mechanics are particularly important in Class II cases to minimize excessive root displacement.[8,13] A multivariate analysis conducted by Liu et al. demonstrated that approx. 51% of the variability in root resorption could be explained by factors such as the tooth type, the sagittal root position, extraction treatment, and the extent of intrusion and extrusion movements.[13]

Age is generally not considered a significant risk factor for root resorption,[5,13] with the exception that teeth with incomplete root development appear to be more resistant to this process.[3] Longer treatment duration and the application of high orthodontic forces are associated with an increased risk of resorption.[5,6] However, treatment duration should be interpreted in relation to the active phases of orthodontic mechanics. The prolonged overall treatment time resulting from the extended intervals between appointments or poor patient compliance does not necessarily increase the risk of root resorption.[5] The more favorable resorption outcomes observed in CAT may be related to the fact that CA are most commonly used in less complex malocclusions, which typically require shorter treatment times and allow for more controlled tooth movements.[5,13] Since root resorption has been reported to be greater in Class II malocclusions than in Class I malocclusions,[5,9] the studies by Liu et al.[13] and by Chen et al.[31] were excluded from the meta-analysis. Similarly, as teeth with incomplete root development are considered more resistant to resorption,[5] the study by Wang et al., which included patients aged 11 years and older, was also excluded.[30]

In the present meta-analysis, the potential influence of malocclusion type and treatment duration on root resorption was minimized, as the included studies demonstrated statistical comparability for these parameters. To account for anatomical variability and differences in the tooth position within the dental arch, subgroup analyses were performed for individual teeth.

The radiographic assessment of root resorption using 2D imaging techniques is considered less reliable because of image distortion and superimposition. More accurate evaluation can be achieved using CBCT; therefore, studies employing imaging methods other than CBCT were excluded from the analysis.[21–24] Since the assessment of root resorption should ideally be performed after the completion of orthodontic treatment, studies evaluating resorption before the end of treatment were also excluded.[24,34] Additionally, studies were excluded if the type of malocclusion was not specified[25] or if only CAT was evaluated without a control group.[8,13,22,25]

This meta-analysis demonstrated that root resorption in CAT is statistically significantly lower than in FAT when assessed using CBCT imaging. Nevertheless, root resorption was observed in both the CAT and FAT groups across all analyzed studies, indicating that treatment with CA does not eliminate the risk of resorption, but rather significantly reduces its extent. Our findings support the hypothesis that the relatively lower and more intermittent forces generated by CA reduce ischemic pressure within PDL, thereby limiting osteoclast and odontoclast activity.[35] Clear aligners apply intermittent forces that activate receptor activator of nuclear factor kappa-B ligand (RANKL) activity through interleukin-1 beta (IL-1β) expression, while minimizing damage to PDL cells.[36] The magnitude of orthodontic force is influenced by the elastic modulus of the appliance material. Steel archwires are highly rigid and possess a high elastic modulus, whereas nickel-titanium archwires exhibit substantially lower stiffness. In comparison, aligners demonstrate an elastic modulus approx. 40–50 times lower than that of nickel-titanium archwires.[37] Consequently, aligners generate lower orthodontic forces. Higher orthodontic forces may induce ischemia within the periodontal capillaries, contributing to root resorption.[22] Fixed appliances typically exert continuous forces of greater magnitude over prolonged periods, potentially intensifying the local inflammatory response and leading to more pronounced root resorption.[38–40] In contrast, the forces applied during CAT are inherently lower, as tooth movement occurs at an average rate of approx. 0.2 mm every 10–14 days as compared to about 1 mm per month during FAT.[9]

Despite its recognized advantages, CAT remains subject to biomechanical limitations that may restrict its effectiveness in the treatment of complex malocclusions. In particular, CAT has demonstrated reduced predictability in achieving certain types of tooth movement, including

significant root torque, bodily translation, extrusion, and the derotation of cylindrical teeth. Unlike FAT, which benefits from the fixed anchorage provided by brackets and archwires, CAT offers less precise control over the application of multidirectional orthodontic forces. As a result, treatment outcomes with CA may be less predictable in more demanding clinical situations. Consequently, cases involving severe crowding, deep bite, anterior open bite, impacted teeth, skeletal discrepancies, or significant transverse deficiencies often require the use of auxiliary devices or hybrid treatment approaches to achieve satisfactory clinical results. Furthermore, CAT in extraction-based treatment should be undertaken with caution, and is generally considered unsuitable for patients with severe skeletal malocclusions or pronounced craniofacial anomalies.[41,42]

The design of CA considerably influences the risk of OIIRR. Key factors such as staging protocols, attachment design, aligner thickness, and trimline morphology determine both the magnitude and direction of the forces applied to the teeth. Small incremental movements (0.2–0.3 mm per aligner) generate lighter, intermittent forces, thereby reducing PDL stress and the risk of resorption, whereas accelerated staging protocols may increase apical loading.[43] Attachments further modify aligner biomechanics. Optimized attachment design intended to facilitate torque, intrusion and rotational movements may improve movement control, but attachments can also concentrate stress at the root apex, particularly when improperly designed or positioned. For example, horizontal rectangular attachments have been associated with greater root stress than beveled or ellipsoidal forms.[44,45] Trimline morphology also affects force delivery. Straight and extended trimlines enhance aligner retention and improve force transmission, especially during intrusive and torque movements.[46] In addition, excessive aligner thickness (>0.95 mm) may increase force magnitude, and consequently elevate the risk of root resorption.[47] Therefore, individualized aligner protocols based on digital treatment planning and the careful evaluation of the root-to-bone relationship are essential for minimizing OIIRR and optimizing treatment outcomes.

Another important advantage of CAT is the precise digital pre-programming of tooth movements, which enables clinicians to sequence individual tooth displacements in a controlled and predictable manner. This approach reduces the need for repeated or overlapping movements, and limits undesirable jiggling forces that may exacerbate apical stress. Furthermore, the intermittent force application characteristic of CAT, resulting from regular appliance removal and reinsertion, may facilitate reparative processes within PDL and cementum, thereby contributing to a lower risk of root resorption.[44,45] Moreover, aligners are digitally engineered to optimize force delivery, including force distribution at the root apex, allowing for more biologically compatible orthodontic mechanics that may support the preservation and healing of the root structure during treatment.[9,10,29]

From a clinical perspective, these findings highlight the potential advantages of selecting CAT for patients at increased risk of root resorption, such as individuals with a thin periodontal biotype or a history of dental trauma. The ability to individualize force application and stage tooth movements more gradually may reduce mechanical stress at the root apex and help preserve long-term tooth integrity. Furthermore, digital workflows and 3D treatment planning enable more precise control of force vectors and tooth movement patterns, thereby minimizing undesirable jiggling or uncontrolled tipping forces that may contribute to root resorption.

Our findings are consistent with those of the meta-analysis conducted by Fang et al., who reported significantly lower root resorption in the CAT group (standardized mean difference (*SMD*): –0.65, 95% *CI*: –0.74 to –0.55; $p < 0.01$).[9] In contrast to our results, which demonstrated the greatest root loss in the maxillary central incisors, Fang et al. identified the maxillary lateral incisors as the teeth most affected by resorption. Nevertheless, both analyses consistently showed lower levels of resorption in all anterior teeth treated with CAT as compared to FAT. The authors attributed these findings to the intermittent nature of orthodontic forces and the digitally planned, controlled tooth movements characteristic of CAT, which reduce undesirable jiggling effects – mechanisms that are also supported by our interpretation. Conversely, Gandhi et al. reported no statistically significant differences between CAT and FAT in their meta-analysis, except for the right maxillary lateral incisor.[4] However, their analysis included studies based on both 2D and 3D imaging modalities, which may have introduced measurement variability. Indeed, their subgroup analysis demonstrated that the root resorption values obtained from 2D imaging were, on average, 0.2 mm greater than those measured using CBCT.[4] By including exclusively CBCT-based studies, the present meta-analysis provides a more standardized and accurate assessment of root resorption. In a meta-analysis focusing exclusively on cases treated with CAT, Butsabul et al. reported that the least resorption occurred in the anterior teeth, with the greatest resorption observed in the maxillary central incisors,[15] which is consistent with our findings. Collectively, these results highlight the clinical relevance of anatomical variability and imaging methodology in the assessment of root resorption, as well as the importance of using standardized CBCT protocols in future research.

A key strength of this meta-analysis is its explicit focus on patients older than 14 years with fully formed root apices and matched malocclusion severity, thereby reducing a major potential source of bias in comparisons between CAT and FAT.

Limitations

This meta-analysis has several limitations:
– the small number of included studies, which limited the ability to perform robust publication bias analyses, such as the funnel plot assessment;
– the absence of RCTs, as studies evaluating root resorption are predominantly retrospective in design;
– the inclusion of relatively uncomplicated malocclusions, with no assessment of more complex cases;
– the use of linear measurements of root resorption, despite the spatial nature of this phenomenon, whereas volumetric assessment of the lost root structure (e.g., in cubic millimetres) would provide a more accurate evaluation;
– the overall moderate risk of bias among the included studies.

Looking ahead, well-designed RCTs incorporating standardized CBCT imaging, uniform force application protocols and a broader range of outcome measures, including the volumetric assessment of root changes and biomarker analyses, are needed to provide more definitive evidence. In addition, the inclusion of patient-centered outcomes, such as treatment satisfaction, pain and discomfort, and long-term periodontal health, would enable a more comprehensive evaluation of CAT vs. FAT, allowing clinicians to balance potential benefits and risks in a more holistic manner.

Conclusions

This meta-analysis demonstrates that, for comparable malocclusion severity and treatment duration, the use of CA is associated with a statistically significantly lower extent of root resorption in anterior teeth as compared with FA. However, given the small absolute difference in resorption between the treatment modalities, it is not appropriate to stratify or preferentially select CAT based on this parameter alone. Further studies are required to confirm these findings across broader and more diverse populations and treatment contexts.

While both CAT and FAT are capable of achieving effective orthodontic outcomes, the ability of aligners to deliver lighter and more intermittent forces may confer a protective effect on the integrity of the root apex. Accordingly, clinicians may consider CAT particularly in patients presenting with increased risk factors for OIIRR. Future research incorporating standardized imaging protocols and comprehensive biological markers will be essential for refining these observations and further supporting evidence-based orthodontic treatment planning.

Trial registration

The study was registered in The International Prospective Register of Systematic Reviews – PROSPERO (CRD42023471622).

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are openly available from https://zenodo.org at doi:10.5281/zenodo.20338555.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Ortiz-Pizarro M, Carruitero-Honores MJ, Bellini-Pereira SA, Aliaga-Del Castillo A. Pain and root resorption due to surgical interventions to accelerate tooth movement in orthodontics: A systematic review and meta-analysis. *Dent Med Probl*. 2024;61(3):427–438. doi:10.17219/dmp/161553
2. Calheiros-Lobo MJ, Costa F, Pinho T. Infraocclusion level and root resorption of the primary molar in second premolar agenesis: A retrospective cross-sectional study in the Portuguese population. *Dent Med Probl*. 2022;59(2):195–207. doi:10.17219/dmp/146256
3. Sameshima GT, Iglesias-Linares A. Orthodontic root resorption. *J World Fed Orthod*. 2021;10(4):135–143. doi:10.1016/j.ejwf.2021.09.003
4. Gandhi V, Mehta S, Gauthier M, et al. Comparison of external apical root resorption with clear aligners and pre-adjusted edgewise appliances in non-extraction cases: A systematic review and meta-analysis. *Eur J Orthod*. 2021;43(1):15–24. doi:10.1093/ejo/cjaa013
5. Yassir YA, McIntyre GT, Bearn DR. Orthodontic treatment and root resorption: An overview of systematic reviews. *Eur J Orthod*. 2021;43(4):442–456. doi:10.1093/ejo/cjaa058
6. Sri SL, Saravanan R, Vikram R, Karthikeyan MK, Satya K, Gershon DB. Root resorption during orthodontic treatment – a review on significant factors and treatment effects. *Int J Sci Res*. 2021;10(10):259–262. doi:10.21275/SR211001131124
7. Villaman-Santacruz H, Torres-Rosas R, Acevedo-Mascarúa AE, Argueta-Figueroa L. Root resorption factors associated with orthodontic treatment with fixed appliances: A systematic review and meta-analysis. *Dent Med Probl*. 2022;59(3):437–450. doi:10.17219/dmp/145369
8. Aman C, Azevedo B, Bednar E, et al. Apical root resorption during orthodontic treatment with clear aligners: A retrospective study using cone-beam computed tomography. *Am J Orthod Dentofacial Orthop*. 2018;153(6):842–851. doi:10.1016/j.ajodo.2017.10.026
9. Fang X, Qi R, Liu C. Root resorption in orthodontic treatment with clear aligners: A systematic review and meta-analysis. *Orthod Craniofac Res*. 2019;22(4):259–269. doi:10.1111/ocr.12337
10. Li Y, Deng S, Mei L, et al. Prevalence and severity of apical root resorption during orthodontic treatment with clear aligners and fixed appliances: A cone beam computed tomography study. *Prog Orthod*. 2020;21(1):1. doi:10.1186/s40510-019-0301-1

11. Samandara A, Papageorgiou SN, Ioannidou-Marathiotou I, Kavvadia-Tsatala S, Papadopoulos MA. Evaluation of orthodontically induced external root resorption following orthodontic treatment using cone beam computed tomography (CBCT): A systematic review and meta-analysis. *Eur J Orthod*. 2019;41(1):67–79. doi:10.1093/ejo/cjy027
12. Alassiry A. Comparative evaluation of orthodontically-induced root resorption using cone beam computed tomography (CBCT) and orthopantomogram (OPG) during en-masse retraction of maxillary anterior teeth. *Cureus*. 2022;14(11):e31219. doi:10.7759/cureus.31219
13. Liu W, Shao J, Li S, et al. Volumetric cone-beam computed tomography evaluation and risk factor analysis of external apical root resorption with clear aligner therapy. *Angle Orthod*. 2021;91(5):597–603. doi:10.2319/111820-943.1
14. Peralta-Mamani M, Rubira CM, López-López J, Honório HM, Rubira-Bullen IR. CBCT vs panoramic radiography in assessment of impacted upper canine and root resorption of the adjacent teeth: A systematic review and meta-analysis. *J Clin Exp Dent*. 2024;6(2):e198–e222. doi:10.4317/jced.61285
15. Butsabul P, Kanpittaya P, Nantanee R. Root resorption in clear aligner treatment detected by CBCT: A systematic review and meta-analysis. *Int Dent J*. 2024;74(6):1326–1336. doi:10.1016/j.identj.2024.04.008
16. Aldeeri A, Alhammad L, Alduham A, Ghassan W, Shafshak S, Fatani E. Association of orthodontic clear aligners with root resorption using three-dimension measurements: A systematic review. *J Contemp Dent Pract*. 2018;19(12):1558–1564. PMID:30713189.
17. Zhang M, Zhang P, Koh JT, Oh MH, Cho JH. Evaluation of aligners and root resorption: An overview of systematic reviews. *J Clin Med*. 2024;13(7):1950. doi:10.3390/jcm13071950
18. Wells GA, Shea B, O'Connell D, et al. The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis. 2011. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed March 1, 2025.
19. Wu Y, Cao L, Cong J. The periodontal status of removable appliances vs fixed appliances: A comparative meta-analysis. *Medicine (Baltimore)*. 2020;99(50):e23165. doi:10.1097/MD.0000000000023165
20. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ*. 2016;355:i4919. doi:10.1136/bmj.i4919
21. Iglesias-Linares A, Sonnenberg B, Solano B, et al. Orthodontically induced external apical root resorption in patients treated with fixed appliances vs removable aligners. *Angle Orthod*. 2017;87(1):3–10. doi:10.2319/02016-101.1
22. Gay G, Ravera S, Castroflorio T, et al. Root resorption during orthodontic treatment with Invisalign®: A radiometric study. *Prog Orthod*. 2017;18(1):12. doi:10.1186/s40510-017-0166-0
23. Alam MK, Habib Alruwaili SF, Alessa MK, Alhamid AA, Albilasi SS, Alanazi SA. Effects of orthodontic mechanics on root resorption: A comparative study. *J Pharm Bioallied Sci*. 2024;16(Suppl 1):S809–S811. doi:10.4103/jpbs.jpbs_1022_23
24. Toyokawa-Sperandio KC, De Castro Ferreira Conti AC, Freire Fernandes TM, De Almeida-Pedrin RR, De Almeida MR, Pedron Oltramari PV. External apical root resorption 6 months after initiation of orthodontic treatment: A randomized clinical trial comparing fixed appliances and orthodontic aligners. *Korean J Orthod*. 2021;51(5):329–336. doi:10.4041/kjod.2021.51.5.329
25. Abd El-Ghafar IS, Hewy Raslan EK, Kilany AM, et al. Clear aligners versus fixed orthodontic appliance on the occurrence of external root resorption in upper teeth; cone-beam study. *Eur Chem Bull*. 2023;12(10):14030–14041. doi:10.48047/ecb/2023.12.10.995
26. Withayanukonkij W, Chanmanee P, Promsawat M, Viteporn S, Leethanakul C. Root resorption during maxillary molar intrusion with clear aligners: A randomized controlled trial. *Angle Orthod*. 2023;93(6):629–637. doi:10.2319/010723-14.1
27. Almagrami I, Almashraqi AA, Almaqrami BS, et al. A quantitative three-dimensional comparative study of alveolar bone changes and apical root resorption between clear aligners and fixed orthodontic appliances. *Prog Orthod*. 2023;24(1):6. doi:10.1186/s40510-023-00458-3
28. Eissa O, Carlyle T, El-Bialy T. Evaluation of root length following treatment with clear aligners and two different fixed orthodontic appliances. A pilot study. *J Orthod Sci*. 2018;7:11. doi:10.4103/jos.JOS_120_17
29. Jyotirmay, Singh SK, Adarsh K, Kumar A, Gupta AR, Sinha A. Comparison of apical root resorption in patients treated with fixed orthodontic appliance and clear aligners: A cone-beam computed tomography study. *J Contemp Dent Pract*. 2021;22(7):763–768. PMID:34615781.
30. Wang G, Yang L, Zhang YF, Luo SL, Zheng JW. A retrospective study on incisor root resorption in patients treated with bracketless invisible appliance and straight wire appliance [in Chinese]. *Shanghai Kou Qiang Yi Xue*. 2017;26(1):121–124. PMID:28474083.
31. Chen H, Liu L, Han M, et al. Changes of maxillary central incisor and alveolar bone in Class II Division 2 nonextraction treatment with a fixed appliance or clear aligner: A pilot cone-beam computed tomography study. *Am J Orthod Dentofacial Orthop*. 2023;163(4):509–519. doi:10.1016/j.ajodo.2022.02.015
32. Levander E, Malmgren O. Evaluation of the risk of root resorption during orthodontic treatment: A study of upper incisors. *Eur J Orthod*. 1988;10(1):30–38. doi:10.1093/ejo/10.1.30
33. Mirabella AD, Artun J. Risk factors for apical root resorption of maxillary anterior teeth in adult orthodontic patients. *Am J Orthod Dentofacial Orthop*. 1995;108(1):48–55. doi:10.1016/s0889-5406(95)70065-x
34. Khalil O, Abouelnour A, Abu-Shahba R. Apical root resorption accompanied orthodontic treatment using clear aligners versus fixed appliances: A CBCT comparative study. *J Pharm Negat Results*. 2023;14(2):750–760. doi:10.47750/pnr.2023.14.S02.92
35. Barbagallo LJ, Jones AS, Petocz P, Darendeliler MA. Physical properties of root cementum: Part 10. Comparison of the effects of invisible removable thermoplastic appliances with light and heavy orthodontic forces on premolar cementum. A microcomputed-tomography study. *Am J Orthod Dentofacial Orthop*. 2008;133(2):218–227. doi:10.1016/j.ajodo.2006.01.043
36. Han JY. A comparative study of combined periodontal and orthodontic treatment with fixed appliances and clear aligners in patients with periodontitis. *J Periodontal Implant Sci*. 2015;45(6):193–204. doi:10.5051/jpis.2015.45.6.193
37. Upadhyay M, Arqub SA. Biomechanics of clear aligners: Hidden truths and first principles. *J World Fed Orthod*. 2022;11(1):12–21. doi:10.1016/j.ejwf.2021.11.002
38. Roscoe MG, Meira JB, Cattaneo PM. Association of orthodontic force system and root resorption: A systematic review. *Am J Orthod Dentofacial Orthop*. 2015;147(5):610–626. doi:10.1016/j.ajodo.2014.12.026
39. Currell SD, Liaw A, Blackmore Grant PD, Esterman A, Nimmo A. Orthodontic mechanotherapies and their influence on external root resorption: A systematic review. *Am J Orthod Dentofacial Orthop*. 2019;155(3):313–329. doi:10.1016/j.ajodo.2018.10.015
40. Simon M, Keilig L, Schwarze J, Jung BA, Bourauel C. Forces and moments generated by removable thermoplastic aligners: Incisor torque, premolar derotation, and molar distalization. *Am J Orthod Dentofacial Orthop*. 2014;145(6):728–736. doi:10.1016/j.ajodo.2014.03.015
41. Najjar HE, Alangary MA, Radwan AN, et al. Indications, limitations, and outcomes of clear aligners in orthodontic treatment. *Int J Community Med Public Health*. 2023;10(7):2604–2608. doi:10.18203/2394-6040.ijcmph20231793
42. Hartogsohn CR, Sonnesen L. Clear aligner treatment: Indications, advantages, and adverse effects – a systematic review. *Dent J (Basel)*. 2025;13(1):40. doi:10.3390/dj13010040
43. Wang Y, Long H, Zhao Z, et al. Expert consensus on the clinical strategies for orthodontic treatment with clear aligners. *Int J Oral Sci*. 2025;17(1):19. doi:10.1038/s41368-025-00350-2
44. Jedliński M, Mazur M, Greco M, Belfus J, Grocholewicz K, Janiszewska-Olszowska J. Attachments for the orthodontic aligner treatment – state of the art: A comprehensive systematic review. *Int J Environ Res Public Health*. 2023;20(5):4481. doi:10.3390/ijerph20054481
45. Li J, Si J, Xue C, Xu H. Seeking orderness out of the orderless movements: An up-to-date review of the biomechanics in clear aligners. *Prog Orthod*. 2024;25(1):44. doi:10.1186/s40510-024-00543-1
46. Nakornnoi T, Srirodjanakul W, Chintavalakorn R, Santiwong P, Sipiyaruk K. The biomechanical effects of clear aligner trimline designs and extensions on orthodontic tooth movement: A systematic review. *BMC Oral Health*. 2024;24(1):1523. doi:10.1186/s12903-024-05274-7
47. Alhasyimi AA, Ayub A, Farmasyanti CA. Effectiveness of the attachment design and thickness of clear aligners during orthodontic anterior retraction: Finite element analysis. *Eur J Dent*. 2024;18(1):174–181. doi:10.1055/s-0043-1761452

Review

Effectiveness of the occlusal stabilization splint in the treatment of myogenous temporomandibular disorders: A systematic review

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

This systematic review aimed to assess the effectiveness of stabilization splints in the treatment of myogenous temporomandibular disorders (TMD).

The review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. An electronic search was conducted in 2 databases – MEDLINE via PubMed and the Web of Science. The final search was conducted in May 2022.

Ten randomized clinical trials (RCTs) were included. The total sample size was 539 participants, with a mean follow-up of 6 months. Four outcomes were assessed: pain reduction (10 studies); mouth opening improvement (5 studies); headache reduction (2 studies); and quality-of-life (QoL) improvement (4 studies). Most trials reported improvement in TMD symptoms in comparison with the baseline measures. Nevertheless, the overall quality of evidence was low due to moderate to high risk of bias and the small sample sizes.

Given the limitations of the systematic review, occlusal stabilization splint therapy appeared to be effective in reducing the signs and symptoms of TMD. However, further large-scale prospective studies are needed to evaluate its long-term effectiveness.

Keywords: systematic review, effectiveness, stabilization splint, TMJ disorders, myogenous

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Highlights

- The stabilization splint represents an effective short-term conservative approach in the management of myogeneous temporomandibular disorders (TMD), demonstrating a clinically significant reduction in pain and functional improvement.
- The long-term superiority of the stabilization splint over other conservative therapies has not been demonstrated.
- Further randomized clinical trials based on standardized diagnostic criteria and assessment parameters are needed to better clarify long-term efficacy of the stabilization splint, optimize therapeutic protocols, and develop individualized approaches based on the underlying ethiopathogenic and pathophysiological mechanisms.

Introduction

Temporomandibular disorders (TMD) comprise a group of neuromuscular and musculoskeletal conditions affecting the masticatory muscles, temporomandibular joints (TMJs), and/or associated structures.[1,2] The prevalence of TMD is estimated to range from 5% to 12% in the general population, with rates reaching up to 30% among young adults.[3] This dysfunction is commonly characterized by pain in the TMJs or masticatory muscles, restricted or deviated mandibular movement, and joint sounds, such as clicking or crepitus, during mandibular function. Additional symptoms may include headaches, earaches, poor sleep quality, and depression.[4] The etiology of TMD is complex and multifactorial. Contributing factors include trauma, parafunctional habits, stress, postural abnormalities, neuromuscular dysfunction, and psychosocial factors.[5–7]

Temporomandibular disorders represent a prevalent condition with substantial effects on quality of life (QoL), often characterized by chronic pain and limitations in daily activities.[8–10] Given the multifactorial nature of TMD, a wide range of treatment approaches has been proposed. Current evidence supports prioritizing the most conservative and reversible interventions aimed at relieving pain, restoring normal function, and improving the patient's physical and psychological well-being.[6] Accordingly, therapeutic approaches are generally classified into 3 categories: non-invasive; semi-invasive; and invasive.

Non-invasive and reversible treatment, also referred to as conservative therapies, may be applied alone or in combination. These include splint therapy, manual therapy, pharmacotherapy, counseling, therapeutic education, postural training, acupuncture, LED therapy, low-level laser therapy (LLLT), and therapeutic ultrasound. More advanced categories involve semi-invasive and surgical interventions.[6,11,12]

There are 2 main categories of dental splints used in splint therapy: occluding splints; and non-occluding splints.[13] The most commonly used types include stabilization splints, anterior repositioning splints and anterior occlusal splints.[14] Each type is intended to address specific clinical symptoms. Stabilization splints, also referred to as hard acrylic splints, are fabricated from rigid acrylic resin.[13,15] These appliances present a flat and smooth occlusal surface that allows the opposing teeth to contact evenly and simultaneously in centric relation.[16,17] In maximum intercuspation, occlusal contacts are designed to be simultaneous and symmetrical. During lateral mandibular movements, canine guidance is maintained. When necessary, additional occlusal adjustments may be performed during follow-up visits.[13] Although the exact mechanism of action of stabilization splints remains unclear, they are believed to reduce masticatory muscle fatigue, promote stable occlusion, minimize occlusal interferences, and decrease neuromuscular activity.

The effectiveness of occlusal stabilization splints in reducing the signs and symptoms of myofascial pain, particularly masticatory muscle pain, has been reported by numerous authors. However, the mechanisms underlying these effects have not been fully elucidated. The literature reports inconsistent findings, which may be attributed to sample heterogeneity, the absence of standardized diagnostic criteria, such as the Research Diagnostic Criteria (RDC) for TMD, and differences in follow-up duration. Only a limited number of studies have demonstrated superior clinical outcomes associated with stabilization splint therapy in the management of TMD.[11] In addition, few investigations have examined the role of stabilization splints in reducing temporalis and masseter muscle activity. Several studies have also reported that the effects of stabilization splints on TMD are comparable to those achieved with non-occluding control splints. Consequently, the literature presents conflicting evidence regarding the effectiveness of stabilization splint therapy, despite promising results reported for muscle energy techniques in the management of TMD.[11,18]

Therefore, this systematic review aimed to evaluate published randomized controlled trials (RCTs) investigating the efficacy of occlusal stabilization splints in the management of orofacial myalgia and myofascial pain in comparison with no treatment or alternative interventions.

The objective of this study was to assess the effectiveness of stabilization splints in reducing the most common myogenic symptoms associated with TMD, including pain, limitation in mouth opening, headaches, and impaired QoL. The null hypothesis was that stabilization splints promote muscle relaxation and are effective in managing these symptoms.

Methodology

Protocol and focused question

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement.[19]

The main objective of this research was to answer the following question: Are stabilization splints effective at treating myogenous TMD?

Criteria for considering studies for the systematic review

The review included trials involving participants diagnosed with myogenous TMD according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) or the updated Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).[20] Participants were eligible if they were older than 18 years, had no history of TMJ surgery, and had no significant comorbidities, such as neurological, rheumatologic or malignant diseases. Individuals with bruxism or sleep apnea were excluded.

The review included clinical trials in which participants received a specific type of occlusal splint – namely stabilization splints – and were compared or not with a control group.

Four outcomes were assessed: (1) pain reduction; (2) mouth opening improvement; (3) headache reduction, and (4) QoL improvement. The maximum follow-up duration was 6 months, and only studies with a minimum follow-up of at least 1 month were included.

Only RCTs were included in this systematic review.

Study selection

An electronic search was carried out in the MEDLINE/PubMed and Web of Science databases, using the following search formula: (((hard flat splint) OR (stabilization splint) OR (occlusal splint) OR (occlusal splint therapy) OR (occlusal appliance)) AND ((effectiveness) OR (efficacy) OR (efficiency))). Randomized clinical trials published between January 2012 and May 2022 were identified. The final electronic search was conducted in May 2022.

The search strategy is presented in Table 1.

Data screening

Article selection was performed by consensus between 2 authors (YB and HBM), based on the predefined and mutually agreed inclusion and exclusion criteria. Both reviewers independently screened the titles and abstracts of all identified studies. When the title or abstract did not provide sufficient information to determine eligibility, the full text was retrieved and assessed prior to making a final decision on inclusion or exclusion. Only studies that fulfilled all eligibility criteria of this systematic review were included.

Table 1. Search strategy

Systematic search strategy	
Focused question	Are stabilization splints effective at treating myogenous TMD?
Search formula terms	1 – hard flat splint OR stabilization splint OR occlusal splint OR occlusal splint therapy OR occlusal appliance 2 – effectiveness OR efficacy OR efficiency
Search combination	1 AND 2
Electronic databases	MEDLINE/PubMed, Web of Science

TMD – temporomandibular disorders.

Inter-rater reliability was assessed using percent agreement between the 2 reviewers, and was estimated at approx. 82%.

Data extraction and management

Data extraction was performed independently by the 2 previously mentioned reviewers. The extracted information included study characteristics such as authors' names, study design (sample size, sex distribution, mean age, and study protocol), TMD diagnostic criteria used, type of intervention, total follow-up duration, comparison, outcomes assessed, and main results. Any discrepancies in data extraction were resolved through consensus between the reviewers.

Inclusion and exclusion criteria

Only studies meeting the following inclusion criteria were considered eligible:

– randomized prospective clinical trials evaluating the efficacy of stabilization splints, with or without comparison to a control group;
– participants were predominantly patients diagnosed with myogenous TMD, aged over 18 years, with no history of prior TMJ surgery or significant comorbidities, including neurological, rheumatologic or malignant diseases, and without bruxism or sleep apnea.

The exclusion criteria were as follows:

– non-randomized or other study designs, including cohort studies, observational studies, and case reports;
– articles not available in English full text;
– inadequate or imprecise diagnostic criteria, where TMD classification was not based on either RDC/TMD or the updated DC/TMD[20];
– studies with unreliable or inadequately reported outcome measurements;
– combination of stabilization splint therapy with another conservative therapy in the treatment group.

Quality of studies

Version 2 of the Cochrane Risk of Bias tool (RoB 2) was used to evaluate the reliability of the results of the included studies.[21] Risk-of-bias and quality assessments were conducted independently by the 2 previously mentioned reviewers as part of the data extraction process. Any discrepancies or disagreement were resolved through discussion with a third reviewer (HB).

Results

Study selection and description

From the initial electronic search conducted in May 2022, 321 articles were identified, of which 10 were ultimately included in this systematic review, as illustrated in the PRISMA flow diagram (Fig. 1).

The main characteristics of the included studies are presented in Table 2. Data extraction and analysis revealed considerable heterogeneity among the RCT samples. In total, 539 patients were included across the studies. Eligible participants were diagnosed with TMD based on RDC/TMD or the updated DC/TMD, including either Axis I or Axis II assessments. Not all clinical trials reported sex distribution. The maximum follow-up duration across the included studies was 6 months.

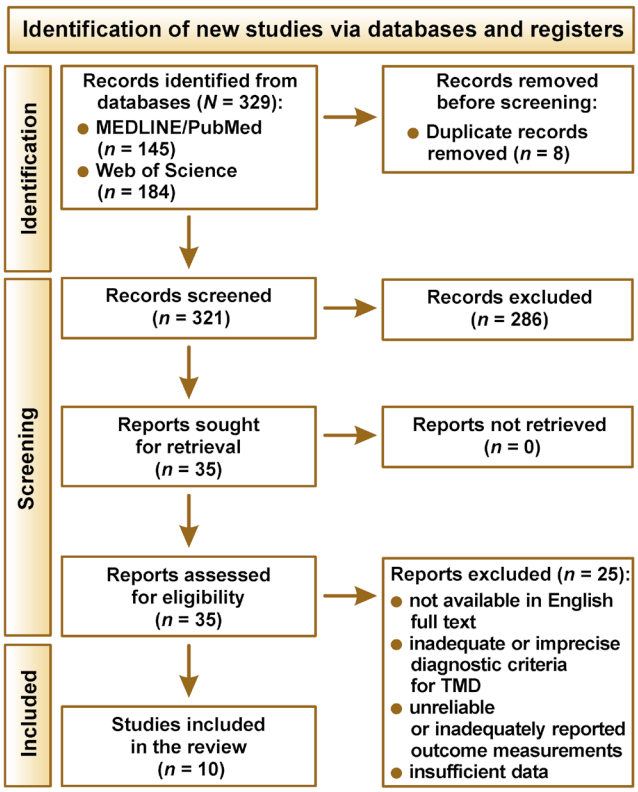

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the study

Risk-of-bias assessment

Risk-of-bias assessment using the Cochrane criteria indicated a moderate risk of bias in most of the included studies, while 2 studies were judged to have a high risk of bias (Fig. 2).

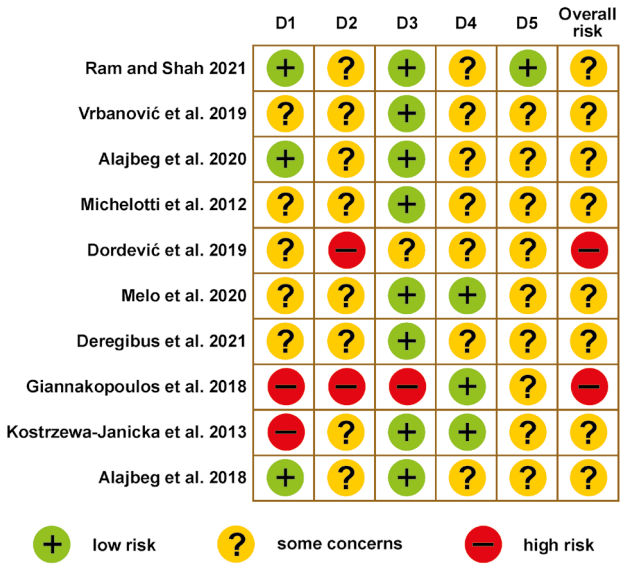

Fig. 2. Risk of bias in the included studies

Cochrane Risk of Bias tool (RoB 2): D1 – randomization process; D2 – deviations from the intended interventions; D3 – missing outcome data; D4 – outcome measurement; D5 – outcome reporting.

Effectiveness of stabilization splints

The effectiveness of stabilization splints was assessed through four outcomes: improvement in pain, mouth opening, quality of life, and headaches. The findings of this systematic review were presented below.

Pain reduction

Pain is the most prevalent symptom reported by patients with TMD.[1,22] It is commonly described as a dull, persistent pain that may be aggravated by jaw function, particularly chewing. In the present systematic review, 10 studies evaluated pain-related outcomes.[4,16,17,23–29] Pain severity was primarily assessed using the visual analog scale (VAS),[16,23–25,29] the numerical rating scale (NRS)[27,28] and/or the Graded Chronic Pain Scale (GCPS).[16,17] In one study, surface electromyography (sEMG) was used.[26] The pain outcomes assessed varied among the included studies. Although pain intensity was the most frequently evaluated parameter, some clinical trials also assessed spontaneous pain, pain during chewing, muscle tenderness, average pain, and the worst pain. Most studies reported that stabilization splints were effective in reducing pain, with significant decreases in the VAS or NRS scores as compared to the baseline values. Michelotti et al.

Table 2. General characteristics of the included studies

Study	Population (age in years)	Overall follow-up duration [months]	Intervention	Comparison	Outcome measures				Results
					pain reduction	mouth opening improvement	headache reduction	QoL improvement	
Ram and Shah[4] 2021 India	*n* = 160	3	SS	MET/ SS + MET/ counseling	–	–	–	–	Pain reduction occurred with SS, but was less pronounced than in the case of combined treatment.
Vrbanović et al.[16] 2019 Croatia	*n* = 30 (all females)	6	SS	–	VAS/ GCPS	MCO	–	QoL: OHIP-14	The treatment with SS was effective.
Alajbeg et al.[17] 2020 Croatia	*n* = 34 (all females) mean age: 36.1 ±11.95	6	SS	PS	GCPS	MCO	–	anxiety: GAD-7 depression: PHQ-9 stress: biochemical stress markers	There was pain and pain-related disability improvement with SS, and SS was superior to PS in terms of the worst pain alleviation; there was slightly greater improvement in the depression scores with SS than PS; there was a reduction in oxidative stress markers with SS.
Michelotti et al.[23] 2012 Italy/ Netherlands/ New Zealand	*n* = 41 (19 females) mean age: SS: 31.1 ±8.8 C: 31.4 ±14.0	3	SS	counseling and education	VAS (spontaneous pain/pain during chewing)	pain-free MMO	VAS	–	SS brought the alleviation of pain (during chewing), mouth opening improvement and headache reduction, but it was slightly less effective in comparison with the control group.
Dordević et al.[24] 2019 Serbia	*n* = 44 (37 females) age range: 25–45	6	SS	NSAID/ the combination of ibuprofen and diazepam	VAS/ a digital algorithm	–	–	–	There was pain reduction without superiority over the control groups.
Melo et al.[25] 2020 Brazil	*n* = 89 (72 females) mean age: 28.0 ±9.34	1	SS	manual therapy/ counseling/ occlusal splint and counseling	VAS	–	–	anxiety: HADS, BAI, STAI	There was pain and anxiety improvement without superiority over the control groups.
Deregibus et al.[26] 2021 Italy	*n* = 40 (27 females) mean age: 47.2 ±12.8	6	maxillary SS	occlusal mandibular splint	sEMG	sEMG	–	–	No significant effects at reducing pain over a 6-month period.
Giannakopoulos et al.[27] 2018 Germany	*n* = 45 (all females) age range: 18–45	3	SS	device-supported sensorimotor training	NRS	–	–	–	There was pain reduction without superiority over the control group.
Kostrzewa-Janicka et al.[28] 2013 Poland	*n* = 43 (38 females) mean age: 32.7 ±9.4	6	SS	–	NRS (orofacial pain intensity)	–	NRS/ headache frequency	–	There was improvement in both signs and symptoms of orofacial pain in 81.4% of patients after 6 months of use.
Alajbeg et al.[29] 2018 Croatia	*n* = 13 mean age: SS: 57.25 ±8.13 C: 46.50 ±18.15	3	SS	amitriptyline/ a placebo pill	VAS	MCO	–	QoL: OHIP-14	There was pain reduction, mouth opening improvement was more significant with SS, but QoL improvement was less significant than with amitriptyline.

QoL – quality of life; SS – stabilization splint; C – control/comparison group; NSAID – non-steroidal anti-inflammatory drug; PS – placebo splint; MET – muscle energy technique; VAS – visual analog scale; NRS – numerical rating scale; GCPS – Graded Chronic Pain Scale; sEMG – surface electromyography; MMO – maximal mouth opening; MCO – maximal comfortable mouth opening; OHIP-14 – Oral Health Impact Profile-14 questionnaire; GAD-7 – Generalized Anxiety Disorder-7 questionnaire; PHQ-9 – Patient Health Questionnaire-9; HADS – Hospital Anxiety and Depression Scale; BAI – Beck Anxiety Inventory; STAI – State-Trait Anxiety Inventory.

reported significant improvement in pain during chewing.[23] Kostrzewa-Janicka et al. observed that, after a 2-month follow-up period, the NRS scores for orofacial pain decreased below 3 points in 77.5% of patients.[28] According to Giannakopoulos et al., chronic pain intensity decreased by 40% after 3 months of splint therapy.[27] In addition, Alajbeg et al. demonstrated a significant reduction in the worst pain scores.[17] Vrbanović et al.[16] and Dordević et al.[24] in 2019, followed by Ram and Shah[4] and Melo et al.,[25] also concluded that stabilization splints were effective in reducing pain. However, despite the general trend toward pain reduction, Deregibus et al. concluded that stabilization splints were not significantly effective in reducing pain over a 6-month period, regardless of whether the splints were applied to the maxillary or mandibular arch.[26]

Mouth opening improvement

Temporomandibular disorders are frequently associated with impaired jaw function. Patients commonly report restricted or asymmetric mandibular movements, including limitations in mouth opening. This systematic review sought to evaluate the evidence regarding the effectiveness of stabilization splints in improving mouth opening. Five studies assessed improvement in mouth opening.[16,17,23,26,29] Various measurement methods were used, the most common being maximum comfortable mouth opening (MCO) or pain-free maximum mouth opening (MMO).[16,17,23,29] This parameter corresponds to the maximum distance a patient can open their mouth without experiencing additional pain or discomfort. The measurement is obtained between the maxillary and mandibular incisal edges, with the overbite value added.[17,23,29] All included studies, except that of Deregibus et al.,[26] reported that stabilization splint produced significantly greater improvement in mouth opening. Most measurements, particularly pain-free MMO, showed significant increases when post-treatment outcomes were compared with the baseline values.

Headache reduction

In addition to pain and impaired jaw function, headaches are among the most common conditions associated with TMD.[1,30,31] Headaches related to TMD are typically localized in the temporal region, preauricular area, and/or masseter muscles.[30,31] Although these headaches may be unilateral, they are often bilateral when both TMJs are involved. Two studies evaluated headache reduction.[23,28] The assessment methods varied, and included VAS, NRS and headache frequency. Overall, stabilization splints appeared to contribute to headache reduction. Kostrzewa-Janicka et al. reported a 3-point reduction in headache intensity in 61.8% of patients after 6 months of treatment.[28] Furthermore, patients suffering from recurrent tension-type headaches reported a significant decrease in headache frequency following stabilization splint therapy.[28]

Quality of life improvement

Chronic TMD may significantly impair the patient's QoL.[29] They can affect social and emotional functioning, and may contribute to various psychological disorders.[9,25,29] In addition, chronic pain may reduce independence in activities of daily living and negatively impact overall health-related quality of life (HRQoL).[16] Consequently, one of the objectives of occlusal splint therapy in TMD management is to improve oral health-related quality of life (OHRQoL). Four studies evaluated this outcome using different questionnaires.[16,17,25,29] Vrbanović et al.[16] and Alajbeg et al.[29] assessed QoL using the Oral Health Impact Profile-14 (OHIP-14) questionnaire, and reported significant improvement following stabilization splint therapy. Improvement in QoL also involves reducing the psychological symptoms associated with TMD. Two clinical trials evaluated changes in anxiety and depression using various instruments, including the Generalized Anxiety Disorder-7 (GAD-7) questionnaire, the Patient Health Questionnaire-9 (PHQ-9),[17] the Hospital Anxiety and Depression Scale (HADS), the Beck Anxiety Inventory (BAI), and the State-Trait Anxiety Inventory (STAI).[25] According to these reports, stabilization splint therapy was effective in reducing depressive symptoms associated with TMD. Finally, the effectiveness of stabilization splints in improving QoL was also investigated through stress reduction. Alajbeg et al. assessed stress-related biochemical markers, including the uric acid levels, and reported a significant reduction in salivary oxidative stress index ratios among patients treated with stabilization splints.[17]

Discussion

Occlusal splints are among the most widely used conservative therapies for alleviating symptoms associated with myogenous TMD. The findings of this review suggest that stabilization splints represent a valuable and effective treatment option for the management of TMD symptoms.

Although the precise mechanism of action of stabilization splints remains unclear, their therapeutic effectiveness is thought to result from several factors. Stabilization splints may reduce electromyographic activity, modify the condylar position or increase the vertical dimension of occlusion. These changes may promote muscle relaxation and reduce stress on TMJS.[26,32] In addition, the adjustment of the splint surface to eliminate occlusal interferences may decrease sensory input to periodontal receptors and relieve pressure on the compromised retrodiscal tissues.

Despite its effectiveness, splint therapy presents several limitations. First, the fabrication of occlusal splints is a time-consuming and costly process for dental technicians, clinicians and patients, which may delay the initiation of treatment.[13,14,23,33] In addition, splints must possess adequate mechanical properties, particularly hardness

and flexural resistance, to withstand excessive occlusal forces.[13,34] They must also demonstrate sufficient resistance to degradation within the oral environment. Although the conventional fabrication method remains the gold standard for achieving successful clinical outcomes, especially for long-term splint use, there is increasing demand for more efficient manufacturing techniques that reduce fabrication and management time. Advances such as tree-dimensional (3D) printing and thermoforming technologies have made this possible. However, further research is still required to improve the properties of the materials used in these techniques and to ensure long-term clinical success.[34]

The additional drawbacks of splint therapy include higher treatment costs and potential complications, such as caries and gingivitis.[24] Moreover, several studies suggest that the effectiveness of stabilization splints is primarily limited to short-term outcomes. Alajbeg et al. found that stabilization splints demonstrated superiority over amitriptyline and placebo splints in improving limited mouth opening during the first 2 weeks.[17,29] Kostrzewa-Janicka et al. suggested that a 2-month observation period was generally sufficient to evaluate the effectiveness of occlusal splint therapy; if satisfactory improvement is not achieved within this timeframe, other contributing factors, such as stress or the underlying systemic conditions, should be investigated.[28] In this regard, Olchowy et al. reported that patients who failed to improve were affected by tetany.[32]

The findings of the present review suggest that occlusal splints mainly provide symptomatic relief. Once TMD symptoms are alleviated, it is essential to identify the underlying etiological factors in order to establish and implement appropriate etiological treatment strategies.

Limitations

Several limitations of the present systematic review should be acknowledged, including heterogeneity among study samples, variability in follow-up periods and differences in the objectives of the included clinical trials. In addition, the absence of an untreated control group in several studies may have limited the ability to more precisely isolate the effects of stabilization splints. Furthermore, the findings cannot be considered definitive or fully generalizable due to the moderate to low methodological quality of the included studies. Most results should therefore be interpreted with caution, as outcome measures are partly subjective and may be influenced by individual pain tolerance thresholds. Consequently, further studies including non-treatment control groups are needed to allow a more rigorous evaluation of treatment effects.

Taking these limitations into account, the possibility that stabilization splints may have a superior therapeutic effect as compared to other treatment modalities cannot be excluded. Larger-scale studies with longer follow-up periods are required to clarify this uncertainty. In the included trials, the maximum overall follow-up duration was 6 months, which is insufficient to draw conclusions regarding the long-term effects of stabilization splint therapy.

Conclusions

This systematic review concluded that occlusal stabilization splint therapy met expectations in terms of effectiveness in reducing signs and symptoms in patients with myogenous TMD. Further large-scale, long-term prospective studies are needed to clarify these findings. Evidence-based conclusions are essential to improve the understanding and management of these and other common orofacial pain disorders.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. National Academies of Sciences, Engineering, and Medicine. *Temporomandibular Disorders: Priorities for Research and Care*. Washington, DC: The National Academies Press; 2020. Available from: https://www.nap.edu/catalog/25652. Accessed September 13, 2024.
2. Ohrbach R, Dworkin SF. The evolution of TMD diagnosis: Past, present, future. *J Dent Res*. 2016;95(10):1093–1101. doi:10.1177/0022034516653922
3. Seweryn P, Orzeszek SM, Waliszewska-Prosół M. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023; 60(4):609–617. doi:10.17219/dmp/171894
4. Ram HK, Shah DN. Comparative evaluation of occlusal splint therapy and muscle energy technique in the management of temporomandibular disorders: A randomized controlled clinical trial. *J Indian Prosthodont Soc*. 2021;21(4):356–365. doi:10.4103/jips.jips_332_21

5. Chisnoiu AM, Picos AM, Popa S, et al. Factors involved in the etiology of temporomandibular disorders – a literature review. *Clujul Med*. 2015;88(4):473–478. doi:10.15386/cjmed-485
6. Kalladka M, Young A, Khan J. Myofascial pain in temporomandibular disorders: Updates on etiopathogenesis and management. *J Bodyw Mov Ther*. 2021;28:104–113. doi:10.1016/j.jbmt.2021.07.015
7. Manfredini D, Lombardo L, Siciliani G. Temporomandibular disorders and dental occlusion. A systematic review of association studies: End of an era? *J Oral Rehabil*. 2017;44(11):908–923. doi:10.1111/joor.12531
8. Machado de Resende CM, Da Silva Rocha LG, De Paiva RP, et al. Relationship between anxiety, quality of life, and sociodemographic characteristics and temporomandibular disorder. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2020;129(2):125–132. doi:10.1016/j.oooo.2019.10.007
9. Felin GC, Da Cunha Tagliari CV, Agostini BA, Collares K. Prevalence of psychological disorders in patients with temporomandibular disorders: A systematic review and meta-analysis. *J Prosthet Dent*. 2024;132(2):392–401. doi:10.1016/j.prosdent.2022.08.002
10. Cigdem Karacay B, Sahbaz T. Investigation of the relationship between probable sleep bruxism, awake bruxism and temporomandibular disorders using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). *Dent Med Probl*. 2023;60(4):601–608. doi:10.17219/dmp/158926
11. Tran C, Ghahreman K, Huppa C, Gallagher JE. Management of temporomandibular disorders: A rapid review of systematic reviews and guidelines. *Int J Oral Maxillofac Surg*. 2022;51(9):1211–1225. doi:10.1016/j.ijom.2021.11.009
12. Hoi NHY, Ip CK, Li DTS, Leung YY. Diagnosis and treatment of myogenous temporomandibular disorders: A clinical update. *Diagnostics (Basel)*. 2022;12(12):2914. doi:10.3390/diagnostics12122914
13. Moufti MA, Lilico JT, Wassell RW. How to make a well-fitting stabilization splint. *Dent Update*. 2007;34(7):398–400. doi:10.12968/denu.2007.34.7.398
14. Dylina TJ. A common-sense approach to splint therapy. *J Prosthet Dent*. 2001;86(5):539–545. doi:10.1067/mpr.2001.118878
15. Gray RJ, Davies SJ, Quayle AA. A clinical approach to temporomandibular disorders. 1. Classification and functional anatomy. *Br Dent J*. 1994;176(11):429–435. doi:10.1038/sj.bdj.4808473
16. Vrbanović E, Alajbeg IZ. Long-term effectiveness of occlusal splint therapy compared to placebo in patients with chronic temporomandibular disorders. *Acta Stomatol Croat*. 2019;53(3):195–206. doi:10.15644/asc53/3/1
17. Alajbeg IZ, Vrbanović E, Lapić I, Alajbeg I, Vuletić L. Effect of occlusal splint on oxidative stress markers and psychological aspects of chronic temporomandibular pain: A randomized controlled trial. *Sci Rep*. 2020;10(1):10981. doi:10.1038/s41598-020-67383-x
18. Orzeszek S, Waliszewska-Prosol A, Ettlin D, et al. Efficiency of occlusal splint therapy on orofacial muscle pain reduction: A systematic review. *BMC Oral Health*. 2023;23(1):180. doi:10.1186/s12903-023-02897-0
19. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *PLoS Med*. 2021;18(3):e1003583. doi:10.1371/journal.pmed.1003583
20. Schiffman E, Ohrbach R, Truelove E, et al.; International RDC/TMD Consortium Network, International Association for Dental Research; Orofacial Pain Special Interest Group, International Association for the Study of Pain. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for clinical and research applications: Recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
21. Sterne JA, Savović J, Page MJ, et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019:366:l4898. doi:10.1136/bmj.l4898
22. Fricton J. Myogenous temporomandibular disorders: Diagnostic and management considerations. *Dent Clin North Am*. 2007;51(1):61–83. doi:10.1016/j.cden.2006.10.002
23. Michelotti A, Iodice G, Vollaro S, Steenks MH, Farella M. Evaluation of the short-term effectiveness of education versus an occlusal splint for the treatment of myofascial pain of the jaw muscles. *J Am Dent Assoc*. 2012;143(1):47–53. doi:10.14219/jada.archive.2012.0018
24. Dordević I, Todorović A, Lazić V, Obradović-Đuričić K, Milekić B, Stamenković D. Occlusal appliances – an alternative in temporomandibular disorders. *Srp Arh Celok Lek*. 2019;147(9–10):541–546. doi:10.2298/SARH190118064D
25. Melo RA, Machado de Resende CM, De Figueirêdo Rêgo CR, De Sousa Leite Bispo A, Seabra Barbosa GA, De Almeida EO. Conservative therapies to treat pain and anxiety associated with temporomandibular disorders: A randomized clinical trial. *Int Dent J*. 2020;70(4):245–253. doi:10.1111/idj.12546
26. Deregibus A, Ferrillo M, Piancino MG, Domini MC, De Sire A, Castroflorio T. Are occlusal splints effective in reducing myofascial pain in patients with muscle-related temporomandibular disorders? A randomized-controlled trial. *Turk J Phys Med Rehabil*. 2021;67(1):32–40. doi:10.5606/tftrd.2021.6615
27. Giannakopoulos NN, Rauer AK, Hellmann D, Hugger S, Schmitter M, Hugger A. Comparison of device-supported sensorimotor training and splint intervention for myofascial temporomandibular disorder pain patients. *J Oral Rehabil*. 2018;45(9):669–676. doi:10.1111/joor.12662
28. Kostrzewa-Janicka J, Mierzwinska-Nastalska E, Rolski D, Szczyrek P. Occlusal stabilization splint therapy in orofacial pain and tension-type headache. *Adv Exp Med Biol*. 2013:788:181–1888. doi:10.1007/978-94-007-6627-3_27
29. Alajbeg IZ, Brakus RB, Brakus I. Comparison of amitriptyline with stabilization splint and placebo in chronic TMD patients: A pilot study. *Acta Stomatol Croat*. 2018;52(2):114–122. doi:10.15644/asc52/2/4
30. International Headache Society (IHS). The International Classification of Headache Disorders 3rd ed. (IHS Classification – ICHD-3). Available from: https://ichd-3.org. Accessed September 14, 2024.
31. Goncalves DA, Camparis CM, Speciali JG, et al. Treatment of comorbid migraine and temporomandibular disorders: A factorial, double-blind, randomized, placebo-controlled study. *J Orofac Pain*. 2013;27(4):325–335. doi:10.11607/jop.1096
32. Olchowy A, Seweryn P, Wieckiewicz M. Assessment of the masseter stiffness in patients during conservative therapy for masticatory muscle disorders with shear wave elastography. *BMC Musculoskelet Disord*. 2022;23(1):439. doi:10.1186/s12891-022-05392-9
33. Berntsen C, Kleven M, Heian M, Hjortsjö C. Clinical comparison of conventional and additive manufactured stabilization splints. *Acta Biomater Odontol Scand*. 2018;4(1):81–89. doi:10.1080/23337931.2018.1497491
34. Wężgowiec J, Małysa A, Więckiewicz M. How does artificial aging affect the mechanical properties of occlusal splint materials processed via various technologies? *Dent Med Probl*. 2025;62(3):527–535. doi:10.17219/dmp/174708

Review

Effectiveness of photobiomodulation therapy and occlusal splints in the treatment of bruxism in children: A systematic review

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Abstract

Untreated sleep bruxism (SB) can cause damage to the stomatognathic system, potentially reducing academic performance in children and negatively affecting their quality of life. Occlusal splints are considered the gold standard for the treatment of bruxism in children; however, they present many limitations, prompting the search for alternative therapies. Laser therapy has been proposed as a more favorable, painless and cost-effective treatment option for pediatric patients. Therefore, the aim of this study was to evaluate the effectiveness of photobiomodulation therapy (PBMT) and occlusal splints in the treatment of bruxism in children.

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Studies were identified through searches of the following databases: MEDLINE (PubMed®); Google Scholar; and the Cochrane Library. The review included articles published between January 2010 and June 2023. Studies evaluating the treatment of bruxism using occlusal splints and/or PBMT in participants under 18 years of age were included.

A total of 67 articles published in English were identified. After applying the eligibility criteria and assessing study quality using the Cochrane risk-of-bias tool, 6 studies were included in the final analysis. Of these, 4 investigated various types of occlusal splints, while 2 compared PBMT with occlusal splints. The papers also examined diagnostic methods, treatment outcomes, salivary cortisol levels, and dopamine levels.

Occlusal splints have limited effectiveness in managing the signs and symptoms of bruxism. Photobiomodulation therapy shows potential as a treatment method, but its effectiveness remains inconclusive. Due to the limited number of available studies, definitive conclusions cannot be drawn, and further research is necessary.

Keywords: photobiomodulation, occlusal splint, bruxism, children, therapy

Highlights

- Occlusal splints, although considered the conventional gold standard, demonstrate limited clinical effectiveness in the management of pediatric bruxism.
- Photobiomodulation therapy (PBMT) represents a promising non-invasive approach with potential therapeutic benefits.
- Current evidence comparing PBMT and occlusal splints remains insufficient to establish clear clinical superiority.
- The available literature is limited by small sample sizes and methodological heterogeneity.
- High-quality randomized controlled trials are needed to support the development of evidence-based treatment guidelines.

Introduction

Bruxism is a repetitive, non-functional activity of the jaw muscles characterized by clenching or grinding of the teeth and/or by thrusting or bracing the mandible.[1,2] Two types of bruxism are distinguished based on the state of occurrence. When it occurs during wakefulness, it is referred to as awake bruxism, also known as secondary bruxism or daytime bruxism, whereas when it occurs during sleep, it is termed sleep bruxism (SB), also known as primary or nighttime bruxism.[1,2] The type of bruxism is typically determined through patient history and physical examination. Diagnostic tools such as the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), Oral Behaviors Checklist (OBC), can be used for the assessment of bruxism.[3] Additionally, polysomnography is considered a reliable method for diagnosing sleep-related conditions.[4]

Sleep bruxism is common among children and adolescents, with reported prevalence rates ranging from 3.5% to 40.6%.[5] Individuals who experience SB during childhood are more likely to continue experiencing it in adulthood.[6] Although early management of bruxism is important to prevent its negative consequences, establishing a definitive diagnosis remains challenging.[7,8] Untreated SB may cause teeth attrition, headaches, muscle pain, temporomandibular disorders (TMD), periodontal diseases, and damage to the stomatognathic system. Moreover, it may negatively affect academic performance and overall quality of life in children and adolescents.[8]

The exact etiology of SB in children remains unknown.[9] However, bruxism is considered a multifactorial condition that may be associated with certain behavioral and psychological factors.[10] Other contributing factors include genetic predisposition, malocclusion, occlusal factors, arterial hypertension, nutritional deficiencies (e.g., 25-hydroxyvitamin D deficiency), oral habits, sleeping habits, allergies, respiratory problems (apnea, snoring), alterations in neurotransmitters (e.g., dopamine), and overall sleep quality.[11–16]

Awake bruxism is often associated with other parafunctional habits, such as excessive gum chewing, biting pens or pencils, or nail biting. These behaviors may begin in early childhood and persist throughout life, often intensifying during periods of stress and being associated with increased muscle tension.[17]

Various treatment approaches for bruxism have been suggested, including dental, psychobehavioral and pharmacological interventions. Pharmacological methods involve the use of medications such as beta-blockers, anticonvulsants, antidepressants, dopamine agents, and muscular relaxants.[18,19] Although drugs such as buspirone, fluoxetine and diazepam have been investigated, they have not demonstrated effectiveness in the treatment of bruxism, and concerns have been raised regarding their potential adverse effects.[20–22]

Botulinum toxin has also been proposed as a treatment for SB; however, it has not demonstrated short-term efficacy.[23] Biofeedback has been used to manage masticatory muscle activity, with studies showing a significant reduction in muscle activity associated with its use.[24] Similarly, injectable platelet-rich fibrin (I-PRF) has exhibited efficacy in alleviating symptoms of internal temporomandibular joint disorders.[25]

Occlusal splints are considered the gold standard for the management of bruxism in both children and adults.[26] Among them, rigid occlusal splints are the most frequently used.[27] However, these appliances have many limitations, which has encouraged the exploration of alternative treatment methods. Recently, photobiomodulation therapy (PBMT) has emerged as a non-pharmacological approach for the treatment of bruxism, following evidence of its effectiveness in patients with TMD.[28]

According to the American Society of Laser Medicine and Surgery (ASLMS), "photobiomodulation therapy is a form of light therapy that utilizes non-ionizing forms of light sources including lasers, LEDs and broadband light, in the visible and near-infrared spectrum".[29] Photobiomodulation therapy is used to accelerate the healing process by stimulating cells and increasing blood circulation.[30,31] Moreover, PBMT exhibits vasodilatory, analgesic and anti-inflammatory effects.[31]

The aim of this study was to evaluate the effectiveness of PBMT and occlusal splints in the treatment of bruxism in children.

Material and methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines for reporting systematic reviews.[32] The terminology and definitions used were consistent with the current international consensus on bruxism.[2]

Search strategy

Studies were identified through searches of the following databases: MEDLINE (PubMed®); Google Scholar; and the Cochrane Library. Articles published between January 2010 and June 2023 were taken into consideration. The following keywords were used: ["Bruxism" OR "Teeth Grinding" OR "Teeth Clenching"] AND ["child" OR "children" OR "childhood" OR "kids" OR/AND "adolescents" OR "teenager"] AND ["therapy" OR "therapeutics"] AND ["laser" OR "laser biomodulation" OR "laser photo biomodulation" OR "splints" OR "occlusal splints"].

The search results were screened by reviewing the titles and/or abstracts to identify studies that met the inclusion and exclusion criteria. This process was conducted independently by 2 reviewers, and the results were then matched.

Eligibility criteria

The review included randomized controlled trials and clinical trials published in English that investigated the treatment of bruxism in children and adolescents (≤18 years of age) using low-level laser therapy (PBMT) and/or occlusal splints.

Studies involving participants older than 18 years, as well as case reports, study protocols and reviews were excluded from consideration.

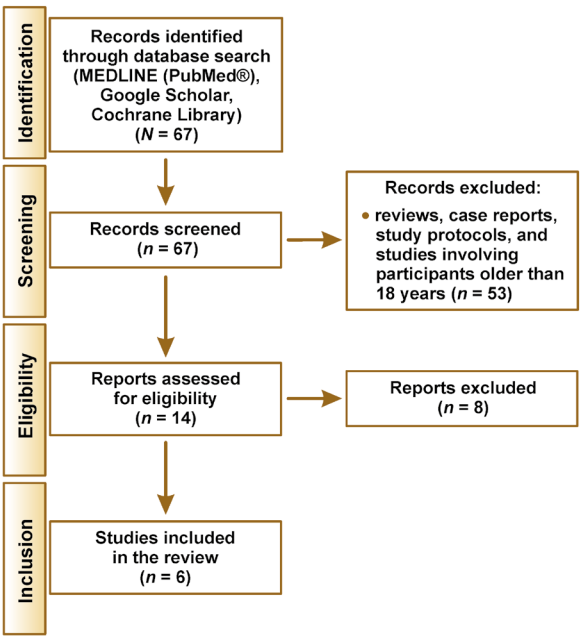

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flowchart of the study

Quality assessment

The quality of the included studies was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) guidelines. Furthermore, the risk of bias was evaluated using the Cochrane risk-of-bias tool.[33]

Results

A total of 67 English-language articles were identified through searches of MEDLINE (PubMed®), Google Scholar and the Cochrane Library. After applying the inclusion and exclusion criteria, 6 studies were included in the final analysis (Fig. 1, Table 1).

Table 1. General characteristics of the included studies

Study	Treatment	Type of study	Sample size, *n*	Patient age [years]
Salgueiro et al. 2021[34]	comparison between PBMT and occlusal splints	randomized controlled trial	76	6–12
Restrepo et al. 2011[27]	occlusal splints	randomized controlled trial	36	3–6
Giannasi et al. 2013[35]	occlusal splints	pilot study with short-term follow-up	9	6–8
Kolcakoglu et al. 2022[36]	occlusal splints	clinical trial	40	6–11
Bortoletto et al. 2014[37]	biteplate therapy	pilot study	12	6–10
Kobayashi et al. 2022[31]	comparison between infrared LED-based PBMT and rigid occlusal splints	randomized clinical trial	30	6–12

PBMT – photobiomodulation therapy; LED – light-emitting diode.

Results of studies evaluating the effectiveness of occlusal splints

Four studies evaluated the effectiveness of occlusal splints alone in the treatment of bruxism. Three studies included children with mixed dentition,[35–37] while 1 study included children with primary dentition.[27] All 4 studies used rigid splints for treatment. Kolcakoglu et al. compared soft and rigid splints and found no significant differences between the 2 types in terms of masseter muscle activity.[36]

Restrepo et al. randomly assigned children with bruxism into 2 groups: an experimental group treated with rigid splints; and a control group.[27] Although rigid splints were used for an extended period (up to 2 years, for at least 14 h per day), no significant differences were observed between the 2 groups when evaluating anxiety, TMD or dental wear.

The study by Bortoletto et al. suggested that bruxism may negatively affect craniocervical posture, and that a rigid occlusal splint (biteplate) may help reduce these negative effects, but only when the appliance is in place.[37]

In a pilot study by Giannasi et al., improvements in the bruxism-related symptoms, such as the absence of grinding sounds, decreased snoring (requiring further investigation) and fewer headaches, were observed after 90 days of nocturnal use of rigid splints.[35] The study involved 9 participants and had a short follow-up period.[35]

Results of studies comparing occlusal splint therapy and photobiomodulation therapy

Two randomized clinical trials evaluating PBMT were included.[31,34] Both studies compared PBMT with occlusal splint therapy (OST).

Salgueiro et al. compared 4 different approaches and evaluated 2 variables, namely bite force, measured using a digital dynamometer, and salivary cortisol levels, assessed using enzyme-linked immunosorbent assay (ELISA) (Table 2).[34]

Kobayashi et al. compared 3 approaches and evaluated muscle activity using electromyography (EMG), as well as salivary dopamine levels measured by ELISA (Table 3).[31] The masseter, anterior temporalis and upper trapezius muscles were assessed using EMG.[31]

Diagnosis of bruxism

All studies included in this review diagnosed bruxism based on parental or guardian reports of tooth grinding

Table 2. Details of the study by Salgueiro et al.[34]

Treatment groups	Laser type and parameters	Characteristics of the occlusal splints	Results
Group 1 (n = 19): experimental group – LLLT applied to acupuncture points Group 2 (n = 19): positive control group – occlusal splint therapy Group 3 (n = 19): placebo group – non-therapeutic laser applied to acupoints Group 4 (n = 19): control group – children without bruxism	• LLLT • wavelength: 786.94 nm • conventional tip • energy density: 25 J/cm^2 • power: 70 mW • intensity: 1.675 mW/cm^2 • spot area: 0.04 cm^2 • 12 irradiation points • dose: 1 J per point for 20 s for a total of 12 sessions • frequency: twice per week • application: direct contact with the skin at 6 acupoints on each side	• maxillary occlusal splints • fabricated from transparent acrylic resin • used on the upper arch with palatal and occlusal coverage • thickness: 3 mm • occlusal contacts: all teeth in centric relation, disocclusion of the posterior teeth during lateral and protrusive movements • usage: nighttime use only for 2 months, with weekly adjustments (one-quarter turn)	Bite force was reduced in group 1 compared to the other groups. Salivary cortisol levels increased in all groups, with the greatest increase observed in group 3. Headache intensity decreased in groups 1 and 2, with no significant differences between these groups.

LLLT – low-level laser therapy.

Table 3. Details of the study by Kobayashi et al.[31]

Treatment groups	Laser type and parameters	Characteristics of the occlusal splints	Results
Group 1 (n = 10): control group – participants without SB Group 2 (n = 10): infrared LED therapy Group 3 (n = 10): treatment with rigid occlusal splints	• infrared LED • wavelength: 850 nm • optical spot size: 5 ±2 mm • dose: 2.675 J/cm^2 • optical output: ~2 mW • target muscles: masseter and temporalis muscles • protocol: single session, 7 min per muscle	• standard rigid occlusal splints • usage: at least 12 h per day for 30 days	Increased muscle activity at rest after interference was observed in the right masseter muscle across all groups. In group 3, increased muscle activity was observed in the right and left temporalis muscles and the left masseter muscle (at rest) after interference. Salivary dopamine levels did not change significantly over time. At baseline, salivary dopamine levels were higher in group 3 than in group 1.

SB – sleep bruxism.

or clenching, combined with clinical signs such as tooth wear. In the study by Restrepo et al., parents or guardians were instructed to sleep next to their children for at least 2 weeks, and bruxism was diagnosed if tooth grinding or clenching occurred on at least 5 nights during this period.[27] Additionally, the presence of bite marks on the buccal mucosa was included as part of the diagnostic criteria.[34] Kobayashi et al. reported additional clinical signs, including dental marks on the tongue, the linea alba on the buccal mucosa, mandibular and/or maxillary tori, gingival recession, and fractures or cracks in the teeth.[31]

Inclusion and exclusion criteria across studies

The majority of the evaluated studies applied similar inclusion criteria, such as a confirmed diagnosis of bruxism, healthy pediatric participants, class I molar and canine relationships according to Angle's classification, no prior treatment for bruxism, and the ability to cooperate during study procedures.

Additionally, the criteria for exclusion encompassed the presence of dental caries, physical or psychiatric conditions, malocclusions, and the use of medications such as antidepressants, analgesics, anti-inflammatory drugs, and muscle relaxants. Kolcakoglu et al. also excluded patients with intestinal parasites,[36] while Restrepo et al. excluded participants with skeletal malocclusions confirmed by cephalometric radiographs.[37]

Discussion

According to the International Classification of Sleep Disorders (ICSD), the minimum criteria for diagnosing bruxism include parental reports of tooth grinding or clenching during sleep.[38] Consequently, such reports are considered a reliable diagnostic method. Although polysomnography is regarded as the most reliable diagnostic tool, with an accuracy of 83.3% for nocturnal bruxism,[39,40] it is also considered the gold standard for diagnosing SB in adults.[2] However, its use is limited by high cost, reduced practicality in pediatric populations, and its potential impact on sleep quality.[41] Additionally, there is no standardized protocol for polysomnography in children.[42] Kolcakoglu et al. introduced the BiteStrip® device as an alternative method for measuring muscle activity before and after treatment, demonstrating its usefulness in both diagnosis and evaluation.[36]

The adverse effects of bruxism are not limited to the stomatognathic system but may also involve the postural system and muscles of the craniofacial–neck–shoulder complex.[43,44] Therefore, early diagnosis is essential to prevent further complications. The ICSD states that 85–90% of the general population experience tooth grinding to varying degrees at some point in their lives; however, only 5% develop a clinically significant condition.[45]

In general, occlusal splints were ineffective in managing all signs and symptoms of bruxism, although some benefits were observed, such as reductions in pain, headache and deviations during mouth opening.[27,36] Prolonged use of splints did not appear to increase their effectiveness. Importantly, careful monitoring and follow-up are required when using occlusal splints in children due to ongoing growth, development and tooth eruption.

The only study reporting significant improvement in bruxism symptoms with occlusal splints was a pilot study with a small sample size of 9 participants.[35] In some cases, occlusal splints produced immediate effects that persisted only when the appliance was worn and disappeared upon removal.[37] This may be explained by the increase in vertical dimension during splint use, which promotes muscle relaxation and contributes to a more stable and functional joint position. Consequently, occlusal splints may help protect teeth, improve the function of the temporomandibular joint, and reduce abnormal muscle activity.[43,46]

All included studies used conventional methods for manufacturing occlusal splints, involving traditional impressions and acrylic materials. Recent studies have introduced 3D-printed materials, including rigid and flexible resins with favorable mechanical properties and resistance.[47,48] Although they may offer advantages over traditional materials, further research is needed to confirm their clinical effectiveness.

In the present review, all studies investigating PBMT compared it with OST, as OST remains the gold standard and most widely used treatment approach. A major limitation of occlusal splints is the need for consistent cooperation from both children and their parents or guardians to ensure proper use.

Bruxism, as a repetitive motor activity, causes muscle contractions that can be measured by bite force. Bite force indicates the magnitude of masticatory muscle strength; a reduction in bite force following treatment may indicate muscle relaxation and diminished damage to anatomical structures.[34] Low-level laser therapy demonstrated effectiveness in reducing bite force and showed superiority over occlusal splints in this parameter.[34]

Salivary cortisol levels were included as a variable to evaluate the effectiveness of bruxism treatment.[49] Cortisol levels have been employed to assess stress-related responses in individuals with muscle disorders.[50] An increase in salivary cortisol reflects an acute response to a stressor. However, the included studies found PBMT to be ineffective in reducing salivary cortisol levels. Therefore, Salgueiro et al. proposed that future studies should analyze hair cortisol instead of salivary cortisol, as salivary cortisol indicates acute responses to stressors, whereas hair cortisol is indicative of cumulative exposure to stress.[34]

Kobayashi et al. introduced salivary dopamine levels as a parameter for evaluating treatment effectiveness.[31] Although their findings showed unfavorable and unexpected results when using occlusal splints, which

manifested as an increase in the activity of the right and left temporal muscles and the left masseter muscles at rest after interference, the study did not clearly demonstrate whether PBMT had produced beneficial outcomes in SB treatment.[31]

Dopamine is a key neurotransmitter mediated by the central nervous system.[51] An association has been identified between dopamine levels and neuromuscular disorders, including Parkinson's disease, where a decrease in the concentration of dopamine was detected.[52] Furthermore, dopamine can be analyzed from saliva swab samples, and an increase in its concentration has been observed in individuals with bruxism compared to controls.[10,31]

Photobiomodulation therapy is considered a promising treatment method due to its ability to stimulate or inhibit cellular processes.[53] It is particularly suitable for pediatric patients, as it is a painless, non-invasive and cost-effecitve procedure with a brief exposure time.[49]

Cerón et al. found that medications such as clonazepam and clonidine showed efficacy in reducing SB[23]; however, their use is limited by potential adverse effects. Similarly, treatments such as botulinum toxin and biofeedback have not demonstrated short-term efficacy and require longer follow-up periods.[23]

The findings of this review are consistent with previous literature indicating that no treatment provides a permanent solution for bruxism.[54] Therefore, management strategies primarily focus on symptom reduction, protection of dental structures, pain relief, and improvement of sleep quality.[18,55]

Due to the limited number of studies investigating PBMT in pediatric populations, no definitive conclusions can be drawn. Further studies are required to evaluate the effectiveness of PBMT in the treatment of bruxism among children. Additionally, restricting this review to English-language publications constitutes a limitation.

Conclusions

The adverse effects of bruxism are not limited to the stomatognathic system but extend to the postural system and the muscles of the craniofacial–neck–shoulder complex. Therefore, early diagnosis is essential to prevent further complications.

Based on the results of the analyzed studies, occlusal splints are not effective in managing all signs and symptoms of bruxism. However, they demonstrate some benefits, particularly in reducing pain, headaches and deviations during mouth opening.

There is a limited number of studies investigating the use of PBMT in the treatment of bruxism in children, which precludes definitive conclusions. Further research is needed to evaluate its clinical effectiveness in this population.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Lobbezoo F, Ahlberg J, Glaros AG, et al. Bruxism defined and graded: An international consensus. *J Oral Rehabil*. 2013;40(1):2–4. doi:10.1111/joor.12011
2. Lobbezoo F, Ahlberg J, Raphael KG, et al. International consensus on the assessment of bruxism: Report of a work in progress. *J Oral Rehabil*. 2018;45(11):837–844. doi:10.1111/joor.12663
3. Karacay BC, Sahbaz T. Investigation of the relationship between probable sleep bruxism, awake bruxism and temporomandibular disorders using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). *Dent Med Probl*. 2023;60(4):601–608. doi:10.17219/dmp/158926
4. Michalek-Zrabkowska M, Wieckiewicz M, Macek P, et al. The relationship between simple snoring and sleep bruxism: A polysomnographic study. *Int J Environ Res Public Health*. 2020;17(23):8960. doi:10.3390/ijerph17238960
5. Manfredini D, Restrepo C, Diaz-Serrano K, Winocur E, Lobbezoo F. Prevalence of sleep bruxism in children: A systematic review of the literature. *J Oral Rehabil*. 2013;40(8):631–642. doi:10.1111/joor.12069
6. Eftekharian A, Raad N, Gholami-Ghasri N. Bruxism and adenotonsillectomy. *Int J Pediatr Otorhinolaryngol*. 2008;72(4):509–511. doi:10.1016/j.ijporl.2008.01.006
7. Machado E, Dal-Fabbro C, Cunali PA, Kaizer OB. Prevalence of sleep bruxism in children: A systematic review. *Dental Press J Orthod*. 2014;19(6):54–61. doi:10.1590/2176-9451.19.6.054-061.oar
8. Guo H, Wang T, Li X, Ma Q, Niu X, Qiu J. What sleep behaviors are associated with bruxism in children? A systematic review and meta-analysis. *Sleep Breath*. 2017;21(4):1013–1023. doi:10.1007/s11325-017-1496-3
9. Ferrari-Piloni C, Barros LAN, Evangelista K, Serra-Negra JM, Silva MAG, Valladares-Neto J. Prevalence of bruxism in Brazilian children: A systematic review and meta-analysis. *Pediatr Dent*. 2022;44(1):8–20. PMID:35232529.
10. Takemura T, Takahashi T, Fukuda M, et al. A psychological study on patients with masticatory muscle disorder and sleep bruxism. *Cranio*. 2006;24(3):191–196. doi:10.1179/crn.2006.031
11. Barclay NL, Gregory AM. Quantitative genetic research on sleep: A review of normal sleep, sleep disturbances and associated emotional, behavioural, and health-related difficulties. *Sleep Med Rev*. 2013;17(1):29–40. doi:10.1016/j.smrv.2012.01.008

12. Martynowicz H, Dymczyk P, Dominiak M, et al. Evaluation of intensity of sleep bruxism in arterial hypertension. *J Clin Med*. 2018;7(10):327. doi:10.3390/jcm7100327
13. Allaf BAW, Abdul-Hak M. Association between bruxism severity and serum concentrations of 25-hydroxyvitamin D levels. *Clin Exp Dent Res*. 2022;8(4):827–835. doi:10.1002/cre2.530
14. Seweryn P, Orzeszek SM, Waliszewska-Prosół M, et al. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023;60(4):609–617. doi:10.17219/dmp/171894
15. Szulc M, Świątkowska-Stodulska R, Pawłowska E, Derwich M. Vitamin D_3 metabolism and its role in temporomandibular joint osteoarthritis and autoimmune thyroid diseases. *Int J Mol Sci*. 2023;24(4):4080. doi:10.3390/ijms24044080
16. Topaloglu-Ak A, Kurtulmus H, Basa S, Sabuncuoglu O. Can sleeping habits be associated with sleep bruxism, temporomandibular disorders and dental caries among children? *Dent Med Probl*. 2022;59(4):517–522. doi:10.17219/dmp/150615
17. Hachmann A, Martins EA, Araujo FB, Nunes R. Efficacy of the nocturnal bite plate in the control of bruxism for 3 to 5 year old children. *J Clin Pediatr Dent*. 1999;24(1):9–15. PMID:10709536.
18. Bader G, Lavigne G. Sleep bruxism; an overview of an oromandibular sleep movement disorder. *Sleep Med Rev*. 2000;4(1):27–43. doi:10.1053/smrv.1999.0070
19. Ierardo G, Mazur M, Luzzi V, Calcagnile F, Ottolenghi L, Polimeni A. Treatments of sleep bruxism in children: A systematic review and meta-analysis. *Cranio*. 2021;39(1):58–64. doi:10.1080/08869634.2019.1581470
20. Sağlam E, Akça ÖF. Treatment of sleep bruxism with a single daily dose of buspirone in a 7-year-old boy. *Clin Neuropharmacol*. 2019;42(4):131–132. doi:10.1097/WNF.0000000000000350
21. Akbaş B, Bilgiç A. Fluoxetine-induced sleep bruxism rapidly treated with once-nightly dosing of buspirone in a 6-year-old girl. *Clin Neuropharmacol*. 2018;41(5):197–198. doi:10.1097/WNF.0000000000000293
22. Mostafavi SN, Jafari A, Hoseini SG, Khademian M, Kelishadi R. The efficacy of low and moderate dosage of diazepam on sleep bruxism in children: A randomized placebo-controlled clinical trial. *J Res Med Sci*. 2019;24:8. doi:10.4103/jrms.JRMS_131_18
23. Cerón L, Pacheco M, Delgado Gaete A, et al. Therapies for sleep bruxism in dentistry: A critical evaluation of systematic reviews. *Dent Med Probl*. 2023;60(2):335–344. doi:10.17219/dmp/156400
24. Florjanski W, Malysa A, Orzeszek S, et al. Evaluation of biofeedback usefulness in masticatory muscle activity management – a systematic review. *J Clin Med*. 2019;8(6):766. doi:10.3390/jcm8060766
25. Manafikhi M, Ataya J, Heshmeh O. Evaluation of the efficacy of platelet rich fibrin (I-PRF) intra-articular injections in the management of internal derangements of temporomandibular joints – a controlled preliminary prospective clinical study. *BMC Musculoskelet Disord*. 2022;23(1):454. doi:10.1186/s12891-022-05421-7
26. Shetty S, Pitti V, Satish Babu CL, Surendra Kumar GP, Deepthi BC. Bruxism: A literature review. *J Indian Prosthodont Soc*. 2010;10(3):141–148. doi:10.1007/s13191-011-0041-5
27. Restrepo CC, Medina I, Patiño I. Effect of occlusal splints on the temporomandibular disorders, dental wear and anxiety of bruxist children. *Eur J Dent*. 2011;5(4):441–450. PMID:21912500.
28. Herpich CM, Leal-Junior ECP, Gomes CAFP, et al. Immediate and short-term effects of phototherapy on pain, muscle activity, and joint mobility in women with temporomandibular disorder: A randomized, double-blind, placebo-controlled, clinical trial. *Disabil Rehabil*. 2018;40(19):2318–2324. doi:10.1080/09638288.2017.1336648
29. American Society for Laser Medicine & Surgery, Inc. Photobiomodulation. https://www.aslms.org/for-the-public/treatments-using-lasers-and-energy-based-devices/photobiomodulation. Accessed November 1, 2023.
30. Dompe C, Moncrieff L, Matys J, et al. Photobiomodulation – underlying mechanism and clinical applications. *J Clin Med*. 2020;9(6):1724. doi:10.3390/jcm9061724
31. Kobayashi FY, Castelo PM, Politti F, et al. Immediate evaluation of the effect of infrared LED photobiomodulation on childhood sleep bruxism: A randomized clinical trial. *Life (Basel)*. 2022;12(7):964. doi:10.3390/life12070964
32. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *J Clin Epidemiol*. 2021;134:178–189. doi:10.1016/j.jclinepi.2021.03.001
33. Munder T, Barth J. Cochrane's risk of bias tool in the context of psychotherapy outcome research. *Psychother Res*. 2018;28(3):347–355. doi:10.1080/10503307.2017.1411628
34. Salgueiro MCC, Kobayashi FY, Motta LJ, et al. Effect of photobiomodulation on salivary cortisol, masticatory muscle strength, and clinical signs in children with sleep bruxism: A randomized controlled trial. *Photobiomodul Photomed Laser Surg*. 2021;39(1):23–29. doi:10.1089/photob.2019.4778
35. Giannasi LC, Santos IR, Alfaya TA, Bussadori SK, de Oliveira LVF. Effect of an occlusal splint on sleep bruxism in children in a pilot study with a short-term follow up. *J Bodyw Mov Ther*. 2013;17(4):418–422. doi:10.1016/j.jbmt.2013.01.001
36. Kolcakoglu K, Dogan S, Tulga Oz F, Aydınbelge M. A comparison of hard and soft occlusal splints for the treatment of nocturnal bruxism in children using the BiteStrip®. *J Clin Pediatr Dent*. 2022;46(3):219–224. doi:10.17796/1053-4625-46.3.8
37. Bortoletto CC, da Silva FC, da Costa Silva PF, et al. Evaluation of cranio-cervical posture in children with bruxism before and after bite plate therapy: A pilot project. *J Phys Ther Sci*. 2014;26(7):1125–1128. doi:10.1589/jpts.26.1125
38. Carlsson GE, Egermark I, Magnusson T. Predictors of bruxism, other oral parafunctions, and tooth wear over a 20-year follow-up period. *J Orofac Pain*. 2003;17(1):50–57. PMID:12756931.
39. Lavigne GJ, Khoury S, Abe S, Yamaguchi T, Raphael K. Bruxism physiology and pathology: An overview for clinicians. *J Oral Rehabil*. 2008;35(7):476–494. doi:10.1111/j.1365-2842.2008.01881.x
40. Rompré PH, Daigle-Landry D, Guitard F, Montplaisir JY, Lavigne GJ. Identification of a sleep bruxism subgroup with a higher risk of pain. *J Dent Res*. 2007;86(9):837–842. doi:10.1177/154405910708600906
41. Kato T, Dal-Fabbro C, Lavigne GJ. Current knowledge on awake and sleep bruxism: Overview. *Alpha Omegan*. 2003;96(2):24–32. PMID:12955779.
42. Restrepo C, Manfredini D, Castrillon E, et al. Diagnostic accuracy of the use of parental-reported sleep bruxism in a polysomnographic study in children. *Int J Paediatr Dent*. 2017;27(5):318–325. doi:10.1111/ipd.12262
43. Vélez AL, Restrepo CC, Peláez-Vargas A, et al. Head posture and dental wear evaluation of bruxist children with primary teeth. *J Oral Rehabil*. 2007;34(9):663–670. doi:10.1111/j.1365-2842.2007.01742.x
44. Armijo-Olivo S, Rappoport K, Fuentes J, et al. Head and cervical posture in patients with temporomandibular disorders. *J Orofac Pain*. 2011;25(3):199–209. PMID:21837287.
45. American Academy of Sleep Medicine. *International Classification of Sleep Disorders: Diagnostic and Coding Manual*. Westchester, IL: American Academy of Sleep Medicine; 2005:148–152.
46. Hirsch C, Hoffmann J, Türp JC. Are temporomandibular disorder symptoms and diagnoses associated with pubertal development in adolescents? An epidemiological study. *J Orofac Orthop*. 2012;73(1):6–8. doi:10.1007/s00056-011-0056-x
47. Paradowska-Stolarz A, Wieckiewicz M, Kozakiewicz M, Jurczyszyn K. Mechanical properties, fractal dimension, and texture analysis of selected 3D-printed resins used in dentistry that underwent the compression test. *Polymers (Basel)*. 2023;15(7):1772. doi:10.3390/polym15071772
48. Paradowska-Stolarz AM, Wieckiewicz M, Mikulewicz M, et al. Comparison of the tensile modulus of three 3D-printable materials used in dentistry. *Dent Med Probl*. 2023;60(3):505–511. doi:10.17219/dmp/166070
49. Salgueiro MCC, Bortoletto CC, Horliana ACRT, et al. Evaluation of muscle activity, bite force and salivary cortisol in children with bruxism before and after low level laser applied to acupoints: Study protocol for a randomised controlled trial. *BMC Complement Altern Med*. 2017;17(1):391. doi:10.1186/s12906-017-1905-y
50. Kobayashi FY, Gavião MBD, Marquezin MCS, et al. Salivary stress biomarkers and anxiety symptoms in children with and without temporomandibular disorders. *Braz Oral Res*. 2017;31:e78. doi:10.1590/1807-3107BOR-2017.vol31.0078
51. Rodríguez Cerdeira C, Sánchez-Blanco E, Sánchez-Blanco B, González-Cespón JL; Working Group of IISGS. Protein biomarkers of mood disorders. *Int J Immunopathol Pharmacol*. 2017;30(1):7–12. doi:10.1177/0394632016681017

52. Kang WY, Yang Q, Jiang XF, et al. Salivary DJ-1 could be an indicator of Parkinson's disease progression. *Front Aging Neurosci*. 2014;6:102. doi:10.3389/fnagi.2014.00102
53. Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys*. 2017;4(3):337–361. doi:10.3934/biophy.2017.3.337
54. Yap AU, Chua AP. Sleep bruxism: Current knowledge and contemporary management. *J Conserv Dent*. 2016;19(5):383–389. doi:10.4103/0972-0707.190007
55. de Lima Bach S, Moreira FP, Goettems ML, et al. Salivary cortisol levels and biological rhythm in schoolchildren with sleep bruxism. *Sleep Med*. 2019;54:48–52. doi:10.1016/j.sleep.2018.09.031

Romanian version of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I: Cross-cultural adaptation, criterion validity, and reliability

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Abstract

Background. Temporomandibular disorders (TMD) are the most common cause of non-dental orofacial pain, affecting about $^1/_3$ of the population worldwide. The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I is recognized as the international gold standard for the clinical and research diagnosis of TMD; however, no validated Romanian version has been available to date.

Objectives. The aim of the present study was to translate and culturally adapt the DC/TMD Axis I into Romanian, and to evaluate its criterion validity and inter- and intra-examiner reliability.

Material and methods. This cross-sectional methodological study followed the Beaton et al. and DC/TMD translation guidelines. The process included forward–backward translation, an expert panel review, and pre-testing on 20 patients to ensure semantic and conceptual equivalence. A total of 140 Romanian-speaking adults (mean age: 34.7 ±12.1 years; 70.7% women) were examined by 2 calibrated clinicians. Criterion validity was assessed against an expert-consensus reference diagnosis. Inter- and intra-examiner reliability for categorical variables was analyzed using Cohen's κ, and for continuous measurements using intraclass correlation coefficients (*ICC*s). The Bland–Altman plots and demographic analyses further evaluated measurement consistency.

Results. The Romanian DC/TMD Axis I showed excellent diagnostic performance, with sensitivity ranging from 85.7% (for disc displacement without reduction) to 91.2% (for myalgia), specificity of 93.5–98.2%, and AUC (the area under the receiver operating characteristic (ROC) curve) $\geq$ 0.91 for all diagnostic categories. Inter-examiner agreement was substantial to almost perfect (κ = 0.78–0.94), and intra-examiner agreement similarly high (κ = 0.80–0.95). Continuous mandibular measurements demonstrated excellent reproducibility (*ICC* = 0.89–0.96). Slightly lower sensitivity for disc displacement without reduction indicates an area for further refinement of diagnostic criteria.

Conclusions. The Romanian version of the DC/TMD Axis I is a valid, reliable and culturally equivalent tool for diagnosing TMD. Its adoption will standardize clinical assessment, enhance interdisciplinary communication, and enable Romanian participation in multicenter and educational initiatives across Central and Eastern Europe.

Keywords: Romania, reproducibility of results, temporomandibular disorders, validation study, cross-cultural comparison

Highlights

- The Romanian Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I demonstrated excellent criterion validity across all major TMD diagnoses.
- Inter- and intra-examiner reliability were substantial to almost perfect.
- Continuous mandibular measurements showed excellent reproducibility.
- The validated Romanian version supports standardized TMD diagnosis in both clinical practice and research.
- This tool facilitates multicenter collaboration and educational initiatives in Central and Eastern Europe.

Introduction

Temporomandibular disorders (TMD) comprise a heterogenous group of musculoskeletal and neuromuscular conditions that affect the temporomandibular joints (TMJs), masticatory muscles, and related structures.[1,2] They are the most common cause of non-dental orofacial pain, affecting approx. 34% of the population worldwide, with geographical variations.[3] They are frequently associated with pain, functional limitation and reduced quality of life (QoL).[4,5] Since TMD have a multifactorial etiology involving biological, psychological and social components, their manifestations often overlap and vary between individuals. Factors such as occlusal and musculoskeletal dysfunctions, emotional stress, parafunctional behaviors, and central sensitization have all been implicated in TMD pathophysiology.[6–13] Therefore, standardized diagnostic criteria are essential to ensure accurate classification, facilitate interdisciplinary management, and enable meaningful comparisons across clinical and research settings.

The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) published in 2014 are considered the international gold standard for the clinical and research diagnosis of TMD.[6] They comprise 2 complementary axes: Axis I, which provides standardized clinical assessment procedures and decision rules for the most common TMD subtypes (e.g., myalgia, arthralgia, disc displacement); and Axis II, which evaluates psychosocial factors and pain-related disability. Axis II includes validated questionnaires that assess domains such as pain intensity (Graded Chronic Pain Scale – GCPS), depressive and anxiety symptoms (Patient Health Questionnaire-9 – PHQ-9, Generalized Anxiety Disorder-7 – GAD-7), and pain catastrophizing. The inclusion of these measures enables a biopsychosocial approach, improving diagnostic accuracy and supporting interdisciplinary treatment planning. Axis I has demonstrated robust psychometric properties, including high reliability and validity across multiple populations.[14]

The cross-cultural adaptation of diagnostic instruments is critical to maintain semantic, idiomatic, experiential, and conceptual equivalence between the original and target versions.[15] Proper adaptation enables clinicians to accurately capture patient-reported symptoms and apply standardized diagnostic procedures in a culturally sensitive manner.[15] Several validated translations of the DC/TMD Axis I have been published, including Brazilian Portuguese, Turkish, and Polish versions, all of which report high inter- and intra-examiner reliability (Cohen's $\kappa > 0.75$) and excellent criterion validity (AUC (the area under the receiver operating characteristic (ROC) curve) ≥ 0.85).[16–19]

However, no validated Romanian version of the DC/TMD Axis I has been available to date, which limits standardized clinical practice, hinders multicenter collaboration and constrains epidemiological surveillance in Romania. Therefore, the present study aims to fill this gap by translating and culturally adapting the DC/TMD Axis I into Romanian in accordance with the internationally accepted guidelines, evaluating its criterion validity against an expert-consensus reference diagnosis, and determining the inter- and intra-examiner reliability of the clinical examination procedures and continuous measurements.

Material and methods

Study design

This was a cross-sectional methodological study designed to translate, culturally adapt, and validate the DC/TMD Axis I for use in Romanian clinical and research settings. The study adhered to the internationally accepted guidelines for the cross-cultural adaptation of clinical instruments, including those outlined by Beaton et al.,[20] and the DC/TMD translation project.[6]

Setting and participants

Participants were recruited between March and September 2024 from the dental clinics and outpatient services affiliated with the Osteo Performance Clinic in Bucharest, Romania, within the framework of the Doctor of Physical Therapy program at the University

of Medical Sciences Arizona (UMSAZ), Honolulu, USA. The eligibility criteria included adults aged 18 years or older who were fluent in Romanian and able to provide written informed consent. The exclusion criteria comprised systemic rheumatic diseases, a history of maxillofacial surgery, acute trauma, cognitive or communication impairment that could interfere with the completion of the protocol, alcohol and/or drug addiction, severe mental illness, active neoplasms, and pregnancy.

The final study group consisted of 140 participants (99 women, 41 men; mean age: 34.7 ±12.1 years; range: 18–65 years), representing a broad cross-section of Romanian adults seeking dental and orofacial care. The pre-test phase included 20 participants who met the same eligibility criteria; it was meant to evaluate the comprehensibility and applicability of the adapted version. Inter-examiner reliability during this phase demonstrated excellent agreement for the diagnostic categories, with an intraclass correlation coefficient (*ICC*) of 0.91, confirming the stability and consistency of the assessment process prior to clinical validation.

The sample size was determined according to methodological recommendations for the reliability and validity studies of clinical diagnostic tools, which suggest that at least 100 participants are required to obtain stable estimates of the κ and *ICC* values with acceptable precision.[21–23] Accordingly, a cohort of 140 participants was considered adequate to ensure sufficient statistical power for detecting reliability coefficients ≥0.80 and for robust criterion validity analysis across diagnostic subgroups. The study protocol was reviewed and approved by the Research Ethics Committee of the National University of Physical Education and Sports, Bucharest, Romania (approval No. 1170/SF/23.09.2025). Written informed consent was obtained from all participants prior to enrollment, and all procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The data was anonymized prior to analysis to ensure confidentiality. Participation was entirely voluntary, and refusal to participate did not affect access to care. The University of Medical Sciences Arizona (UMSAZ) reviewed the protocol and formally acknowledged the Romanian ethics committee approval as sufficient ethical oversight for this project.

Translation and cross-cultural adaptation

The translation and cultural adaptation of the DC/TMD Axis I followed the DC/TMD guidelines,[6] and included the following steps:

1. Forward translation: Two independent bilingual translators with medical backgrounds translated the original English version into Romanian to ensure the accurate rendering of clinical terminology.
2. Synthesis: The 2 forward translations were reconciled into a single synthesized version to resolve discrepancies and achieve linguistic consistency.
3. Back translation: Two native English speakers, blinded to the original version, back-translated the synthesized Romanian version into English. This step verified semantic and conceptual equivalence between the original and translated versions.
4. Expert committee review: A multidisciplinary expert panel, consisting of the authors themselves (AM, DGB, AP, TS), reviewed all versions to ensure semantic, idiomatic, experiential, and conceptual equivalence, and to confirm that clinical terms were culturally appropriate for Romanian practice.
5. Pre-testing: The pre-final version was tested on 20 patients to evaluate clarity, cultural relevance and comprehension. Minor adjustments were made as needed, primarily involving phrasing and terminology changes to enhance clarity and cultural relevance (e.g., replacing dental jargon with commonly used Romanian equivalents). This stage ensured that the adapted version was easily understood by patients and consistent with everyday language use.
6. Finalization: The expert panel approved the final Romanian version after confirming that all adaptations preserved the intent and diagnostic meaning of the original DC/TMD Axis I.

Reference standard diagnosis

The reference diagnosis was determined by a consensus panel of 3 TMD specialists (AM, MI and AP), who independently reviewed all available clinical information and, when indicated, imaging findings. The panel members were blinded to the Romanian DC/TMD results. Discrepancies were resolved through discussion until consensus was reached.

Clinical examination procedures

Two examiners (DGB and TS) were trained and calibrated according to the DC/TMD guidelines prior to data collection.[6] Calibration included joint training sessions involving clinical demonstrations, supervised practice, and consensus discussions with a senior DC/TMD instructor (TG) until complete agreement was reached on all diagnostic procedures. Inter- and intra-examiner reliability was confirmed in pilot assessments before the main study. Any diagnostic discrepancies during data collection were resolved through re-examination and discussion, with final decisions determined by consensus within the expert panel. Examinations followed the standardized DC/TMD Axis I protocol, including a structured patient history, the clinical palpation of the masticatory muscles and TMJs, joint noise assessment, and the measurement of the mandibular range of motion.

Reliability assessment

Inter-examiner reliability was evaluated by having both examiners independently assess each participant on the same day, with each examiner blinded to the other's findings. Intra-examiner reliability was determined by having one examiner repeat the complete assessment after 7–10 days under comparable conditions, blinded to their initial results.

Statistical analysis

All data was analyzed using IBM SPSS Statistics for Windows, v. 29 (IBM Corp., Armonk, USA). Inter- and intra-examiner agreement for categorical diagnostic variables was evaluated using Cohen's κ with 95% confidence intervals (*CI*s), interpreted according to the Landis and Koch benchmarks (κ = 0.61–0.80 indicating substantial agreement; κ = 0.81–1.00 indicating almost perfect agreement). The reliability of continuous mandibular mobility measurements was assessed using *ICC*s (two-way mixed-effects model, absolute agreement) with 95% *CI*, with *ICC* values greater than 0.75 considered excellent.

To provide a more comprehensive assessment of measurement consistency, the Bland–Altman plots were generated for continuous mandibular movement data to visualize agreement and potential systematic bias between the examiners. Additionally, the potential influence of demographic variables (age and sex) on inter-examiner agreement was explored using correlation and subgroup analyses.

Criterion validity was determined by calculating sensitivity, specificity and AUC, comparing Romanian DC/TMD diagnoses against the expert-consensus reference standard.

Results

Participant characteristics

A total of 140 participants were included (99 women (70.7%), 41 men (29.3%)), with a mean age of 34.7 ±12.1 years (range: 18–65 years). The mean symptom duration was 26.4 ±18.7 months. Myalgia was the most frequent diagnosis (38.6%), followed by arthralgia (22.1%) and disc displacement with reduction (20.7%). Disc displacement without reduction and degenerative joint disease were less common (10.7% and 7.9%, respectively).

Criterion validity

The Romanian DC/TMD Axis I demonstrated excellent criterion validity when compared with the expert-consensus reference diagnosis. Sensitivity ranged from 85.7% for disc displacement without reduction to 91.2% for myalgia, with specificity from 93.5% to 98.2% and AUC values ≥0.91 for all diagnostic categories (Table 1). Although all diagnostic values were high, the slightly lower sensitivity for disc displacement without reduction suggests potential variability in the clinical recognition of this condition, and indicates an area that may warrant further research to optimize diagnostic criteria and examiner calibration.

Inter- and intra-examiner reliability

Inter-examiner reliability was substantial to almost perfect across all diagnostic categories (κ = 0.78–0.94), with similar results for intra-examiner reliability (κ = 0.80–0.95) (Table 2).

Reliability of continuous measures

Mandibular mobility measurements demonstrated excellent reproducibility. Inter-examiner *ICC*s ranged from 0.89 for maximum protrusion to 0.94 for maximum unassisted mouth opening. Intra-examiner *ICC*s ranged from 0.92 to 0.96, confirming high consistency across the repeated measurements.

Discussion

This study translated, culturally adapted, and validated the DC/TMD Axis I for use in Romanian clinical and research settings. Our findings demonstrate that the Romanian version possesses excellent criterion validity and high inter- and intra-examiner reliability for all major diagnostic categories, confirming that it is a robust tool for diagnosing TMD in Romania.

Table 1. Criterion validity of the Romanian Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I (*N* = 140)

Diagnosis	Sensitivity [%]	Specificity [%]	AUC
Myalgia	91.2	93.5	0.94
Arthralgia	88.6	95.0	0.92
Disc displacement with reduction	90.3	94.1	0.93
Disc displacement without reduction	85.7	97.0	0.91
Degenerative joint disease	87.5	98.2	0.96

AUC – area under the receiver operating characteristic (ROC) curve.

Table 2. Inter- and intra-examiner reliability (Cohen's κ)

Diagnosis	Inter-examiner κ	Intra-examiner κ
Myalgia	0.88	0.90
Arthralgia	0.86	0.89
Disc displacement with reduction	0.84	0.87
Disc displacement without reduction	0.78	0.80
Degenerative joint disease	0.94	0.95

The high sensitivity (85.7–91.2%), specificity (93.5–98.2%) and AUC values (≥0.91) observed in this study are consistent with those reported in other validated translations of the DC/TMD Axis I.[16–18] Antunes Da Cunha et al. (the Brazilian Portuguese version) reported similar diagnostic accuracy, with AUC values above 0.85 for most categories.[18] Our reliability coefficients (κ = 0.78–0.95) were comparable to or slightly higher than those reported in the Brazilian and Turkish adaptations,[17,18] which also found substantial to almost perfect inter- and intra-examiner agreement.

In relation to the Polish study, our findings closely mirror those reported by Osiewicz et al., who demonstrated high criterion validity and substantial to almost perfect agreement for the Polish version of the DC/TMD Axis I.[16] In our sample, sensitivity and specificity were slightly higher across several diagnostic categories, with AUC values consistently ≥0.91 in comparison with their somewhat wider range. Similarly, our inter- and intra-examiner κ coefficients (0.78–0.94 and 0.80–0.95, respectively) were comparable to or marginally higher than those observed in the Polish validation, particularly for disc displacement categories. These findings suggest that examiner calibration and balanced diagnostic representation in our cohort may have contributed to the slightly stronger reliability estimates. Together with the Brazilian, Turkish and Polish validations, our results reinforce the robustness of the DC/TMD Axis I, and support its applicability across diverse cultural and linguistic contexts.

Our *ICC* values for continuous measurements (0.89–0.96) also align closely with those reported in previous studies, including the original English version by Schiffman et al.,[6] indicating excellent reproducibility for mandibular range-of-motion measurements. Notably, our sample included a balanced distribution of common TMD diagnoses, which likely contributed to the stable reliability estimates across categories, even for less prevalent conditions such as disc displacement without reduction and degenerative joint disease.

Clinical implications

The availability of the validated Romanian DC/TMD Axis I enables standardized diagnosis of TMD in dental, physical therapy, and multidisciplinary pain clinics across Romania. The use of uniform diagnostic criteria can shorten diagnostic time by providing clear decision rules, reducing the likelihood of misdiagnosis through consistent clinical definitions, and supporting timely referral decisions to appropriate specialists, such as physical therapists, orofacial pain experts, or psychologists. This standardization will also facilitate better patient stratification, more efficient treatment planning and improved communication among healthcare providers. Moreover, its integration into research will allow Romanian data to be reliably compared with international findings, fostering multicenter collaboration and epidemiological surveillance.

Limitations

The study has several limitations. The participants were recruited using convenience sampling from a single metropolitan area, which may limit the representativeness of the findings and their generalizability to other regions of Romania, as well as to individuals from different age groups or socioeconomic backgrounds. Although the overall sample size was adequate for reliability and validity analyses, it may not fully capture the demographic diversity of the national population.

Imaging procedures were not systematically applied across all the participants, particularly for the suspected cases of degenerative joint disease, which may have reduced the precision of the diagnostic reference standard. This limitation reflects the pragmatic design of the study, which followed the DC/TMD clinical protocol used in routine clinical practice. Future studies should incorporate consistent imaging protocols (e.g., magnetic resonance imaging (MRI) or cone-beam computed tomography (CBCT)) to strengthen diagnostic confirmation, especially for joint-related disorders.

Finally, the study was cross-sectional and did not assess the instrument's responsiveness to clinical change over time.

Future directions

Future studies should aim to improve the representativeness of the sample through multicenter recruitment across diverse Romanian regions, including both urban and rural populations. In addition, longitudinal follow-up studies are needed to evaluate the responsiveness and longitudinal validity of the Romanian DC/TMD Axis I, assessing its ability to detect clinically meaningful changes following treatment. Further research should also examine its applicability in pediatric and geriatric populations. Moreover, the Romanian version could serve as a standardized tool for multicenter studies across Central and Eastern Europe, enhancing regional collaboration and the comparability of diagnostic data. Finally, the translation and validation of the Axis II psychosocial assessment into Romanian would further support a comprehensive biopsychosocial approach to the management of TMD.

Conclusions

The Romanian version of the DC/TMD Axis I is a valid and reliable diagnostic tool for TMD. Its implementation is recommended for both clinical practice and research in Romania, as it will harmonize diagnostic standards and facilitate international collaboration. In addition, the availability of a standardized and validated instrument provides an important educational resource for dentists, physical therapists, and other healthcare professionals, enabling training to be based on uniform, evidence-based diagnostic criteria.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Research Ethics Committee of the National University of Physical Education and Sports, Bucharest, Romania (approval No. 1170/SF/23.09.2025). Written informed consent was obtained from all participants prior to enrollment, and all procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The data was anonymized prior to analysis to ensure confidentiality. Participation was entirely voluntary, and refusal to participate did not affect access to care. The University of Medical Sciences Arizona (UMSAZ), Honolulu, USA, reviewed the protocol and formally acknowledged the Romanian ethics committee approval as sufficient ethical oversight for this project.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

AI-assisted technology (ChatGPT, OpenAI) was used solely for improving the English language and manuscript readability. No AI tools were used for study design, data collection, statistical analysis, the interpretation of results, or scientific conclusions. All scientific content, as well as the final approval of the manuscript, were the responsibility of the authors.

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References

1. Ohrbach R, Dworkin SF. The evolution of TMD diagnosis: Past, present, future. *J Dent Res*. 2016;95(10):1093–1101. doi:10.1177/0022034516653922
2. Okeson JP. *Bell's Oral and Facial Pain*. Hanover Park, IL: Quintessence Publishing Co. Inc.; 2014:546.
3. Zieliński G. Quo vadis temporomandibular disorders? By 2050, the global prevalence of TMD may approach 44%. *J Clin Med*. 2025;14(13):4414. doi:10.3390/jcm14134414
4. Schiffman E, Ohrbach R. Executive summary of the Diagnostic Criteria for Temporomandibular Disorders for clinical and research applications. *J Am Dent Assoc*. 2016;147(6):438–445. doi:10.1016/j.adaj.2016.01.007
5. Dahlstrm L, Carlsson GE. Temporomandibular disorders and oral health-related quality of life. A systematic review. *Acta Odontol Scand*. 2010;68(2):80–85. doi:10.3109/00016350903431118
6. Schiffman E, Ohrbach R, Truelove E, et al.; International RDC/TMD Consortium Network, International Association for Dental Research; Orofacial Pain Special Interest Group, International Association for the Study of Pain. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for clinical and research applications: Recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
7. Dos Reis Ferreira N, Machado Marto CM, Tôrres de Oliveira AT, Rodrigues MJ, DosSantos MF. Development of core outcome sets for clinical trials in temporomandibular disorders: A study protocol. *PLoS One*. 2022;17(4):e0267722. doi:10.1371/journal.pone.0267722
8. Fillingim RB, Slade GD, Greenspan JD, et al. Long-term changes in biopsychosocial characteristics related to temporomandibular disorder: Findings from the OPPERA study. *Pain*. 2018;159(11):2403–2413. doi:10.1097/j.pain.0000000000001348
9. Wieckiewicz M, Jenca Jr. A, Seweryn P, et al. Determination of pain intensity, pain-related disability, anxiety, depression, and perceived stress in Polish adults with temporomandibular disorders: A prospective cohort study. *Front Integr Neurosci*. 2022;16:1026781. doi:10.3389/fnint.2022.1026781
10. Orzeszek S, Martynowicz H, Smardz J, et al. Assessment of the relationship between sleep bruxism, reported pain and headache, selected health factors, and general health conditions among temporomandibular disorder patients: A preliminary report. *Dent Med Probl*. 2025;62(2):393–399. doi:10.17219/dmp/192824
11. Seweryn P, Orzeszek SM, Waliszewska-Prosół M, et al. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023;60(4):609–617. doi:10.17219/dmp/171894
12. Tomczyk J, Olczak-Kowalczyk D, Turska-Szybka A, Studnicki M. Oral health behaviors and tooth decay at the age of 12 and 15–18 years in Poland. *Dent Med Probl*. 2025;62(1):13–22. doi:10.17219/dmp/184054
13. Friedman Rubin P, Eli I, Greenbaum T, Shapira K, Emodi-Perlman A, Winocur E. Potential orofacial hazards of resistance training: A controlled comparative study. *Cranio*. 2019;37(1):45–52. doi:10.1080/08869634.2017.1370566
14. Reiter S, Jazmawi S, Winocur E, Winocur Arias O, Kats L, Manor Y. Axis I diagnosis profile according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD): Comparison between hospital-based orofacial pain clinic and dental academic-based orofacial pain clinic. *J Oral Facial Pain Headache*. 2024;38(4):76–84. doi:10.22514/jofph.2024.040
15. Wild D, Grove A, Martin M, et al.; ISPOR Task Force for Translation and Cultural Adaptation. Principles of good practice for the translation and cultural adaptation process for patient-reported outcomes (PRO) measures: Report of the ISPOR Task Force for Translation and Cultural Adaptation. *Value Health*. 2005;8(2):94–104. doi:10.1111/j.1524-4733.2005.04054.x
16. Osiewicz M, Ciapała B, Bolt K, Kołodziej P, Więckiewicz M, Ohrbach R. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD): Polish assessment instruments. *Dent Med Probl*. 2024;61(1):5–8. doi:10.17219/dmp/181149
17. Arikan H, Citaker S, Ucok C. Cross-cultural adaptation, reliability, and validity of the Turkish version of the Craniofacial Pain and Disability Inventory (CF-PDI/T) for individuals with temporomandibular disorders. *Disabil Rehabil*. 2023;45(3):523–533. doi:10.1080/09638288.2022.2028909
18. Antunes Da Cunha T, Alstergren P, Pereira Jr. FJ, Gonçalves DAdG, Chaves TC, Biasotto-Gonzalez DA. Brazilian Portuguese version of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I: Cross-cultural adaptation, criterion validity, and reliability. *Braz J Phys Ther*. 2025;29(2):101187. doi:10.1016/j.bjpt.2025.101187
19. Gałczyńska-Rusin M, Pobudek-Radzikowska M, Czajka-Jakubowska A. Polish language adaptation and validation of the Fonseca Anamnestic Index for individuals with temporomandibular disorders. *Dent Med Probl*. 2024;61(5):705–711. doi:10.17219/dmp/177287
20. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine (Phila Pa 1976)*. 2000;25(24):3186–3191. doi:10.1097/00007632-200012150-00014

21. Mokkink LB, De Vet HC, Prinsen CA, et al. COSMIN risk of bias checklist for systematic reviews of patient-reported outcome measures. *Qual Life Res*. 2018;27(5):1171–1179. doi:10.1007/s11136-017-1765-4
22. Terwee CB, Bot SD, De Boer MR, et al. Quality criteria were proposed for measurement properties of health status questionnaires. *J Clin Epidemiol*. 2007;60(1):34–42. doi:10.1016/j.jclinepi.2006.03.012
23. Mondal D, Vanbelle S, Cassese A, Candel MJ. Review of sample size determination methods for the intraclass correlation coefficient in the one-way analysis of variance model. *Stat Methods Med Res*. 2024;33(3):532–553. doi:10.1177/09622802231224657

Research letter

Altered circadian patterns of diurnal blood pressure in clinical phenotypes of bruxism: A pilot study on autonomic imbalance in healthy young adults

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Abstract

Background. Bruxism, a repetitive masticatory muscle activity, is increasingly recognized as a behavioral marker with possible systemic implications. While its association with autonomic dysregulation has been proposed, the relationship between bruxism phenotypes and variations in circadian blood pressure (BP) in healthy individuals remains unclear.

Objectives. The aim of the study was to explore whether distinct behavioral bruxism phenotypes (sleep vs. awake, morning vs. evening) are associated with altered diurnal BP profiles, particularly postprandial patterns, in healthy normotensive young adults.

Material and methods. A total of 59 healthy young adults (mean age: 23.4 ±3.8 years) with probable bruxism were classified into 4 phenotypes: sleep bruxism (SB); morning awake bruxism (AB); evening AB; and undefined. The participants underwent 3 standardized BP measurements throughout the day (morning, postprandial and afternoon), alongside assessment using the Pittsburgh Sleep Quality Index (PSQI), the Insomnia Severity Index (ISI) and the Epworth Sleepiness Scale (ESS). Bruxism classification was based on self-reports, clinical signs and timing patterns. Data was analyzed using analysis of variance (ANOVA) and post hoc tests. Confidence intervals and effect sizes were calculated, and boxplots were used to illustrate group differences.

Results. Participants with SB exhibited a significantly steeper postprandial diastolic BP decline compared to other phenotypes ($p = 0.045$), deviating from the expected parasympathetic post-meal dip observed in healthy profiles. Sleep bruxism was also associated with poorer sleep quality (higher PSQI scores), greater insomnia severity (higher ISI scores) and a lack of significant increase in daytime sleepiness. Individuals with morning AB showed a biphasic BP pattern suggestive of autonomic lability, while participants with evening AB exhibited a blunted profile. Questionnaire data reinforced a chronic hyperarousal profile among SB participants.

Conclusions. Behavioral bruxism phenotypes, particularly SB, may reflect underlying autonomic imbalance and altered circadian BP modulation, even in clinically normotensive individuals. These findings support the hypothesis that bruxism may serve as a behavioral marker of subclinical cardiovascular risk. Further studies incorporating objective autonomic markers and longitudinal data are warranted.

Keywords: bruxism, phenotypes, blood pressure, circadian

Highlights

- This pilot study assessed circadian blood pressure (BP) variation across different bruxism phenotypes in healthy young adults.
- None of the bruxism subgroups exhibited the expected physiological BP rhythm, suggesting altered autonomic regulation.
- Participants with sleep bruxism showed significantly lower postprandial diastolic BP, poorer sleep quality and greater insomnia severity.
- Bruxism phenotyping may serve as a behavioral marker of early cardiovascular risk and autonomic imbalance.

Introduction

Bruxism is a repetitive masticatory muscle activity involving clenching or grinding of the teeth and/or bracing or thrusting of the mandible. It is currently understood as a behavior, rather than a disorder, with possible functional or dysfunctional consequences depending on its intensity, duration and systemic context. A recent consensus has emphasized the importance of distinguishing between awake bruxism (AB) and sleep bruxism (SB).[1] Interestingly, this classification primarily reflects a state-dependent distinction (i.e., wakefulness vs. sleep) rather than a strict circadian division in the physiological sense. While the timing of bruxism expression may be influenced by circadian processes, referring to AB and SB as circadian profiles may overstate the role of endogenous circadian regulation and should therefore be interpreted cautiously. Beyond the partial synchrony observed between some SB episodes and transient increases in blood pressure (BP),[2] it is also possible that moment-to-moment variability in masticatory muscle activity throughout the 24-hour cycle contributes to cardiovascular dynamics. This is particularly relevant given the observed high intraindividual variability in SB behavior and the absence of a consistent correlation between the SB index and total sleep-time masseter muscle activity, as reported in recent electromyographic studies.[3] Such findings suggest that bruxism-related motor fluctuations may operate independently of classic circadian markers, yet still exert physiologically significant effects on autonomic and hemodynamic regulation. Moreover, previous studies have proposed that individual chronotype may modulate the systemic impact of bruxism, particularly through its influence on autonomic tone and cardiovascular stress reactivity, further supporting the view that bruxism should be interpreted not only as a motor phenomenon, but as a potential biomarker of chronobiological and cardiometabolic dysregulation.[4]

In recent years, growing interest has emerged regarding non-pathological autonomic markers in youth, particularly those related to BP variability and circadian patterning, as potential indicators of early cardiovascular and neuroregulatory disturbances. Even in otherwise healthy individuals, daytime and circadian BP variation may reflect subclinical autonomic imbalance or altered sympathetic–vagal tone preceding overt cardiovascular dysfunction.[5] It is plausible that behavioral and physiological cues, including sleep-related behaviors, modulate these patterns. Thus, assessing bruxism phenotypes in relation to diurnal BP rhythms may offer novel insights into early dysautonomia, especially in normotensive young adults. Recent investigations have emphasized sex-related and behavioral differences in self-reported bruxism and obstructive sleep apnea (OSA) symptoms, which may further influence the phenotypic expression and clinical relevance of bruxism-related cardiovascular outcomes.[6]

Blood pressure exhibits a predictable circadian rhythm in healthy individuals, characterized by a morning surge, a parasympathetically driven postprandial dip and afternoon stabilization.[5,7] However, the influence of bruxism phenotypes on this physiological BP pattern remains unexplored. This pilot study aimed to characterize BP variations in young adults with distinct bruxism profiles and to explore whether these variations could reflect behavioral circadian phenotypes (i.e., behavioral expressions of bruxism timing) potentially associated with autonomic imbalance. We hypothesized that different bruxism subtypes, particularly SB, would show altered circadian BP profiles.

Material and methods

Study design and participants

A total of 178 healthy university students (mean age: 21.3 ±1.5 years; 74.6% female) were evaluated. All participants provided informed consent. The inclusion criteria encompassed self-reported tooth grinding or clenching, the presence of at least 1 clinical sign (e.g., tongue indentation, linea alba), and the absence of current medical conditions or use of medications affecting cardiovascular or sleep function. The exclusion criteria included hypertension, diabetes, heavy smoking, inflammatory conditions, and the use of medications influencing the autonomic nervous system. A cross-sectional analysis was conducted among 59 participants (mean age: 23.4 ±3.8 years; mean body mass index (BMI): 26.07 ±2.72 kg/m^2) with probable bruxism, as confirmed by diaries and clinical signs.

Bruxism classification

Bruxism was assessed using 2 self-report questionnaires translated into Portuguese, based on the studies by Winocur et al. and Pintado et al.[8,9] In the first questionnaire, bruxism was considered probable when participants answered affirmatively to question 1 and/or question 2 (concerning awareness of grinding or clenching) and reported at least 1 symptom-related item in question 3 (e.g., jaw discomfort or tooth wear).[8] In the second questionnaire, bruxism was classified as present when at least 2 items received a positive response.[9]

Participants were then classified according to different circadian profiles: AB (morning – reported in the first 3 h after awakening; evening – reported in the last 3 h before bedtime); SB (bruxism reported during sleep only); and undefined (bruxism reported inconsistently throughout the day, including overlap among different types or patterns that could not be clearly categorized).

Blood pressure measurement

Blood pressure was measured with a validated oscillometric device (Physiogard TM 910; SCHILLER AG, Baar, Switzerland), according to the European Society of Hypertension (ESH) guidelines.[10] Each participant underwent 6 seated BP measurements daily (2 readings at each of the 3 timepoints: morning, postprandial and afternoon) over the course of 3 days. Readings were taken in a quiet, temperature-controlled room (22–24°C) by trained personnel using appropriately sized cuffs after 5 min of rest and in the absence of recent caffeine intake or exercise.

Sleep and lifestyle questionnaires

All participants completed a battery of self-report questionnaires that had been previously validated in Portuguese and widely applied in sleep research and clinical practice.

Chronotype was assessed using the morningness–eveningness questionnaire (MEQ).[11] Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI),[12] insomnia symptoms were analyzed with the Insomnia Severity Index (ISI),[13] and daytime sleepiness was assessed with the use of the Epworth Sleepiness Scale (ESS).[14] The risk of OSA was evaluated using the Berlin Questionnaire.[15] These instruments were analyzed descriptively and explored for associations with BP and bruxism profiles.

Statistical analysis

Descriptive statistics were computed for all variables, including mean (*M*) and standard deviation (*SD*). The Shapiro–Wilk test was applied to assess normality, and Levene's test was used to evaluate the homogeneity of variance.

Group comparisons for continuous variables were performed using one-way analysis of variance (ANOVA) or Kruskal–Wallis tests, as appropriate, followed by post hoc analyses. Categorical variables were analyzed using the χ^2 test. Effect sizes (Cohen's *d*) were calculated for two-group comparisons, and 95% confidence intervals (95% *CI*s) were reported to better evaluate the clinical relevance of the findings. A post hoc power analysis indicated sufficient power (0.78) to detect medium effect sizes ($f = 0.25$).

All statistical analyses were conducted using the IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA). A two-tailed *p*-value of <0.05 was considered statistically significant.

Results

Among the 59 individuals with probable bruxism, 20 were classified as having SB, 15 with morning AB, 12 with evening AB, and 12 as undefined. While none of the participants met the criteria for hypertension during the standardized BP assessments, distinct BP variation patterns emerged across phenotypes. None of the bruxism subgroups displayed the expected physiological circadian BP pattern, characterized by a morning surge, a postprandial dip and afternoon stabilization. Instead, each phenotype exhibited a deviant trajectory (Fig. 1).

The SB group showed a significantly lower mean postprandial diastolic BP (74.1 ±6.2 mmHg) compared to the morning AB group (79.6 ±5.8 mmHg; $p = 0.045$), with a Cohen's *d* of 0.91 (95% *CI*: 0.10–1.72), indicating a large effect size (Fig. 2). Morning systolic BP values were as follows: SB – 113.4 ±9.2 mmHg; morning AB – 117.1 ±8.7 mmHg; evening AB – 114.6 ±9.1 mmHg. Postprandial systolic BP averaged 110.3 ±8.9 mmHg in SB, 114.5 ±9.6 mmHg in morning AB and 113.0 ±8.4 mmHg in evening AB. Afternoon systolic BP values were 112.7 ±9.4 mmHg in SB, 118.0 ±10.1 mmHg in morning AB and 112.8 ±9.8 mmHg in evening AB.

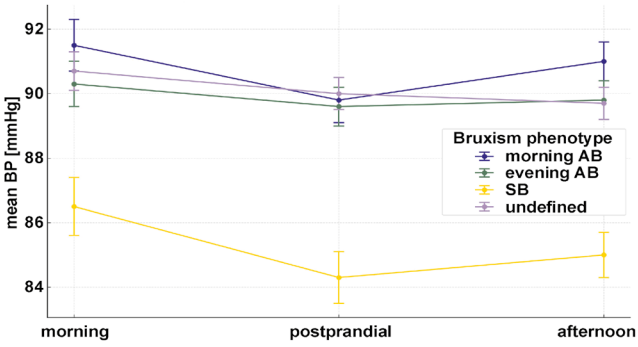

Fig. 1. Circadian variation in mean blood pressure (BP) according to bruxism phenotype

A distinct pattern of postprandial diastolic BP reduction observed in the SB group suggests a possible alteration in cardiovascular autonomic regulation in this subgroup.
SB – sleep bruxism; AB – awake bruxism.

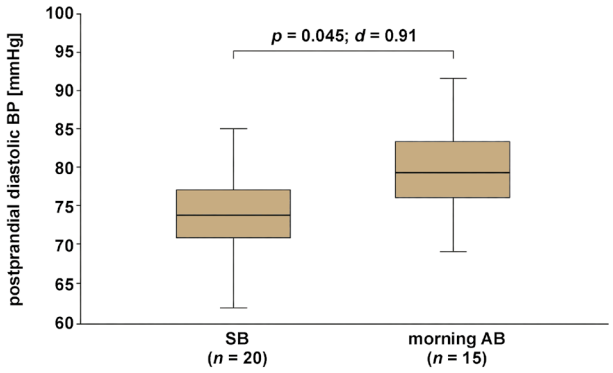

Fig. 2. Comparison of mean postprandial diastolic blood pressure (BP) between individuals with sleep bruxism (SB) and morning awake bruxism (AB)

In a complementary analysis comparing participants with probable bruxism (n = 59) to those without (n = 119), no significant differences were found in morning or afternoon systolic and diastolic BP values. However, participants with probable bruxism showed a non-significant trend toward lower postprandial diastolic BP (76.2 ±6.9 mmHg vs. 78.4 ±6.5 mmHg; p = 0.071, Cohen's d = 0.33, 95% *CI*: −0.5–0.02). No significant differences were observed in systolic BP at any timepoint (all $p > 0.10$). These results support the phenotypic distinctions observed across subgroups rather than a global bruxism-related BP alteration.

Participants with SB also showed significantly higher insomnia severity scores (ISI = 10.4 ±3.3) and poorer sleep quality (PSQI = 7.8 ±2.1), further supporting the presence of sleep-related vulnerability.

In terms of sleep parameters, the SB group exhibited significantly higher scores for insomnia severity (ISI = 10.4 ±3.3) compared to the morning AB group (ISI = 7.6 ±2.9; p = 0.021, Cohen's d = 0.91, 95% *CI*: 0.12–1.70) and the evening AB group (ISI = 6.8 ±2.7; p = 0.008, Cohen's d = 1.18, 95% *CI*: 0.35–2.01). Sleep quality assessed using the PSQI was also poorer in the SB group (7.8 ±2.1) compared to the morning AB group (5.3 ±1.8; p = 0.009, Cohen's d = 1.28, 95% *CI*: 0.40–2.16) and the evening AB group (5.1 ±1.7; p = 0.007, Cohen's d = 1.40, 95% *CI*: 0.49–2.31). No statistically significant differences were observed for excessive daytime sleepiness (ESS) or OSA risk (Berlin Questionnaire). The SB group tended to show higher risk classifications in the Berlin Questionnaire, although without reaching statistical significance.

Discussion

This pilot study suggests that distinct clinical bruxism phenotypes may be associated with altered circadian BP variation in healthy normotensive young adults. While all participants were clinically normotensive during baseline assessment, none of the bruxism subgroups displayed the expected physiological circadian BP pattern, characterized by a morning surge, a postprandial dip and afternoon stabilization. The absence of a normal rhythmic profile across all phenotypes suggests a systemic alteration of autonomic regulation rather than isolated or subgroup-specific anomalies, highlighting the potential role of subclinical dysautonomia even in healthy individuals.

The lower postprandial BP observed in the SB group, when interpreted in conjunction with poor sleep quality and higher insomnia scores, may point toward a maladaptive autonomic response rather than robust vagal tone. Previous studies have shown that SB is associated with sympathetic surges during sleep,[2] and these nocturnal events may disrupt daytime vascular regulation through impaired baroreflex sensitivity or increased arterial stiffness.[16] Furthermore, the higher ISI and PSQI scores among SB individuals reinforce the hypothesis of a chronic hyperarousal state, which could contribute to both nocturnal motor activity and diurnal hemodynamic rigidity. While one might interpret lower postprandial BP as a marker of preserved or even increased parasympathetic tone, the co-occurrence of poor sleep quality and elevated insomnia severity supports the view of a maladaptive or dysregulated response rather than a protective physiological adaptation. The lack of differences in daytime sleepiness suggests that these alterations occur despite preserved subjective daytime alertness.

The biphasic BP response observed in individuals with morning AB may represent a more labile autonomic profile, potentially driven by psychological hyperarousal or stress anticipation.[17] Individuals with evening AB, in turn, showed a rigid BP profile, which may be associated with decreased autonomic adaptability, a phenomenon observed in both late chronotypes and individuals at higher risk for hypertension and cardiometabolic dysfunction.[18] However, these mechanistic hypotheses, though plausible, remain speculative due to the absence of objective autonomic or endocrine measures. As such, these interpretations should be framed cautiously and validated in future studies using physiological or neuroendocrine biomarkers.

Importantly, however, when comparing all participants with probable bruxism to those without, no significant differences in overall systolic or diastolic BP were found. This suggests that the presence of bruxism per se may not be associated with broad alterations in BP profiles. However, the distinct circadian BP patterns observed across bruxism phenotypes support the notion that behavioral timing and subtype may be more informative than the binary classification of bruxism presence or absence. These findings reinforce the need for more granular phenotyping in sleep and autonomic research.

The results of the present study support the notion that bruxism, far from being a purely local orofacial activity, may reflect broader autonomic and circadian

dysregulation. This is consistent with research reporting associations between bruxism, cardiovascular stress load and allostatic overload,[19] and aligns with evidence indicating that markers of systemic physiological rhythm, such as circadian and autonomic indicators, are increasingly relevant even in complex critical contexts.[20] This is not a diagnostic sign, but rather a signal of altered regulation worthy of further study. In fact, circadian clock disruption has been proposed as a contributor to cardiometabolic dysfunction in both acute and chronic settings,[21] while bruxism-related dysautonomia may represent an early behavioral signal of such disruption.[21,22] Beyond the autonomic nervous system, other key physiological systems involved in BP regulation, such as the renin–angiotensin–aldosterone system (RAAS), endothelial nitric oxide signaling, vascular reactivity, and low-grade inflammation, may contribute to the BP variation patterns observed across bruxism phenotypes. Although not directly measured herein, their relevance is well-established and supports a broader framework for interpreting our findings.[23] Additionally, external behavioral and environmental variables (e.g., caffeine intake, smoking, stress exposure, and light–dark cycle alignment) were not controlled for, potentially contributing to interindividual BP variability. This underscores the exploratory nature of the study, which is not confirmatory but hypothesis-generating.

Mechanistically, circadian clock gene dysregulation and sleep fragmentation, which may occur in patients with high-frequency SB, can disturb cardiometabolic status,[24] eventually affecting autonomic sensitivity and the expected diurnal BP decline.[25] These findings align with the broader literature on chronobiology and provide a relevant foundation for future investigations.

On the other hand, the multidimensional nature of bruxism, including its potential origins in central sensitization, social context and biopsychosocial vulnerability, underscores the relevance of integrated phenotyping approaches that go beyond surface-level behavioral classification. Notably, while self-reported bruxism may lack polysomnographic precision, its integration with validated questionnaires can provide valuable behavioral insight, particularly in populations with sleep complaints or risk factors, as demonstrated in large-scale survey studies.[6,26]

Apart from the cardiovascular and autonomic implications, bruxism phenotypes may also present with associated somatic symptoms, such as headaches, jaw muscle pain and dental clenching behaviors, which further reflect dysregulated neurosensory processing. Evidence indicates that individuals with SB often report tension-type or temporomandibular-related headaches, potentially mediated by sustained muscle hyperactivity or trigeminal sensitization.[27] Moreover, behavioral features such as diurnal clenching may signify an expression of stress-coping strategies or emotional dysregulation, consistent with findings linking bruxism to central sensitization and somatization syndromes.[28] These overlapping mechanisms confirm that bruxism is not solely a local motor event but may represent a systemic neurobehavioral condition influenced by affective, neuroendocrine and circadian factors.

From a clinical standpoint, recognizing bruxism phenotypes as potential early indicators of abnormal BP regulation could inform preventive strategies in sleep medicine and cardiovascular care. Patients presenting with bruxism, particularly SB and evening AB profiles, may benefit from additional screening for sleep fragmentation, stress exposure and circadian misalignment. Such integrative approaches could be further strengthened by considering the co-occurrence of bruxism and OSA, especially in light of emerging endotypic models and pathophysiological overlaps.[29] Still, bruxism may become a valuable behavioral marker in future models of cardiovascular risk assessment.

Limitations

This is a preliminary study with several limitations. The sample size was modest, particularly for subgroup analyses. Bruxism was not confirmed with polysomnography or electromyography. Additionally, we did not use 24-hour ambulatory BP monitoring, which limits the interpretation of full circadian variation and nocturnal dipping profiles. The cross-sectional design prevents causal inferences between bruxism phenotypes and BP alterations. Moreover, potential confounding factors such as perceived stress, anxiety, caffeine consumption, and lifestyle-related variables were not assessed. These are known to influence both bruxism expression and BP variability and may have impacted the observed associations. Future studies should control for these elements to better delineate the specific contribution of bruxism-related mechanisms.

Nevertheless, the standardized methodology, repeated BP measurements under controlled conditions and the use of validated sleep and chronotype questionnaires contribute to the robustness of our observations. On the other hand, although self-reported bruxism showed limited correlation with laboratory-based measurements of masticatory muscle activity, it should not be dismissed outright. Subjective complaints often reflect the patient's perceived burden and may capture broader psychophysiological disturbances, including stress sensitivity, arousal patterns and sleep fragmentation. These factors may be omitted when using objective indices alone. Thus, incorporating self-reported bruxism remains relevant, especially in the assessment of behavioral phenotypes with potential cardiovascular implications. These findings should serve as a basis for further investigations using larger samples, more standardized criteria[30] and objective autonomic measures.

To the best of our knowledge, this pilot study is one of the first to investigate behavioral phenotypes of bruxism as potential markers of altered circadian BP profiles in a healthy young population. By integrating a multidimensional assessment, including time-specific bruxism subtypes, structured BP measurements and validated sleep-related questionnaires, this research presents a novel perspective on how seemingly benign oral behaviors may reflect broader autonomic and chronobiological dysregulation. The findings support the growing paradigm that bruxism should be evaluated not only in dental or musculoskeletal contexts but also as a systemic indicator with potential cardiometabolic implications. When validated in larger samples using objective physiological markers, this approach could contribute to early screening and preventive strategies targeting at-risk individuals before the onset of overt cardiovascular disease.

Conclusions

Distinct circadian BP patterns across bruxism phenotypes may reflect subtle autonomic differences, even in healthy individuals. While these findings are exploratory and based on a limited sample without objective confirmation of bruxism, they raise the hypothesis that behavioral phenotypes may offer a novel window into circadian–cardiovascular interactions. These observations should be interpreted cautiously but may nonetheless support future research using larger cohorts and objective physiological methods to clarify whether self-reported bruxism can serve as an early marker of autonomic dysregulation and cardiovascular risk.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Coimbra, Portugal (reference No. CE-012/2019). All participants provided written informed consent prior to participation.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

AI-assisted tools were used for language editing and manuscript organization. The authors are responsible for all scientific content, interpretation and final approval.

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References

1. Verhoeff MC, Lobbezoo F, Ahlberg J, et al. Updating the bruxism definitions: Report of an International Consensus Meeting. *J Oral Rehabil*. 2025;52(9):1335–1342. doi:10.1111/joor.13985
2. Nashed A, Lanfranchi P, Rompré P, et al. Sleep bruxism is associated with a rise in arterial blood pressure. *Sleep*. 2012;35(4):529–536. doi:10.5665/sleep.1740
3. Ohlmann B, Bömicke W, Behnisch R, Rammelsberg P, Schmitter M. Variability of sleep bruxism-findings from consecutive nights of monitoring. *Clin Oral Investig*. 2022;26(4):3459–3466. doi:10.1007/s00784-021-04314-8
4. Meira E Cruz M, Winocur E, Gozal D, Lavigne GJ. Chronotype and bruxism: Should we look further and get it from the heart? *Cranio*. 2021;39(5):457–458. doi:10.1080/08869634.2021.1956786
5. Yano Y, Reis JP, Lewis CE, et al. Association of blood pressure patterns in young adulthood with cardiovascular disease and mortality in middle age. *JAMA Cardiol*. 2020;5(4):382–389. doi:10.1001/jamacardio.2019.5682
6. Emodi-Perlman A, Eli I, Morad D, Turchin A, Friedman-Rubin P. Sex differences in a self-report questionnaire related to obstructive sleep apnea: An online cross-sectional survey. *Dent Med Probl*. 2025;62(3):513–520. doi:10.17219/dmp/195345
7. Kulkarni S, Parati G, Bangalore S, et al. Blood pressure variability: A review. *J Hypertens*. 2025;43(6):929–938. doi:10.1097/HJH.0000000000003994
8. Winocur E, Uziel N, Lisha T, Goldsmith C, Eli I. Self-reported bruxism – associations with perceived stress, motivation for control, dental anxiety and gagging. *J Oral Rehabil*. 2011;38(1):3–11. doi:10.1111/j.1365-2842.2010.02118.x
9. Pintado MR, Anderson GC, DeLong R, Douglas WH. Variation in tooth wear in young adults over a two-year period. *J Prosthet Dent*. 1997;77(3):313–320. doi:10.1016/s0022-3913(97)70189-6
10. Stergiou GS, Palatini P, Parati G, et al.; European Society of Hypertension Council and the European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability. 2021 European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement. *J Hypertens*. 2021;39(7):1293–1302. doi:10.1097/HJH.0000000000002843
11. Rodrigues PFS, Vagos P, Pandeirada JNS, Marinho PI, Randler C, Silva CF. Initial psychometric characterization for the Portuguese version of the Morningness-Eveningness-Stability-Scale improved (MESSi). *Chronobiol Int*. 2018;35(11):1608–1618. doi:10.1080/07420528.2018.1495646
12. Del Rio João KA, Becker NB, de Neves Jesus S, Santos Martins RI. Validation of the Portuguese version of the Pittsburgh Sleep Quality Index (PSQI-PT). *Psychiatry Res*. 2017;247:225–229. doi:10.1016/j.psychres.2016.11.042
13. Clemente V, Ruivo Marques D, Miller-Mendes M, Morin CM, Serra J, Allen Gomes A. The European Portuguese version of the insomnia severity index. *J Sleep Res*. 2021;30(1):e13198. doi:10.1111/jsr.13198
14. Sargento P, Perea V, Ladera V, Lopes P, Oliveira J. The Epworth Sleepiness Scale in Portuguese adults: From classical measurement theory to Rasch model analysis. *Sleep Breath*. 2015;19(2):693–701. doi:10.1007/s11325-014-1078-6
15. Vaz AP, Drummond M, Mota PC, Severo M, Almeida J, Winck JC. Translation of Berlin Questionnaire to Portuguese language and its application in OSA identification in a sleep disordered breathing clinic. *Rev Port Pneumol*. 2011;17(2):59–65. PMID:21477567.
16. Silvani A. Physiological sleep-dependent changes in arterial blood pressure: Central autonomic commands and baroreflex control. *Clin Exp Pharmacol Physiol*. 2008;35(9):987–994. doi:10.1111/j.1440-1681.2008.04985.x
17. AlQatari AA, Alturki JA, Abdulali KA, et al. Changes in heart rate variability and baroreflex sensitivity during daytime naps. *Nat Sci Sleep*. 2020;12:661–669. doi:10.2147/NSS.S270191

18. Vondrasek JD, Alkahtani SA, Al-Hudaib AA, et al. Heart rate variability and chronotype in young adult men. *Healthcare (Basel)*. 2022;10(12):2465. doi:10.3390/healthcare10122465
19. Michalek-Zrabkowska M, Martynowicz H, Wieckiewicz M, Smardz J, Poreba R, Mazur G. Cardiovascular implications of sleep bruxism – a systematic review with narrative summary and future perspectives. *J Clin Med*. 2021;10(11):2245. doi:10.3390/jcm10112245
20. Jiménez-Pastor JM, Rodríguez-Cortés F, López-Soto P, López-Coleto L, Meira E Cruz M. Cardiorespiratory and circadian clock markers in intensive care unit patients. *Dent Med Probl*. 2024;61(5):797–801. doi:10.17219/dmp/191537
21. Martynowicz H, Dymczyk P, Dominiak M, et al. Evaluation of intensity of sleep bruxism in arterial hypertension. *J Clin Med*. 2018;7(10):327. doi:10.3390/jcm7100327
22. Martynowicz H, Wieckiewicz M, Poreba R, et al. The relationship between sleep bruxism intensity and renalase concentration – an enzyme involved in hypertension development. *J Clin Med*. 2020;9(1):16. doi:10.3390/jcm9010016
23. Mehta JK, Kaur G, Buttar HS, et al. Role of the renin–angiotensin system in the pathophysiology of coronary heart disease and heart failure: Diagnostic biomarkers and therapy with drugs and natural products. *Front Physiol*. 2023;14:1034170. doi:10.3389/fphys.2023.1034170
24. Meira E Cruz M, Acuña-Castroviejo D. Cardiometabolic impact of changing internal time during daylight saving time: A window for a deleterious role within sleep-related breathing disorders. *Intern Emerg Med*. 2018;13(8):1345–1346. doi:10.1007/s11739-018-1934-7
25. Meira E Cruz M, Guimarães AS. A window to consolidate the "functional hypothesis of bruxism" through cardiovascular autonomic link. *Oral Dis*. 2019;25(4):1237–1238. doi:10.1111/odi.13077
26. Ekman A, Tiisanoja A, Näpänkangas R, Sipilä K. Association of health-related factors with self-reported sleep and awake bruxism in Northern Finland Birth Cohort 1966 – a cross-sectional study. *Cranio*. 2025;43(3):510–520. doi:10.1080/08869634.2023.2198462
27. Dibello V, Lozupone M, Sardone R, et al. Temporomandibular disorders as contributors to primary headaches: A systematic review. *J Oral Facial Pain Headache*. 2023;37(2):91–100. doi:10.11607/ofph.3345
28. Câmara-Souza MB, Carvalho AG, Figueredo OMC, Bracci A, Manfredini D, Rodrigues Garcia RCM. Awake bruxism frequency and psychosocial factors in college preparatory students. *Cranio*. 2023;41(2):178–184. doi:10.1080/08869634.2020.1829289
29. Meira e Cruz M. A novel method to identify endotypes and risk factors related to co-occurring obstructive sleep apnea and sleep bruxism. *Sleep*. 2026;49(1):zsaf238. doi:10.1093/sleep/zsaf238
30. Thymi M, Farzan A, Ahlberg J, Manfredini D, Lobbezoo F. Qualitative suggestions for the further development of the Standardized Tool for the Assessment of Bruxism (STAB). *Dent Med Probl*. 2024;61(3):323–333. doi:10.17219/dmp/183692

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