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Oncogenic viruses in the genomic era: Integration, instability, and cancer evolution

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Keywords: HBV, EBV, oncogenic viruses, instability, cancer evolution

Emerging evidence suggests that viral integration into non-coding and genomically vulnerable regions may represent one of several mechanisms contributing to oncogenesis, warranting further investigation through long-read whole-genome sequencing (WGS).

Oncogenic viruses are no longer viewed merely as isolated infectious causes of cancer, but as context-dependent drivers and cofactors that shape tumor evolution through viral persistence, host genome integration, genomic instability, and the disruption of coding and non-coding regulatory landscapes. This modern understanding, however, emerged from a much simpler early idea: that cancer might, at least in some cases, be caused by a transmissible biological agent.

At the beginning of the 20th century, cancer lacked a unifying causal framework. In 1911, Peyton Rous demonstrated that a transmissible agent, Rous sarcoma virus (RSV), could induce tumors in chickens, providing the first experimental evidence that cancer could arise from a defined transmissible biological agent.¹

This hypothesis gained momentum in the mid-20th century with advances in virology and molecular biology. Oncogenic viruses were progressively identified, beginning with the discovery of Epstein–Barr virus (EBV) in Burkitt lymphoma cells in 1964, followed by the demonstration of its ability to immortalize human lymphocytes in 1967, and the recognition of hepatitis B virus (HBV) as the causative agent of serum hepatitis in 1965.² Over time, several human tumor viruses were firmly established, including human herpesvirus 8 (HHV-8; Kaposi sarcoma), human papillomavirus (HPV; cervical and other anogenital cancers), HBV and hepatitis C virus (HCV) (hepatocellular carcinoma), EBV (Burkitt lymphoma, nasopharyngeal carcinoma, and selected lymphomas), and human T-cell lymphotropic virus 1 (adult T-cell leukemia/lymphoma).^{3–6} Collectively, these oncogenic viruses are now estimated to account for 10–15% of cancers worldwide.^{2,7}

These discoveries translated into tangible clinical benefits – prophylactic vaccines against HPV and HBV became the first effective tools for cancer prevention. Their impact is particularly evident for HPV-associated malignancies. A recent global analysis of cervical, penile, vulvar, vaginal, laryngeal, oropharyngeal, oral, and anal cancers estimated 831,204 HPV-attributable cancer cases and 422,935 deaths in 2022.⁸ In oral oncogenesis, however, the role of HPV appears more heterogeneous than in cervical or oropharyngeal cancer.

Human papillomavirus has been detected by genetic testing in 0.0–74.5% of oral squamous cell carcinoma (OSCC) cases, with a weighted mean odds ratio of 17.1 for the detection of HPV-16 or HPV-18 in patients with OSCC.⁵

However, the early expectation that cancer might be explained by a single viral cause gradually gave way to a more complex model. Most cancers arise through the accumulation of genetic alterations driven by defective DNA repair and environmental exposures. Viruses remain important, but as components of a broader oncogenic landscape, acting as initiators, cofactors or catalysts rather than universal drivers. This broader cofactor-based view is supported by systematic evidence from colorectal cancer, where gut microbiota dysbiosis may contribute to carcinogenesis through chronic inflammation, carcinogenic metabolites and altered host immune responses.⁹

Today, in the era of genomics, attention has returned to the ways in which viral DNA integrates into the host genome: which chromatin contexts are permissive; how integration sites intersect with genomic instability; and how defects in DNA repair pathways influence these processes. The original search for a singular cause has thus evolved into a deeper insight – viruses are not the universal explanation for cancer, but they provide a powerful lens through which the mechanisms of oncogenesis can be understood.

Among human oncogenic viruses, EBV has emerged as a paradigmatic model linking viral persistence to malignant transformation. Epstein–Barr virus infects more than 90% of the global population and establishes lifelong latency, yet only a small fraction of infected individuals develop malignancy, underscoring the importance of host, environmental and genomic modifiers.¹⁰ Epstein–Barr virus is etiologically associated with a broad spectrum of lymphoid and epithelial malignancies, including Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, and EBV-associated gastric carcinoma, collectively accounting for approx. 1% of all cancers worldwide.¹¹ Despite this well-established association, the mechanisms by which EBV contributes to oncogenesis remain incompletely understood and likely extend beyond a single unifying pathway.¹⁰

Traditionally, EBV-driven tumorigenesis has been attributed to latent infection programs, in which viral gene products, such as EBNA1, LMP1 and LMP2, modulate host signaling pathways, promote cell survival and induce epigenetic reprogramming.¹² However, accumulating evidence suggests that viral–host genome interactions represent an additional, yet underappreciated, layer of oncogenic regulation.

Although EBV genomes are typically maintained as episomes, integration into the host genome has been repeatedly documented in both lymphoid and epithelial malignancies, where it may disrupt gene expression, alter chromatin architecture and contribute to genomic instability.¹¹ Notably, integration events have been identified near oncogenes such as *REL* and *BCL11A* in Burkitt

lymphoma, suggesting a potential role in the deregulation of key oncogenic pathways.¹³

Moreover, EBV integration has been linked to regions of chromosomal fragility and instability, raising the possibility that viral insertion may both exploit and exacerbate vulnerable genomic contexts.¹⁴ These observations are consistent with emerging genomic evidence showing that integration sites are enriched in regions prone to DNA damage and microhomology-mediated repair, implicating defective DNA repair pathways as key determinants of viral insertion and its oncogenic consequences.¹¹

Intriguingly, the “hit-and-run” hypothesis further expands this framework by proposing that EBV may initiate oncogenic transformation through transient genomic or epigenetic alterations, even if viral genomes are subsequently lost from tumor cells.¹⁰

Building on this framework, recent advances in high-throughput genomic technologies are beginning to redefine our understanding of viral–host interactions in cancer. Conventional approaches, largely based on targeted sequencing or short-read platforms, have provided important insights into viral presence and integration, but remain limited in resolving complex structural events, repetitive regions and deeply intronic insertions. In contrast, whole-genome sequencing (WGS), particularly when incorporating long-read sequencing technologies, now enables a more comprehensive and unbiased interrogation of the cancer genome, including previously inaccessible genomic regions.

These technologies are particularly relevant in the context of EBV, where integration events are relatively rare, heterogeneous, and frequently located outside protein-coding regions. Emerging data indicate that viral DNA often integrates within intronic or intergenic regions, including loci harboring long non-coding RNAs (lncRNAs), regulatory elements, or chromatin domains involved in transcriptional control. Such events may not directly disrupt protein-coding genes, but can nonetheless alter transcriptional programs through enhancer hijacking, epigenetic remodeling, or the modulation of three-dimensional (3D) chromatin architecture.

Our recent work provides an illustrative example of this paradigm.¹⁵ Using high-resolution genomic approaches in lymphomas arising in the context of Nijmegen breakage syndrome, we identified multiple EBV-associated genomic alterations within non-coding regions, including 3 distinct loci characterized by deep intronic integration signals. Notably, these regions were enriched for lncRNA elements, suggesting that EBV may preferentially target regulatory genomic compartments rather than canonical oncogenes.¹⁵ This observation aligns with a broader shift in cancer genomics, in which non-coding regions are increasingly recognized as critical drivers of oncogenesis.

Importantly, similar patterns have been observed beyond EBV-associated malignancies. In hepatocellular carcinoma, HBV integration has been reported within non-coding

regions, including loci associated with regulatory RNAs, where it may influence gene expression and promote tumorigenesis through cis-regulatory effects.¹⁶ These parallels suggest that viral integration into the non-coding genome may represent a conserved oncogenic mechanism across distinct virus–tumor systems.

Taken together, the integration landscape revealed by modern genomic technologies supports a model in which viral insertion is not merely a stochastic event, but rather one shaped by chromatin accessibility, DNA repair dynamics, and higher-order genome organization. By enabling the precise mapping of integration breakpoints and their genomic context, long-read WGS provides critical tools to dissect these processes at unprecedented resolution. Ultimately, this integrative approach may bridge the gap between viral infection and malignant transformation, offering new insights into how alterations within the non-coding genome contribute to cancer development.

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References

1. Rous P. A sarcoma of the fowl transmissible by an agent separable from the tumor cells. *J Exp Med*. 1911;13(4):397–411. doi:10.1084/jem.13.4.397
2. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer*. 2010;10(12):878–679. doi:10.1038/nrc2961
3. Mortazavi H, Rahbani Nobar B, Shafiei S, Ahmadi N. Drug-related cancers: Analyses of head and neck cases reported in the literature. *Dent Med Probl*. 2021;58(2):267–280. doi:10.17219/dmp/131116
4. Duś-Illicka 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
5. Rąpalska I, Chęciński M, Kaczmarzyk T. Impact of human papillomavirus (HPV) infection on the development of oral squamous cell carcinoma (OSCC): A systematic review. *Dent Med Probl*. 2025;62(6):1211–1221. doi:10.17219/dmp/186354
6. Xiao Q, Liu Y, Li T, et al. Viral oncogenesis in cancer: From mechanisms to therapeutics. *Signal Transduct Target Ther*. 2025;10(1):151. doi:10.1038/s41392-025-02197-9
7. GBD 2023 Cancer Collaborators. The global, regional, and national burden of cancer, 1990–2023, with forecasts to 2050: A systematic analysis for the Global Burden of Disease Study 2023. *Lancet*. 2025;406(10512):1565–1586. doi:10.1016/S0140-6736(25)01635-6
8. Zhang J, Ke Y, Chen C, et al. HPV cancer burden by anatomical site, country, and region in 2022. *Sci Rep*. 2025;15(1):21048. doi:10.1038/s41598-025-06700-8
9. Minervini G, Shivakumar S, Ronsiville V, Franco R, Cicciù M, Marrapodi MM. Microbiological aspects of cancer progression: A systematic review conducted according to the PRISMA 2020 guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. *Dent Med Probl*. 2024;61(5):739–746. doi:10.17219/dmp/183712
10. Mangiaterra T, Chabay P, Bellan C, et al. Hit-and-run hypothesis: Revisiting the impact of EBV in malignancies. *Front Immunol*. 2026;17:1742214. doi:10.3389/fimmu.2026.1742214
11. Xu M, Zhang WL, Zhu Q, et al. Genome-wide profiling of Epstein–Barr virus integration by targeted sequencing in Epstein–Barr virus associated malignancies. *Theranostics*. 2019;9(4):1115–1124. doi:10.7150/thno.29622
12. Xie M, Yao D, Lei L, Tang C, Mo B. Epstein–Barr virus-driven molecular pathogenesis of primary pulmonary lymphoepithelial carcinoma. *Front Oncol*. 2025;15:1630415. doi:10.3389/fonc.2025.1630415
13. Luo WJ, Takakuwa T, Ham MF, et al. Epstein–Barr virus is integrated between *REL* and *BCL-11A* in American Burkitt lymphoma cell line (NAB-2). *Lab Invest*. 2004;84(9):1193–1199. doi:10.1038/labinvest.3700152
14. Jox A, Rohen C, Belge G, et al. Integration of Epstein–Barr virus in Burkitt's lymphoma cells leads to a region of enhanced chromosome instability. *Ann Oncol*. 1997;8(Suppl 2):131–135. PMID:9209656.
15. Pastorczak A, Szmyd B, Braun M, et al. Clinical and laboratory diversity of diffuse large B-cell lymphomas in children with Nijmegen breakage syndrome. *Haematologica*. 2023;108(10):2808–2813. doi:10.3324/haematol.2022.282325
16. Li M, Du M, Cong H, et al. Characterization of hepatitis B virus DNA integration patterns in intrahepatic cholangiocarcinoma. *Hepatol Res*. 2021;51(1):102–115. doi:10.1111/hepr.13580

Could this be about the clocks? Rethinking insomnia, sleep bruxism and circadian misalignment

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Insomnia symptoms reported in sleep bruxism may partly reflect circadian misalignment rather than insomnia disorder per se, highlighting the need to integrate chronobiological assessment into both bruxism research and clinical practice.

Introduction

There are moments in science when a familiar association, long accepted, repeatedly described, almost taken for granted, suddenly reveals an unexpected contour. It often happens quietly – not in the results section of a paper, but in the unease that arises while reading it, in the intuition that something important may be missing from the framework we habitually use. It was precisely this feeling that accompanied me as I examined a recent study exploring the relationship between insomnia symptoms and probable sleep bruxism.¹ The findings were sound and clinically relevant, yet one question persisted, like a faint rhythmic pulse beneath the explicit conclusions: perhaps our interpretation is too firmly anchored in insomnia, while overlooking a more fundamental temporal dimension that shapes both sleep quality and nocturnal motor activity. From this unease emerged a single, deceptively simple possibility – one that may broaden the conventional interpretation of such findings. Could it be that what we call insomnia in this context is, at least in part, the consequence of living out of synchrony with our biological time?

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Bruxism as a temporally structured phenomenon

Sleep bruxism is not temporally random. It is currently defined as a centrally mediated masticatory muscle activity during sleep, conceptualized as a behavior rather than a disease entity, and characterized by rhythmic or non-rhythmic activity, in accordance with the most recent international consensus on bruxism.² Its distribution across the night follows recognizable patterns that mirror key features of sleep architecture and autonomic regulation. Bruxism episodes cluster preferentially in the first half of the sleep period, occur around sleep-stage transitions, and are preceded by

characteristic fluctuations in cortical and autonomic activity.³ These observations are commonly interpreted within the frameworks centered on arousal mechanisms, emotional processing and sleep instability.² Yet the consistency of these temporal peaks also points to broader rhythmicity, suggesting that bruxism is shaped not only by micro-arousal dynamics, but potentially also by circadian modulation. The circadian system regulates sympathetic tone, parasympathetic balance, motor readiness, and arousal thresholds. Together, these processes define periods of greater or lesser vulnerability to motor events during sleep. If bruxism were merely a peripheral or mechanical phenomenon, one would not expect its occurrence to conform so consistently to 24-hour rhythmic patterns. The fact that it does invites us to consider that sleep bruxism may emerge at the interface between sleep regulation and circadian timing.

Insomnia or misalignment? A conceptual shift

In the study under discussion, patients with probable sleep bruxism reported insomnia symptoms, a finding that the authors rightly interpret as reflecting psychological burden, sleep fragmentation, and possibly hyperarousal.¹ However, insomnia symptoms – particularly difficulties initiating sleep, non-restorative sleep and daytime dysfunction – are phenomenologically nonspecific and frequently overlap with those observed in circadian rhythm sleep–wake disorders. Delayed sleep–wake phase disorder (DSWPD) is especially relevant in this context, as it often presents with a remarkably similar clinical picture while arising from a fundamentally different mechanism, i.e., the inability to fall asleep at a desired or socially imposed time due to a biologically delayed circadian phase.³ Individuals with DSWPD often report prolonged sleep latency on work or school nights, yet experience deep and restorative sleep when allowed to follow their natural schedule. The resulting fatigue and cognitive impairment reflect not intrinsic insomnia, but the consequences of chronically mistimed sleep. This pattern is particularly common among younger individuals,⁴ a population that is also overrepresented in studies on sleep bruxism. Consequently, the insomnia signal detected in the study may partly reflect circadian misalignment rather than insomnia disorder as defined by the International Classification of Sleep Disorders, Third Edition (ICSD-3). If so, sleep bruxism may be interacting with, rather than causing or resulting from, a misaligned biological clock, giving rise to a clinical presentation that resembles insomnia while emerging from a different upstream mechanism. Asking whether circadian misalignment is the true underlying driver fundamentally reframes the interpretation.

Arousal, autonomic activation, and the role of the clock

Bruxism is tightly linked to autonomic fluctuations. Micro-arousals, increases in the heart rate, sympathetic surges, changes in respiratory stability, and cortical micro-deactivations precede or accompany bruxism episodes. All of these physiological processes are themselves modulated by the circadian phase. The circadian system regulates arousal thresholds, cardiovascular variability, stress responsivity, and the propensity for sleep fragmentation. If the biological night is shifted later, the sleep obtained at an earlier, socially imposed time becomes more unstable, more fragile, and more susceptible to arousal. This instability increases the likelihood of motor events, particularly those emerging during transitional non-rapid eye movement (NREM) sleep, when arousability is highest. Within this framework, bruxism and insomnia-like complaints may both arise as downstream consequences of a single upstream disturbance, namely sleeping “out of phase”. Circadian misalignment may promote sleep instability, autonomic activation, and ultimately the expression of sleep bruxism. The proposed sequence can be summarized as follows: circadian misalignment → unstable sleep → increased arousals → increased bruxism → perceived poor sleep → self-reported “insomnia” (Fig. 1). Although circadian variables were not assessed in the studies included in the systematic review, this model is compatible with the reported findings, and offers an integrated explanation for why bruxism and insomnia symptoms co-occur so consistently, at times beyond what psychological variables alone would predict.

Why the distinction matters clinically

Distinguishing insomnia from circadian misalignment is not merely a theoretical exercise. It has direct implications for treatment selection, prognosis, and patient education. Treating DSWPD with cognitive behavioral therapy for insomnia (CBT-I) may provide partial relief, but often fails to address the underlying mechanism and, in some cases, may inadvertently reinforce circadian misalignment. Conversely, interventions targeting the circadian phase, such as morning bright light exposure, evening light restriction, appropriately timed melatonin, consistent wake schedules, and better alignment of social obligations with biological time, can substantially improve sleep continuity, reduce perceived insomnia and stabilize autonomic function. Importantly, if sleep bruxism is amplified by unstable or mistimed sleep, circadian realignment may reduce its frequency or intensity, even when the intervention does not target bruxism directly.⁵ In this sense, sleep bruxism may represent a marker of circadian vulnerability or instability rather than a condition driven solely by stress, hyperarousal or mechanical factors.

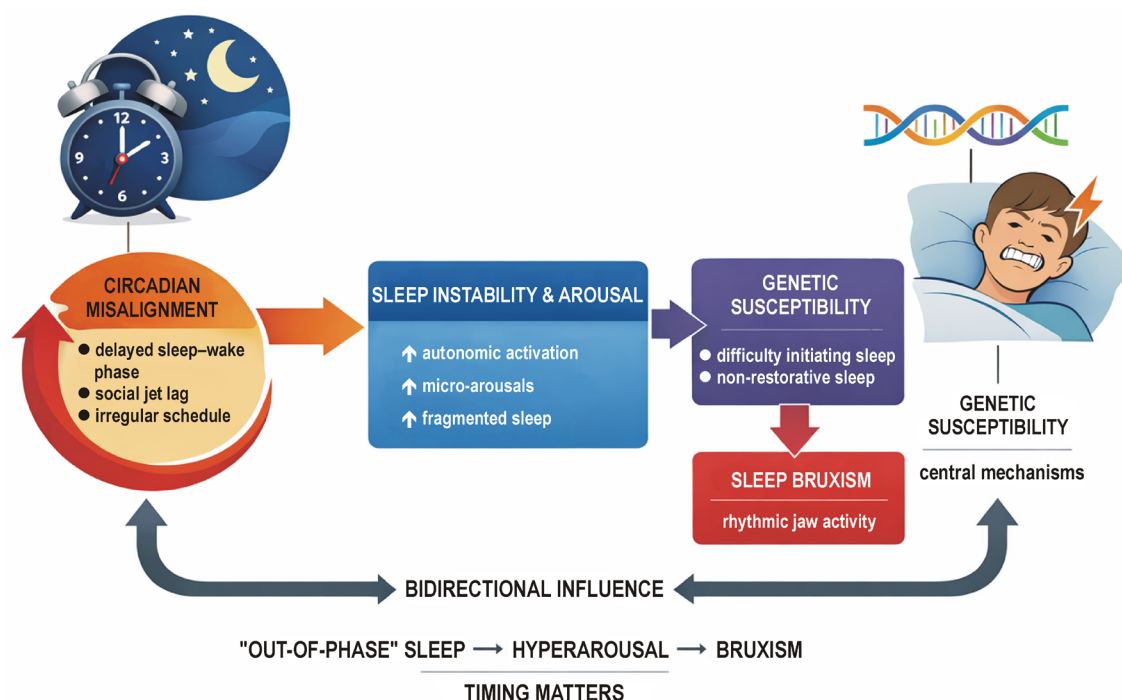


Fig. 1. Conceptual framework linking circadian misalignment, autonomic activation and sleep bruxism

This figure illustrates a model in which sleep bruxism, conceptualized as a centrally mediated behavior with a genetic predisposition, is modulated by circadian timing and chronotype. Circadian misalignment, particularly delayed sleep–wake phase and social jet lag, promotes sleep instability and increases the frequency of micro-arousals, leading to heightened autonomic activation. These processes facilitate the expression of sleep bruxism and may give rise to insomnia-like symptoms (e.g., difficulty initiating sleep and non-restorative sleep) that reflect biological mistiming rather than insomnia disorder per se.

Interpreting insomnia symptoms without assessing circadian parameters therefore carries a risk of misclassifying patients and selecting suboptimal interventions. Recent clinical studies further support this perspective, demonstrating that the management of both insomnia and sleep bruxism benefits from precise phenotyping and individualized therapeutic approaches. Emerging evidence suggests that interventions integrating behavioral, sleep-focused and chronobiological components may be more effective than symptom-based treatment alone, particularly in patients exhibiting heightened arousal and increased motor activity during sleep.^{6,7}

The methodological gap: Missing circadian variables

The systematic review by Machado et al. provides valuable insights, but does not include measures of chronotype, sleep timing, social jet lag, actigraphy-derived sleep parameters, or biological circadian phase markers, such as dim-light melatonin onset (DLMO).¹ Without these assessments, distinguishing true insomnia disorder from circadian misalignment remains difficult.⁵ Given the high prevalence of delayed sleep–wake phase tendencies in the age groups commonly represented in bruxism research, the absence of circadian assessments may lead to an overestimation of insomnia prevalence and an underrecognition

of circadian contributors. Incorporating even simple chronobiological tools, such as the Morningness–Eveningness Questionnaire (MEQ) or the Munich Chronotype Questionnaire (MCTQ), would enable future studies to better distinguish circadian misalignment from hyperarousal. Actigraphy could further strengthen this approach by providing objective measures of sleep timing and regularity, and phase variability. Together, these assessments could determine whether the insomnia-like symptoms observed in bruxism cohorts primarily reflect circadian mistiming rather than intrinsic difficulties with sleep initiation or maintenance.⁸ Such a distinction would not only refine diagnostic interpretation, but also deepen our understanding of the mechanisms underlying sleep bruxism.

Bruxism as a marker of systemic desynchrony

If circadian misalignment contributes to hyperarousability, autonomic dysregulation and sleep fragmentation, then sleep bruxism might represent an early physiological marker of systemic desynchronization, with potentially important clinical implications.⁵ Many of the processes implicated in sleep bruxism, such as stress reactivity, emotional regulation, cardiovascular variability, and muscle tone dynamics, are strongly influenced by circadian timing. When the circadian system is out of synchrony with

behavioral schedules, these physiological processes may interact in unpredictable or unstable ways, creating conditions that favor nocturnal motor events. Within this framework, sleep bruxism may be viewed not as an isolated disorder, but as a manifestation of broader physiological dysregulation. Such interpretation is consistent with recent reconceptualizations of sleep bruxism as a centrally mediated, multifactorial behavior rather than a strictly peripheral jaw-related condition. This perspective also opens new avenues for research by encouraging the study of sleep bruxism through a chronobiological lens, integrating sleep timing, the circadian phase, autonomic balance, and environmental zeitgebers. Importantly, sleep bruxism is increasingly recognized as a centrally regulated behavior with a significant genetic contribution. Neurophysiological and genetic studies suggest that individual susceptibility is partly determined by central arousal networks and heritable traits influencing autonomic responsiveness and sleep–wake regulation.⁹ This central and genetic framework provides a plausible biological substrate through which circadian misalignment may amplify the expression of sleep bruxism, rather than acting as an isolated peripheral trigger.¹⁰

Returning to the central question

Considering all these factors, it becomes increasingly plausible that the insomnia symptoms observed in the abovementioned study may reflect circadian misalignment in at least a subset of participants. If so, the associated findings on sleep bruxism acquire a different interpretation – one rooted less in insomnia per se than in the destabilizing effects of sleeping at a biologically inappropriate time. A similar conceptual challenge has emerged in obstructive sleep apnea (OSA) research, particularly in the context of comorbid insomnia and sleep apnea (COMISA), where circadian misalignment is increasingly recognized as a modifier of symptom expression, disease burden and treatment response. In these patients, insomnia complaints frequently coexist with delayed or irregular sleep timing, and growing evidence suggests that at least some reported “insomnia” may reflect circadian mistiming rather than primary insomnia disorder. This parallel reinforces the broader importance of circadian phenotyping across sleep-related conditions and supports the notion that biological timing may shape symptom expression beyond disorder-specific mechanisms.^{11,12}

The question that inspired this reflection therefore returns with greater force. Could the observed association be an artefact of timing rather than the expression of insomnia? Could aligning the circadian clock, rather than focusing exclusively on sleep, provide a more effective strategy for reducing both insomnia complaints and sleep bruxism severity? These questions lie beyond the scope of the original study, but they may help shape future research directions.

Conclusion

By integrating chronobiological assessment into future research, we may uncover mechanisms that remain invisible yet clinically decisive. Bruxism, insomnia complaints and sleep fragmentation may represent different manifestations of a deeper disturbance in biological timing. From this perspective, the interplay between bruxism and insomnia is not merely a coexistence of two diagnoses, but a potential consequence of a misaligned circadian system. Exploring this hypothesis may reveal novel therapeutic pathways and provide a more nuanced understanding of how nocturnal motor activity intersects with the fundamental temporal organization of human physiology.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Machado E, Knorst JK, Zanatta FB, Iglesias TP, Dal Fabbro C, Poyares D. Is insomnia linked to sleep bruxism in adults? A systematic review and meta-analysis. *J Oral Rehabil.* 2026;53(1):257–264. doi:10.1111/joor.70068
2. 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
3. Lavigne GJ, Kato T, Kolta A, Sessle BJ. Neurobiological mechanisms involved in sleep bruxism. *Crit Rev Oral Biol Med.* 2003;14(1):30–46. doi:10.1177/154411130301400104
4. Gloston GF, Ward KC, Rodriguez-Torres GC, Gamble KL, Thomas SJ. Integrating assessment of circadian rhythmicity to improve treatment outcomes for circadian rhythm sleep–wake disorders: Updates on new treatments. *Curr Sleep Med Rep.* 2025;11(1):8. doi:10.1007/s40675-025-00325-z
5. Meira e Cruz M, Pereira S, Rodrigues MJ, Gozal D. Altered circadian patterns of diurnal blood pressure in clinical phenotypes of bruxism: A pilot study on autonomic imbalance in healthy young adults. *Dent Med Probl.* 2026;63(2):533–539. doi:10.17219/dmp/211745
6. Więckiewicz M, Smardz J, Martynowicz H. Lifestyle, daily habits, sleep hygiene, and diet: Proposal of a new approach for sleep bruxism management. *Dent Med Probl.* 2025;62(1):5–7. doi:10.17219/dmp/191517
7. Meira e Cruz M, Parreira E, Attarian H. Headache phenotypes in insomnia, obstructive sleep apnea, and COMISA: Impact on diagnosis and therapy. *Dent Med Probl.* 2025;62(6):1009–1012. doi:10.17219/dmp/207666
8. Meira e Cruz M. Clinical relevance of circadian clocks within the orofacial scope of sleep medicine. Implications for diagnosis, therapeutic and prophylactic measures. *Sleep Med.* 2026;138:108683. doi:10.1016/j.sleep.2025.108683

9. Wieckiewicz M, Bogunia-Kubik K, Mazur G, et al. Genetic basis of sleep bruxism and sleep apnea – response to a medical puzzle. *Sci Rep.* 2020;10(1):7497. doi:10.1038/s41598-020-64615-y
10. Smardz J, Martynowicz H, Wojakowska A, et al. Lower serotonin levels in severe sleep bruxism and its association with sleep, heart rate, and body mass index. *J Oral Rehabil.* 2022;49(4):422–429. doi:10.1111/joor.13295
11. Karuga FF, Jaromirska J, Sochal M, Białasiewicz P, Gabryelska A. Association between glucose metabolism, the circadian cycle and hypoxia: Evaluation of the NPAS2 and Rev-Erb- α protein serum levels in obstructive sleep apnea patients – a pilot study. *Dent Med Probl.* 2024;61(3):465–469. doi:10.17219/dmp/185718
12. Gabryelska A, Turkiewicz S, Gajewski A, et al. Investigating the link between circadian clock gene expressions, chronotype, insomnia, and daytime sleepiness in patients with obstructive sleep apnea. *Int J Mol Sci.* 2024;25(16):9062. doi:10.3390/ijms25169062

Relation of the body mass index to salivary cortisol and periodontal indices in children: A comparative analysis

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Abstract

Background. Obesity is a chronic disease resulting from the interaction of genetic, environmental, socio-economic, and behavioral factors, and is associated with the development of numerous comorbidities. Its prevalence is increasing worldwide, making obesity one of the most significant public health challenges.

Objectives. The aim of the present study was to investigate the relationship between obesity and the cortisol levels and periodontal status in children.

Material and methods. Ninety eligible children aged 8–12 years were enrolled in the study and allocated into 3 groups: normal-weight; overweight; and obesity. After the parents completed a demographic questionnaire, the children underwent periodontal examinations and saliva collection. The salivary cortisol levels were measured using the ELISA method. Data were analyzed using ANOVA, Pearson's correlation test, the χ^2 test, linear regression, and the Kruskal–Wallis test.

Results. The children's body mass index (BMI) was significantly associated with the salivary cortisol levels and the gingival index (GI), bleeding on probing (BoP) and periodontal probing depth (PPD) ($p = 0.010$, $p < 0.001$, $p < 0.001$, and $p = 0.005$, respectively). However, BMI was not significantly associated with father's education, mother's education, the parental smoking addiction status, total family income, the frequency of mouthwash use per week, toothbrushing frequency per day, or daily study duration ($p = 0.599$, $p = 0.143$, $p = 0.480$, $p = 0.875$, $p = 0.884$, $p = 0.203$, and $p = 0.338$, respectively). The salivary cortisol levels were also significantly associated with age and GI, BOP and PPD ($p = 0.010$, $p = 0.001$, $p = 0.018$, and $p = 0.016$, respectively). In the multivariable linear regression analysis, the salivary cortisol levels remained significantly associated with age ($\beta = 0.276$; $p = 0.004$), obesity ($\beta = 0.269$; $p = 0.006$), father's education level ($\beta = 0.393$; $p < 0.001$), and the parental smoking addiction status ($\beta = 0.238$; $p = 0.026$).

Conclusions. The salivary cortisol levels and periodontal indices were higher in overweight and obese children. Therefore, encouraging children and adolescents to adopt a healthier lifestyle and achieve weight reduction may help reduce the salivary cortisol levels and improve periodontal health.

Keywords: body mass index, periodontal pocket, cortisol, gingival index, periodontal indices

Cite as

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Highlights

- Childhood obesity was associated with higher salivary cortisol levels and a poorer periodontal status.
- Higher salivary cortisol levels were significantly associated with increased periodontal inflammation.
- Weight management may contribute to improved periodontal health and help reduce physiological stress in children.

Introduction

Obesity refers to the abnormal or excessive accumulation of adipose tissue in the body.¹ It is considered not only an esthetic concern, but also a chronic disease resulting from the complex interaction of genetic, environmental, socioeconomic, and behavioral factors.² It has been suggested that the genes associated with sweet and bitter taste perception may influence both obesity and oral health.³ In recent years, obesity has replaced smoking as one of the most important lifestyle-related risk factors for premature death because of its association with numerous health problems and chronic diseases.^{1,4}

According to the World Health Organization (WHO), more than 340 million children and adolescents aged 5–19 years were overweight or obese in 2016.⁴ Akbari and Mohammadi reported an overall obesity prevalence of 11.4% among Iranian children.⁵ The diagnosis of obesity in children is usually based on the calculation of the body mass index (BMI). Although BMI has limitations as a direct measure of body fat, it remains the most widely used and practical method for diagnosing obesity in children.⁶

Cortisol is commonly known as the body's stress hormone.⁷ Studies have demonstrated alterations in cortisol secretion, function and metabolism in individuals with obesity.⁸ Subclinical hypercortisolism increases the risk of osteoporotic fractures, hyperglycemia, hypertension, dyslipidemia, and obesity.^{9,10} In addition, hypercortisolism may promote insulin resistance, thereby perpetuating a vicious cycle.¹¹

However, the available evidence is conflicting. Several studies have reported that the mean salivary and serum cortisol levels are lower during the daytime in children with obesity.^{12–14} In contrast, other studies have demonstrated a positive association between the cortisol levels and BMI in children.^{15–17} Similarly, conflicting findings have been reported regarding the relationship between the serum cortisol levels and BMI in adults.^{18–20}

Severe periodontal disease is the 11th most prevalent disease worldwide.²¹ It often begins as gingivitis, which is characterized by gingival bleeding, swelling and pain. If left untreated, gingivitis may progress to periodontitis, resulting in the loss of periodontal attachment, alveolar bone, and ultimately teeth, thereby impairing mastication, esthetics, self-confidence, and quality of life.²²

Concern about periodontal disease lies not only in its high prevalence, but also in its well-established association

with several systemic conditions, including cardiovascular disease, diabetes mellitus, and adverse pregnancy outcomes, such as low birth weight.^{23–25} Perinatal exposures and pregnancy outcomes have also been associated with an increased predisposition to obesity.²⁶

The association between obesity and inflammatory processes has long been recognized.²⁷ Obesity may be considered a risk factor for periodontal disease, as it alters immune function and promotes a chronic low-grade inflammatory state associated with excess adipose tissue.²⁸ Although numerous studies have investigated periodontal health in adults with obesity,^{29,30} only limited studies have been conducted in children and adolescents, and these suggest a possible association between obesity and periodontal health.^{2,28,31} Given the increasing prevalence of overweight and obesity among children, understanding the early periodontal changes associated with obesity is of considerable importance.^{32,33}

Considering the insufficient number of studies and the conflicting findings regarding the relationship between obesity and periodontal health in children and adolescents, further research in this field is warranted. Therefore, the aim of the present study was to investigate the relationship between overweight and obesity, the salivary cortisol levels, and the periodontal status in children.

Material and methods

Study design and participants

This cross-sectional comparative descriptive study was conducted from 2021 to 2022 in 3 downtown schools in Qom, Iran. The study protocol was developed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

According to a study by Putignano et al., considering the average salivary cortisol levels in 2 groups of individuals with obesity and normal weight, with an alpha level of 5% and a beta level of 10%, the required sample size was calculated as 30 participants per group.³⁴ Therefore, a total of 90 participants were included in this study, using the non-random convenience sampling method.

The inclusion criteria were children aged 8–12 years with total dmft and DMFT (decayed, missing and filled teeth in primary and permanent dentition, respectively) scores <3.

The exclusion criteria comprised a history of any systemic disease (such as epilepsy, diabetes, hypothyroidism, cancer, psychological disorders, precocious puberty, or short stature), severe periodontitis, the use of any medication, a history of surgery, trauma, or other stressful events within the previous 3 months, participation in professional sports activities, living with only one parent (due to parental divorce or death), saliva sampling during the menstrual period in female participants, and resistance or the lack of cooperation during saliva collection and clinical examinations.

After obtaining informed consent from the children's parents, eligible children were enrolled in the study and equally divided into 3 groups: 30 children with normal weight; 30 children with overweight; and 30 children with obesity. The groups were matched based on age and sex. The participants were also divided into 2 groups according to sex, including 45 males and 45 females. Each sex group consisted of 15 children with obesity, 15 children with overweight and 15 children with normal weight.

Measurements and the questionnaire

The children's weight was measured while wearing light clothing (such as a T-shirt and pants) and without shoes or belts, using a digital scale (SBS 4414; Sinbo Precision Industry, Shenzhen, China) with an accuracy of 100 g. Height was measured using a wall-mounted stadiometer with an accuracy of 0.5 cm. The body mass index was then calculated by dividing weight in kilograms by the square of height in meters.

Based on the BMI values and the standard BMI charts developed by WHO,^{6,35} the 90 eligible children were included in the study and equally divided into 3 groups – normal weight, overweight, and obesity – with 30 participants in each group. According to the WHO standards for children and adolescents aged 2–20 years, a BMI percentile of 85–97% was considered overweight, while a BMI above the 97th percentile was considered obesity.^{6,35}

The children and their parents were instructed on proper toothbrushing and flossing techniques 1 month before data collection. The parents were also encouraged to carefully supervise their children's oral hygiene practices during this period.

Then, the designed questionnaire was completed by the children's parents, and the children's demographic characteristics, parental smoking status, daily activity status, medical and dental history, frequency of mouthwash use per week, toothbrushing frequency per day, and daily study duration in hours were recorded.

Collection of saliva samples

Before saliva collection, the participants were instructed to refrain from toothbrushing, eating, drinking, and vigorous physical activities for 1 h. During this period, they were allowed to relax through activities

such as reading, writing and drawing.¹⁵ Due to circadian variations in cortisol concentrations, saliva samples were collected at noon (12:00). Saliva samples were obtained using the spitting method.³⁶ After rinsing the mouth with water and swallowing saliva once, each participant was instructed to allow saliva to accumulate in the floor of the mouth, and then transfer it into a Falcon tube 1–2 times per minute for 5 min while sitting with the head and neck positioned at a 45-degree angle.³⁷

Oral examinations

All oral examinations were performed by a trained dentist. To avoid bias related to the incomplete eruption of some permanent teeth, periodontal indices were assessed only on first permanent molars and maxillary and mandibular central and lateral incisors. Primary teeth were not included in the periodontal evaluation.²

Gingival index

The gingival index (GI) was scored from 0 to 3 according to the Löe and Silness criteria as follows³⁸:

- score 0: no bleeding on probing (BoP); normal gingiva without signs of inflammation;
- score 1: presence of non-bleeding gingival changes; mild inflammation of the marginal gingiva detected during a clinical examination (the marginal gingiva is slightly redder than normal, with slight edema, and colorless gingival fluid may be observed at the entrance of the sulcus);
- score 2: bleeding of the marginal gingiva after stimulation with a dental probe or air spray; moderate gingival inflammation (the gingiva appears red or bluish-red and shiny, with the edema of the marginal gingiva);
- score 3: spontaneous gingival bleeding; severe gingival inflammation (the gingiva is markedly red or bluish-red, enlarged, and swollen).

Bleeding on probing

To evaluate BoP, a Williams probe (Smart Instrument, model MS5201; Hu-Friedy Manufacturing, Chicago, USA) was gently inserted into the sulcus of all teeth up to the base of the pocket or sulcus without applying additional pressure. After 10–15 s, the number of teeth exhibiting gingival bleeding was recorded.³⁹

Periodontal probing depth

To assess periodontal probing depth (PPD), a Williams probe (Smart Instrument, model MS5201) was inserted into the sulcus or periodontal pocket with gentle force while maintaining contact with the tooth surface and keeping the probe parallel to the root surface (the long axis of the tooth). The probe was advanced until the tip

reached the base of the pocket and encountered increased resistance, indicating the limit of further penetration. The distance between the base of the pocket (the point where the probe stopped) and the gingival margin was measured and recorded.⁴⁰

Measurements were performed at 6 sites for each tooth (mesiobuccal, mid-buccal, distobuccal, distolingual, midlingual, and mesiolingual). A pocket depth greater than 3 mm was considered indicative of an inflammatory condition.² The mean PPD values for each tooth were then calculated and recorded.⁴¹

Salivary cortisol levels

The cortisol levels can be measured using 3 types of samples: blood; saliva; and urine. The salivary cortisol levels have a direct and strong correlation with the serum cortisol levels and follow the circadian pattern of serum cortisol secretion. Due to the simplicity and non-invasive nature of saliva collection, as well as the ease of saliva transport and storage, the salivary cortisol measurement is widely used in pediatric research. In addition, blood sampling can be stressful for children and may result in elevated serum cortisol levels.^{42,43}

The salivary cortisol levels were measured using a salivary cortisol kit (DKO020; DiaMetra, Spello, Italy) based on the enzyme-linked immunosorbent assay (ELISA) method.⁴⁴

Bias

The risk of bias was minimized by excluding children with total dmft and DMFT scores >3 from the study, providing oral hygiene instructions to the participants and their parents, and assessing the periodontal status using reliable methods (GI, BoP and PPD). The salivary cortisol levels were analyzed in a specialized laboratory in Qom, Iran.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, v. 26 (IBM Corp., Armonk, USA). Descriptive and analytical statistics were applied. The Kolmogorov–Smirnov test and the Q–Q plot were used to assess the normality of data distribution. Differences between the groups were analyzed using the one-way analysis of variance (ANOVA) and the Kruskal–Wallis test according to the distribution of data (normal and not normal, respectively). The χ^2 test was used for categorical variables, and Pearson's correlation test was used to assess correlations between variables. The multivariable linear regression analysis was performed to assess the associations between the salivary cortisol levels (the quantitative dependent variable) and the independent variables while controlling for potential confounding factors.

Results

Characteristics of the participants

In this study, 90 eligible children, including 45 males and 45 females, were enrolled. The mean age was 10.00 ± 1.48 years in the obese and normal-weight groups, and 10.16 ± 1.39 years in the overweight group. There were no significant differences among the 3 groups regarding age and sex ($p = 1.000$).

Correlation between BMI and the demographic status

The BMI status was not significantly associated with the paternal or maternal education level (χ^2 test; $p = 0.599$ and $p = 0.143$, respectively). The relationship between the children's BMI status and total family income was also non-significant (χ^2 test; $p = 0.875$).

In addition, 50% of parents of normal-weight children reported smoking addiction, compared with 36.7% of parents of children from the overweight and obesity groups. Therefore, parental smoking was more prevalent among the parents of normal-weight children than among those of overweight and obese children. However, no significant association was found between the children's BMI status and the parental smoking addiction status (χ^2 test; $p = 0.480$).

The BMI status was not significantly associated with the frequency of mouthwash use per week, toothbrushing frequency per day or daily study duration in hours (Kruskal–Wallis test; $p = 0.884$, $p = 0.203$ and $p = 0.338$, respectively) (Table 1).

Correlation between BMI and the salivary cortisol levels and periodontal indices

The mean salivary cortisol levels in the normal-weight, overweight and obesity groups were 2.42 ± 0.74 , 2.93 ± 1.32 and 3.68 ± 2.26 ng/mL, respectively. Thus, the mean salivary cortisol levels were highest in children with obesity, followed by overweight children and normal-weight children. There was a significant association between the BMI status and the salivary cortisol levels (one-way ANOVA; $p = 0.010$) (Table 2).

The mean GI values in the normal-weight, overweight and obesity groups were 0.42 ± 0.13 , 0.51 ± 0.16 and 0.61 ± 0.15 , respectively. Thus, the mean GI was highest in children with obesity, followed by overweight children and normal-weight children.

The mean BoP values in the normal-weight, overweight and obesity groups were $17.83 \pm 7.20\%$, $21.50 \pm 7.61\%$ and $26.00 \pm 6.82\%$, respectively. Accordingly, the mean BoP was highest in children with obesity, followed by overweight children and normal-weight children.

The mean PPD values in the normal-weight, overweight and obesity groups were 1.52 ± 0.22 , 1.60 ± 0.24 and 1.72 ± 0.23 mm, respectively. Similarly, the mean PPD was

highest in children with obesity, followed by overweight children and normal-weight children.

There was a significant association between the children's BMI status and GI, BoP and PPD (one-way ANOVA; $p < 0.001$, $p = 0.001$ and $p < 0.005$, respectively) (Table 2).

The pairwise comparisons of the salivary cortisol levels among the study groups showed a significant difference only between the obesity and normal-weight groups (post hoc test; $p = 0.003$). No significant differences were observed between the obesity and overweight groups, or between the overweight and normal-weight groups (post hoc test; $p = 0.069$ and $p = 0.209$, respectively) (Table 3).

The pairwise comparisons of GI showed significant differences between the obesity and overweight groups, the obesity and normal-weight groups, and the overweight and normal-weight groups (post hoc test; $p = 0.011$, $p < 0.001$ and $p = 0.017$, respectively) (Table 3).

For BoP, significant differences were observed between the obesity group and both the overweight and normal-weight groups (post hoc test; $p = 0.018$ and $p < 0.001$, respectively). However, no significant difference was found

between the overweight and normal-weight groups (post hoc test; $p = 0.052$) (Table 3).

For PPD, a significant difference was observed only between the obesity and normal-weight groups (post hoc test; $p = 0.001$). No significant differences were found between the obesity and overweight groups, or between the overweight and normal-weight groups (post hoc test; $p = 0.054$ and $p = 0.166$, respectively) (Table 3).

According to Pearson's correlation analysis, there were significant positive correlations between GI and BoP ($r = 0.948$; $p < 0.001$), and between GI and PPD ($r = 0.680$; $p < 0.001$). A significant positive correlation was also observed between BoP and PPD ($r = 0.674$; $p < 0.001$). In addition, the salivary cortisol levels were positively correlated with GI ($r = 0.340$; $p = 0.001$), BoP ($r = 0.250$; $p = 0.018$) and PPD ($r = 0.253$; $p = 0.016$) (Table 4).

Based on the results of the univariable linear regression analysis, the salivary cortisol levels were significantly and positively associated with BMI ($\beta = 0.116$; $p = 0.001$), obesity ($\beta = 1.221$; $p = 0.005$), GI ($\beta = 3.325$; $p = 0.001$), and age ($\beta = 0.311$; $p = 0.010$). However, no significant association

Table 1. Relationship between the body mass index (BMI) status and the frequency of mouthwash use per week, toothbrushing frequency per day and daily study duration (Kruskal–Wallis test)

Variable	N	M ±SD	min	max	Percentile			p-value
					25 th	50 th (Me)	75 th	
Frequency of mouthwash use per week	90	0.1556 ±0.4719	0.00	3.00	0.0000	0.0000	0.0000	0.884
Toothbrushing frequency per day	90	0.9333 ±0.6670	0.00	3.00	1.0000	1.0000	1.0000	0.203
Daily study duration [h]	90	1.22 ±1.24	0	6	0.00	1.00	2.00	0.338
BMI status [kg/m ²]	90	21.34 ±4.91	13.15	33.07	17.20	21.54	24.69	–

M – mean; SD – standard deviation; min – minimum; max – maximum; Me – median.

Table 2. Relationship between the body mass index (BMI) status and the salivary cortisol levels and periodontal indices (one-way ANOVA)

Variable	BMI status	N	M ±SD	SE	95% CI		min	max	p-value
					lower bound	upper bound			
Salivary cortisol level [ng/mL]	normal weight	30	2.4167 ±0.7438	0.1358	2.1389	2.6944	0.70	3.60	0.010*
	overweight	30	2.9300 ±1.3231	0.2416	2.4360	3.4240	0.90	5.80	
	obesity	30	3.6767 ±2.2583	0.4123	2.8334	4.5199	0.40	9.50	
	total	90	3.0078 ±1.6380	0.1727	2.6647	3.3508	0.40	9.50	
GI	normal weight	30	0.4193 ±0.1309	0.0239	0.3705	0.4682	0.22	0.82	<0.001*
	overweight	30	0.5130 ±0.1606	0.0293	0.4530	0.5730	0.25	0.95	
	obesity	30	0.6133 ±0.1533	0.0280	0.5561	0.6706	0.25	0.84	
	total	90	0.5152 ±0.1673	0.0176	0.4802	0.5503	0.22	0.95	
BoP [%]	normal weight	30	17.8333 ±7.1959	1.3138	15.1463	20.5203	8.00	40.00	<0.001*
	overweight	30	21.5000 ±7.6090	1.3892	18.6588	24.3412	8.00	45.00	
	obesity	30	25.9833 ±6.8172	1.2446	23.4378	28.5289	12.50	41.00	
	total	90	21.7722 ±7.8812	0.8308	20.1215	23.4229	8.00	45.00	
PPD [mm]	normal weight	30	1.5203 ±0.2163	0.0395	1.4396	1.6011	1.20	1.98	0.005*
	overweight	30	1.6020 ±0.2369	0.0433	1.5135	1.6905	1.18	2.15	
	obesity	30	1.7163 ±0.2257	0.0412	1.6320	1.8006	1.33	2.21	
	total	90	1.6129 ±0.2381	0.0251	1.5630	1.6627	1.18	2.21	

SE – standard error; CI – confidence interval; GI – gingival index; BoP – bleeding on probing; PPD – periodontal probing depth; * statistically significant ($p < 0.05$).

Table 3. Pairwise comparisons of the salivary cortisol levels and periodontal indices in the study groups (post hoc statistical analysis – the least significant difference (LSD) test)

Dependent variable	Obesity status (I)	Obesity status (J)	Mean difference (I–J)	SE	95% CI		p-value
					lower bound	upper bound	
Salivary cortisol level [ng/mL]	normal weight	overweight	–0.5133	0.4056	–1.3195	0.2929	0.209
		obesity	–1.2600	0.4056	–2.0662	–0.4538	0.003*
	overweight	normal weight	0.5133	0.4056	–0.2929	1.3195	0.209
		obesity	–0.7467	0.4056	–1.5529	0.0595	0.069
	obesity	normal weight	1.2600	0.4056	0.4538	2.0662	0.003*
		overweight	0.7467	0.4056	–0.0595	1.5529	0.069
GI	normal weight	overweight	–0.0937	0.0384	–0.1700	–0.0173	0.017*
		obesity	–0.1940	0.0384	–0.2704	–0.1176	<0.001*
	overweight	normal weight	0.0937	0.0384	0.0173	0.1700	0.017*
		obesity	–0.1003	0.0384	–0.1767	–0.0240	0.011*
	obesity	normal weight	0.1940	0.0384	0.1176	0.2704	<0.001*
		overweight	0.1003	0.0384	0.0240	0.1767	0.011*
BoP [%]	normal weight	overweight	–3.6667	1.8628	–7.3692	0.0359	0.052
		obesity	–8.1500	1.8628	–11.8525	–4.4475	<0.001*
	overweight	normal weight	3.6667	1.8628	–0.0359	7.3692	0.052
		obesity	–4.4833	1.8628	–8.1859	–0.7808	0.018*
	obesity	normal weight	8.1500	1.8628	4.4475	11.8525	<0.001*
		overweight	4.4833	1.8628	0.7808	8.1859	0.018*
PPD [mm]	normal weight	overweight	–0.0817	0.0585	–0.1979	0.0346	0.166
		obesity	–0.1960	0.0585	–0.3122	–0.0798	0.001*
	overweight	normal weight	0.0817	0.0585	–0.0346	0.1979	0.166
		obesity	–0.1143	0.0585	–0.2306	0.0019	0.054
	obesity	normal weight	0.1960	0.05847	0.0798	0.3122	0.001*
		overweight	0.1143	0.05847	–0.0019	0.2306	0.054

* statistically significant ($p < 0.05$).**Table 4.** Correlation between the salivary cortisol levels and periodontal indices (Pearson's correlation analysis)

Variable	Pearson's correlation analysis	GI	BoP	PPD	Cortisol test result
Cortisol test result	r	0.340	0.250	0.253	1
	p -value (two-tailed)	0.001**	0.018*	0.016*	–
	N	90	90	90	90
GI	r	1	0.948	0.680	0.340
	p -value (two-tailed)	–	<0.001**	<0.001**	0.001**
	N	90	90	90	90
BoP	r	0.948	1	0.674	0.250
	p -value (two-tailed)	<0.001**	–	<0.001**	0.018*
	N	90	90	90	90
PPD	r	0.680	0.674	1	0.253
	p -value (two-tailed)	<0.001**	<0.001**	–	0.016*
	N	90	90	90	90

 r – Pearson's correlation coefficient; * significant correlation at $p < 0.05$ (two-tailed); ** significant correlation at $p < 0.01$ (two-tailed).

was observed between the salivary cortisol levels and the overweight status ($\beta = 0.474$; $p = 0.263$) (Table 5).

Based on the results of the multivariable linear regression analysis, the salivary cortisol levels were significantly and positively associated with age ($\beta = 0.276$; $p = 0.004$), obesity ($\beta = 0.269$; $p = 0.006$), father's education level

($\beta = 0.393$; $p < 0.001$), and the parental smoking addiction status ($\beta = 0.238$; $p = 0.026$) (Table 6). These findings indicate that obesity remained independently associated with the salivary cortisol levels after adjusting for age, father's education level and the parental smoking addiction status.

Table 5. Univariable linear regression analysis

Variable	β	Standardized β	p -value	R^2
BMI	0.116	0.348	0.001*	0.121
Obesity status	normal-weight	reference		
	overweight	0.138	0.263	0.093
	obese	0.354	0.005*	
Age	0.311	0.269	0.010*	0.072
GI	3.325	0.340	0.001*	0.115

* statistically significant ($p < 0.05$).**Table 6.** Multivariable linear regression analysis for the salivary cortisol level variable

Model	Unstandardized coefficients		Standardized coefficient	t	p -value	R^2
	B	SE	β			
1	(constant)	−3.301	1.925	−	−1.715	0.090
	GI	1.485	1.102	0.149	1.347	0.182
	age	0.281	0.116	0.239	2.410	0.018
	overweight	0.298	0.396	0.087	0.752	0.454
	obesity	0.875	0.444	0.254	1.971	0.052
	father's education level	0.672	0.216	0.381	3.110	0.003
	total family income	0.029	0.386	0.009	0.074	0.941
	parental smoking addiction	0.806	0.353	0.242	2.282	0.025
2	(constant)	−3.197	1.312	−	−2.437	0.017
	GI	1.475	1.088	0.148	1.356	0.179
	age	0.280	0.115	0.238	2.427	0.017
	overweight	0.301	0.392	0.087	0.768	0.445
	obesity	0.880	0.435	0.255	2.024	0.046
	father's education level	0.664	0.185	0.376	3.590	0.001
	parental smoking addiction	0.809	0.348	0.243	2.321	0.023
3	(constant)	−3.066	1.298	−	−2.363	0.021
	GI	1.665	1.056	0.168	1.577	0.119
	age	0.273	0.115	0.232	2.377	0.020
	obesity	0.690	0.357	0.200	1.935	0.056
	father's education level	0.672	0.184	0.381	3.648	0.000
	parental smoking addiction	0.785	0.346	0.236	2.268	0.026
4	(constant)	−2.863	1.303	−	−2.197	0.031
	age	0.324	0.111	0.276	2.922	0.004*
	obesity	0.927	0.326	0.269	2.842	0.006*
	father's education level	0.693	0.185	0.393	3.738	<0.001*
	parental smoking addiction	0.793	0.349	0.238	2.271	0.026*

* statistically significant ($p < 0.05$).

Discussion

Correlation between the salivary cortisol levels and age

In this study, the salivary cortisol levels were measured to investigate physiological changes in cortisol metabolism during childhood and adolescence.

The present study found that the salivary cortisol levels increased significantly with increasing age in children. According to Kiess et al., the cortisol levels are age-dependent, such that after the age of 6 years, they are significantly correlated with maturation stages.¹⁶ Törnåge⁴⁵ and Larsson et al.⁴⁶ also reported higher salivary cortisol levels in older children. This positive association between age and increased cortisol levels has been reported in other

studies as well.⁴⁷ However, these findings are inconsistent with those of Kjölhede et al.¹² and Knutsson et al.⁴⁸ The discrepancies between the present findings and those of the aforementioned studies may be attributed to differences in the sampling location and time, the methods used to measure salivary cortisol, and the age range of the study populations.

Correlation between the salivary cortisol levels and obesity

The present study found that the salivary cortisol levels were positively and significantly associated with increasing BMI and obesity. Chu et al. also reported that obese children had higher morning salivary and urinary cortisol levels.¹⁵ Several other studies have likewise demonstrated a positive relationship between the salivary, serum and urinary cortisol levels and obesity.^{16,17,43,49} These studies suggest that cortisol concentrations increase long before a child becomes obese.¹⁵ Yu et al. also found that the salivary nocturnal cortisol levels were positively correlated with weight gain, abdominal fat distribution (the waist-to-height ratio (WHtR)), and body fat percentage in all participants,⁴³ consistent with the findings of the present study. However, the present findings are inconsistent with those reported by Kjölhede et al., who found lower morning and evening salivary cortisol levels in overweight and obese children aged 6–12 years,¹² as well as with the studies by Hillman et al.¹³ and Chalew et al.,¹⁴ which reported lower plasma cortisol levels in obese children. Hillman et al. further showed a negative association between the daytime cortisol levels and BMI z-score and central obesity, but a positive association during the night.¹³ The discrepancies between the present findings and those of these studies may be attributed to differences in the type of cortisol measured (e.g., plasma rather than salivary cortisol), the cortisol measurement methods and the timing of saliva sample collection.

Cortisol concentrations are not elevated in obese adults with peripheral fat distribution, whereas individuals with central obesity exhibit increased cortisol levels.⁵⁰ Unfortunately, due to the limitations of the present study, fat distribution was not assessed using waist circumference or other anthropometric measures. Therefore, based on the present findings, no definitive conclusions can be drawn regarding fat distribution and its relationship with the cortisol levels in children. Further studies are needed to investigate this association.

Correlation between obesity and the periodontal status

According to several studies, a high BMI may be a risk factor for periodontal disease in adolescents and young adults, as assessed by the plaque index (PI), BoP, PPD, clinical attachment loss (CAL), and alveolar bone loss.⁵¹

Several hypotheses have been proposed to explain biological interactions between obesity and periodontal disease, including alterations in inflammatory and immune responses, impaired glucose tolerance, dyslipidemia, changes in host immunity, increased macrophage activation, impaired microvascular function, physiological responses, psychosocial stress, and the secretion of pro-inflammatory cytokines (tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6, and IL-8), adipokines (leptin, adiponectin, resistin, and plasminogen activator inhibitor-1 (PAI-1)), and other bioactive substances, such as reactive oxygen species (ROS) from adipose tissue and C-reactive protein (CRP).^{52,53} However, the precise molecular and cellular mechanisms underlying these interactions remain unclear, and further studies are needed to elucidate these mechanisms, which may contribute to the development of preventive and therapeutic strategies.

According to some reports, periodontal disease in obese individuals is associated with alterations in the composition of the oral microbiota.^{54,55} However, most studies investigating the relationship between obesity and periodontitis have primarily considered immunological and metabolic alterations as the main predisposing factors for periodontal disease.

Several mechanisms have been proposed to explain this relationship. Adipocytes may contribute to a systemic pro-inflammatory state through the release of various inflammatory mediators.^{56,57} Some studies have shown that obesity is associated with adverse postoperative outcomes, including infectious complications and impaired wound healing.^{58,59} Therefore, the altered immune responses and impaired wound healing associated with obesity may influence the clinical response to periodontal treatment. However, Suvan et al.³⁰ and Chaffee and Weston⁶⁰ report that the evidence regarding the association between BMI, overweight or obesity and the clinical response to periodontal treatment remains limited.

In the present study, the GI, BoP and PPD indices were used to assess the periodontal status. The findings showed that obesity was positively and significantly associated with all 3 indices.

Although the association between obesity and periodontal disease has been widely investigated in adults, it has been less extensively studied in the pediatric population.

De Castilhos et al. reported a statistically significant association in young adults between obesity and GI and calculus, both of which are indicators associated with periodontal disease; however, no significant association was observed between obesity and periodontitis.⁶¹ On the other hand, Suvan et al. confirmed the association between increasing BMI and the severity and extent of periodontitis,⁶² consistent with the findings of the present study.

Other researchers have shown that obesity in children is associated with increased PI, gingival inflammation and

BoP, and that BMI is significantly correlated with periodontal indices, including PI, BoP, PPD, CAL, the oral hygiene index (OHI), and the community periodontal index (CPI).^{2,41,51,63–65} Martens et al. likewise demonstrated a positive association between overweight and obesity and the prevalence of periodontal disease.⁵³ They further reported that obesity was associated with less frequent daily toothbrushing and flossing, a higher prevalence of dental caries, a lower parental education level, a higher socioeconomic status, and less healthy dietary habits.⁵³

The increased BoP observed in obese participants may be attributed to elevated levels of pro-inflammatory cytokines in gingival crevicular fluid (GCF).³¹

Vaziri et al. reported that the mean PPD was higher in obese than in non-obese participants; although this difference was statistically significant, it was not considered clinically significant.⁴¹ Similarly, Mod  r et al.³¹ and Scorzetti et al.⁶³ found that participants in the obese groups had a significantly higher prevalence of PPD ≥ 4 mm.

In a study by Vallogini et al., the findings were inconsistent with those of the present study and the aforementioned studies.⁶⁶ The obese group showed lower visible PI values and gingival bleeding index (GBI) scores, as well as a better periodontal status in terms of PPD. In their study, obese pediatric participants were recruited from a children's hospital, whereas non-obese participants were recruited from a school.⁶⁶ Therefore, the discrepancies between the findings of that study and the present study may be attributed to differences in the sampling location, as participants recruited from a hospital setting may have received specific dietary and oral hygiene counseling, potentially resulting in improved periodontal parameters.

Kim et al. showed that individuals with a BMI higher than 25 (classified as overweight or obese) had a lower risk of periodontitis as compared to individuals of normal weight, whereas those with abdominal obesity had a significantly higher risk of periodontal disease.⁶⁷ These findings suggest that metabolic syndrome may play an important role in periodontal inflammation.^{68–70}

Reeves et al. concluded that the onset of periodontitis may be associated with weight gain and increased waist circumference.⁷¹ They found that among individuals aged 17–21 years, each 1-kilogram increase in body weight was associated with a 6% increase in the risk of periodontal disease, while each 1-inch increase in waist circumference was associated with a 5% increase in the risk of periodontitis. In contrast, no significant association was observed between these parameters and periodontal disease among younger children aged 13–16 years.⁷¹

The findings of most of the aforementioned studies indicate an adverse effect of obesity on periodontal health in young individuals, which is likely due to a combination of factors, including metabolic and inflammatory changes, inadequate attention to oral and dental hygiene practices and preventive measures, an unbalanced diet, and irregular dental examinations.^{72,73}

Correlation between the salivary cortisol levels and the periodontal status

The findings of this study showed that the salivary cortisol levels were positively and significantly associated with the GI, BoP and PPD indices. Various studies have investigated the role of chronic stress as an underlying factor in the development of oral and dental health problems. Based on substantial scientific evidence, stress and psychological disorders may cause individuals to neglect oral and dental hygiene, thereby accelerating plaque accumulation and the progression of periodontal disease.⁷⁴ Academic stress has also been reported as a risk factor for gingival inflammation, through increased levels of IL-1 β in GCF, as well as reduced oral hygiene.^{75–77}

It has been hypothesized that stress induces behavioral changes, such as overeating and an increased preference for high-fat diets, which may contribute to immunosuppression through increased cortisol production.⁷⁴ Furthermore, chronic stress can alter the salivary flow rate, pH and chemical composition.^{74,78} Rai et al. demonstrated that stress-related biomarkers, including the salivary cortisol concentration, were increased in children with dental caries.⁷⁹ In the present study, children with dmft/DMFT scores above 3 were excluded due to the potential association between the cortisol levels and dental caries.^{80–82} Therefore, the influence of this confounding variable was reduced in the current study, which represents one of its strengths.

It appears that periodontal disease and stress have a bi-directional relationship. Several studies have reported that the chronic elevation of the cortisol levels may be a potential risk factor for the onset or severity of periodontal disease in adults.^{74,83,84}

Consistent with the findings of the present study, Schmidt et al. showed that the salivary cortisol levels were significantly higher in adolescents with periodontitis than in those without periodontitis.⁸⁵ Individuals with elevated cortisol levels appear to be at a higher risk of developing periodontitis. In contrast, Panagiotou et al. found no association between stress and dental caries or periodontal disease.⁸⁶ One possible explanation for this discrepancy may be the lack of adherence by parents and participants to the scheduled saliva collection times at home.

Considering the complex nature of periodontal disease and numerous confounding factors that influence its development, comprehensive longitudinal studies are recommended in the future to further clarify and confirm the potential relationship between periodontal disease, BMI and the cortisol levels.

Limitations

The difficulty in obtaining participants' cooperation during oral examinations or saliva collection under calm conditions resulted in the need for repeated sampling.

Conclusions

The findings of the present study showed that:

- children's BMI was positively associated with the salivary cortisol levels and the GI, BOP and PPD indices, indicating that overweight and obese children have a poorer periodontal status compared with children of normal weight;
- the salivary cortisol levels, as an indicator of the serum cortisol levels, were higher in overweight and obese children than in children of normal weight.

These findings highlight the importance of encouraging healthy lifestyle modifications and weight management from early childhood and adolescence to prevent the long-term adverse effects of elevated cortisol levels on the body, and to improve periodontal health.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki after obtaining approval from the Ethics Committee of Qom University of Medical Sciences, Iran (No. IR.MUQ.REC.1400.231). All methods were carried out in accordance with the relevant guidelines and regulations. Written informed consent was obtained from the parents or legal guardians of all participating children.

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

- Panuganti KK, Nguyen M, Kshirsagar RK, Doerr C. *Obesity (Nursing)*. StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2021. Updated 2023. <https://www.ncbi.nlm.nih.gov/books/NBK568702>.
- Sfasciotti GL, Marini R, Pacifici A, Ierardo G, Pacifici L, Polimeni A. Childhood overweight–obesity and periodontal diseases: Is there a real correlation? *Ann Stomatol (Roma)*. 2016;7(3):65–72. doi:10.11138/ads/2016.7.3.065
- Kiliç M, Gurbuz T, Kahraman CY, Cayir A, Bilgiç A, Kurt Y. Relationship between the *TAS2R38* and *TAS1R2* polymorphisms and the dental status in obese children. *Dent Med Probl*. 2022;59(2):233–240. doi:10.17219/dmp/143252
- McPhee PG, Singh S, Morrison KM. Childhood obesity and cardiovascular disease risk: Working toward solutions. *Can J Cardiol*. 2020;36(9):1352–1361. doi:10.1016/j.cjca.2020.06.020
- Akbari H, Mohammadi M. The Prevalence of obesity in Iranian children: A systematic review and meta-analysis. *J Pediatr Rev*. 2022;10(2):93–102. doi:10.32598/jpr.10.2.875.2
- Tyson N, Frank M. Childhood and adolescent obesity definitions as related to BMI, evaluation and management options. *Best Pract Res Clin Obstet Gynaecol*. 2018;48:158–164. doi:10.1016/j.bpobgyn.2017.06.003
- Kaur J, Gandhi J, Sharma S. *Physiology, Cortisol*. StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2021. Updated 2025. <https://www.ncbi.nlm.nih.gov/books/NBK538239>.
- Silva Morais JB, Severo JS, Beserra JB, et al. Association between cortisol, insulin resistance and zinc in obesity: A mini-review. *Biol Trace Elem Res*. 2019;191(2):323–330. doi:10.1007/s12011-018-1629-y
- Di Dalmazi G, Pasquali R, Beuschlein F, Reincke M. Subclinical hypercortisolism: A state, a syndrome, or a disease? *Eur J Endocrinol*. 2015;173(4):M61–M71. doi:10.1530/EJE-15-0272
- Whitworth JA, Williamson PM, Mangos G, Kelly JJ. Cardiovascular consequences of cortisol excess. *Vasc Health Risk Manag*. 2005;1(4):291–299. doi:10.2147/vhrm.2005.1.4.291
- Van Rossum EF. Obesity and cortisol: New perspectives on an old theme. *Obesity (Silver Spring)*. 2017;25(3):500–501. doi:10.1002/oby.21774
- Kjölhede EA, Gustafsson PE, Gustafsson P, Nelson N. Overweight and obese children have lower cortisol levels than normal weight children. *Acta Paediatr*. 2014;103(3):295–299. doi:10.1111/apa.12499
- Hillman JB, Dorn LD, Loucks TL, Berga SL. Obesity and the hypothalamic–pituitary–adrenal axis in adolescent girls. *Metabolism*. 2012;61(3):341–348. doi:10.1016/j.metabol.2011.07.009
- Chalew SA, Lozano RA, Armour KM, Zadik Z, Kowarski AA. Reduction of plasma cortisol levels in childhood obesity. *J Pediatr*. 1991;119(5):778–780. doi:10.1016/s0022-3476(05)80302-6
- Chu L, Sheng K, Liu P, et al. Increased cortisol and cortisone levels in overweight children. *Med Sci Monit Basic Res*. 2017;23:25–30. doi:10.12659/msmbr.902707
- Kiess W, Meidert A, Dressendörfer RA, et al. Salivary cortisol levels throughout childhood and adolescence: Relation with age, pubertal stage, and weight. *Pediatr Res*. 1995;37(4 Pt 1):502–506. doi:10.1203/00006450-199504000-00020
- Rosmalen JG, Oldehinkel AJ, Ormel J, De Winter AF, Buitelaar JK, Verhulst FC. Determinants of salivary cortisol levels in 10–12 year old children; a population-based study of individual differences. *Psychoneuroendocrinology*. 2005;30(5):483–495. doi:10.1016/j.psyneuen.2004.12.007
- Filipovský J, Ducimetière P, Eschwege E, Richard JL, Rosselin G, Claude JR. The relationship of blood pressure with glucose, insulin, heart rate, free fatty acids and plasma cortisol levels according to degree of obesity in middle-aged men. *J Hypertens*. 1996;14(2):229–235. doi:10.1097/00004872-199602000-00012
- Walker BR, Soderberg S, Lindahl B, Olsson T. Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. *J Intern Med*. 2000;247(2):198–204. doi:10.1046/j.1365-2796.2000.00609.x
- Ward AM, Fall CH, Stein CE, et al. Cortisol and the metabolic syndrome in South Asians. *Clin Endocrinol (Oxf)*. 2003;58(4):500–505. doi:10.1046/j.1365-2265.2003.01750.x
- GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1211–1259. doi:10.1016/S0140-6736(18)31891-9
- 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
- Martins Chávayry NG, Vettore MV, Sansone C, Sheiham A. The relationship between diabetes mellitus and destructive periodontal disease: A meta-analysis. *Oral Health Prev Dent*. 2009;7(2):107–127. PMID:19583037.

24. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: A systematic review. *BJOG*. 2006;113(2):135–143. doi:10.1111/j.1471-0528.2005.00827.x
25. Humphrey LL, Fu R, Buckley DJ, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: A systematic review and meta-analysis. *J Gen Intern Med*. 2008;23(12):2079–2086. doi:10.1007/s11606-008-0787-6
26. Nardi GM, Grassi R, Ndokaj A, et al. Maternal and neonatal oral microbiome developmental patterns and correlated factors: A systematic review – does the apple fall close to the tree? *Int J Environ Res Public Health*. 2021;18(11):5569. doi:10.3390/ijerph18115569
27. Zermeno-Ibarra JA, Delgado-Pastrana S, Patiño-Marín N, Loyola-Rodríguez JP. Relationship between overweight–obesity and periodontal disease in Mexico. *Acta Odontol Latinoam*. 2010;23(3):204–209. PMID:21638960.
28. Perez MM, Pessoa JS, Ciamponi AL, et al. Correlation of salivary immunoglobulin A with body mass index and fat percentage in overweight/obese children. *J Appl Oral Sci*. 2018;27:e20180088. doi:10.1590/1678-7757-2018-0088
29. De Moura-Grec PG, Marsicano JA, Paz de Carvalho CA, De Carvalho Sales-Peres SH. Obesity and periodontitis: Systematic review and meta-analysis. *Cien Saude Colet*. 2014;19(6):1763–1772. doi:10.1590/1413-81232014196.13482013
30. Suvan J, D'Aiuto F, Moles DR, Petrie A, Donos N. Association between overweight/obesity and periodontitis in adults. A systematic review. *Obes Rev*. 2011;12(5):e381–e404. doi:10.1111/j.1467-789X.2010.00808.x
31. Modéer T, Blomberg C, Wondimu B, Lindberg TY, Marcus C. Association between obesity and periodontal risk indicators in adolescents. *Int J Pediatr Obes*. 2011;6(2–2):e264–e270. doi:10.3109/17477166.2010.495779
32. López R, Fernández O, Baelum V. Social gradients in periodontal diseases among adolescents. *Community Dent Oral Epidemiol*. 2006;34(3):184–196. doi:10.1111/j.1600-0528.2006.00271.x
33. Pari A, Ilango P, Subbareddy V, Katamreddy V, Parthasarthy H. Gingival diseases in childhood – a review. *J Clin Diagn Res*. 2014;8(10):ZE01–ZE04. doi:10.7860/JCDDR/2014/9004.4957
34. Putignano P, Dubini A, Toja P, et al. Salivary cortisol measurement in normal-weight, obese and anorexic women: Comparison with plasma cortisol. *Eur J Endocrinol*. 2001;145(2):165–171. doi:10.1530/eje.0.1450165
35. Kumar S, Kelly AS. Review of childhood obesity: From epidemiology, etiology, and comorbidities to clinical assessment and treatment. *Mayo Clin Proc*. 2017;92(2):251–265. doi:10.1016/j.mayocp.2016.09.017
36. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci*. 1993;694:72–77. doi:10.1111/j.1749-6632.1993.tb18343.x
37. Arhakis A, Karagiannis V, Kalfas S. Salivary alpha-amylase activity and salivary flow rate in young adults. *Open Dent J*. 2013;7:7–15. doi:10.2174/1874210601307010007
38. Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol*. 1967;38(Suppl 6):610–616. doi:10.1902/jop.1967.38.6.610
39. Joss A, Adler R, Lang NP. Bleeding on probing. A parameter for monitoring periodontal conditions in clinical practice. *J Clin Periodontol*. 1994;21(6):402–408. doi:10.1111/j.1600-051x.1994.tb00737.x
40. Hefti AF. Periodontal probing. *Crit Rev Oral Biol Med*. 1997;8(3):336–356. doi:10.1177/10454411970080030601
41. Vaziri F, Bahrololoomi Z, Savabieh Z, Sezavar K. The relationship between children's body mass index and periodontal status. *J Indian Soc Periodontol*. 2022;26(1):64–68. doi:10.4103/jisp.jisp_899_20
42. Blair J, Adaway J, Keevil B, Ross R. Salivary cortisol and cortisone in the clinical setting. *Curr Opin Endocrinol Diabetes Obes*. 2017;24(3):161–168. doi:10.1097/MED.0000000000000328
43. Yu T, Zhou W, Wu S, Liu Q, Li X. Evidence for disruption of diurnal salivary cortisol rhythm in childhood obesity: Relationships with anthropometry, puberty and physical activity. *BMC Pediatr*. 2020;20(1):381. doi:10.1186/s12887-020-02274-8
44. Shimada M, Takahashi K, Ohkawa T, Segawa M, Higurashi M. Determination of salivary cortisol by ELISA and its application to the assessment of the circadian rhythm in children. *Horm Res*. 1995;44(5):213–217. doi:10.1159/000184628
45. Törnhaage CJ. Reference values for morning salivary cortisol concentrations in healthy school-aged children. *J Pediatr Endocrinol Metab*. 2002;15(2):197–204. doi:10.1515/jpem.2002.15.2.197
46. Larsson CA, Gullberg B, Råstam L, Lindblad U. Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: A cross-sectional study. *BMC Endocr Disord*. 2009;9:16. doi:10.1186/1472-6823-9-16
47. Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, Kirschbaum C. HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: Impact of age and gender. *Psychoneuroendocrinology*. 2004;29(1):83–98. doi:10.1016/s0306-4530(02)00146-4
48. Knutsson U, Dahlgren J, Marcus C, et al. Circadian cortisol rhythms in healthy boys and girls: Relationship with age, growth, body composition, and pubertal development. *J Clin Endocrinol Metab*. 1997;82(2):536–540. doi:10.1210/jcem.82.2.3769
49. Reinehr T, Kulle A, Wolters B, et al. Steroid hormone profiles in prepubertal obese children before and after weight loss. *J Clin Endocrinol Metab*. 2013;98(6):E1022–E1030. doi:10.1210/jc.2013-1173
50. Ljung T, Andersson B, Bengtsson BA, Björntorp P, Mårin P. Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: A dose–response study. *Obes Res*. 1996;4(3):277–282. doi:10.1002/j.1550-8528.1996.tb00546.x
51. Khan S, Barrington G, Bettiol S, Barnett T, Crocombe L. Is overweight/obesity a risk factor for periodontitis in young adults and adolescents? A systematic review. *Obes Rev*. 2018;19(6):852–883. doi:10.1111/obr.12668
52. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. *Periodontol 2000*. 2013;62(1):59–94. doi:10.1111/j.1600-0757.2012.00457.x
53. Martens L, De Smet S, Yusof MY, Rajasekharan S. Association between overweight/obesity and periodontal disease in children and adolescents: A systematic review and meta-analysis. *Eur Arch Paediatr Dent*. 2017;18(2):69–82. doi:10.1007/s40368-017-0272-1
54. Goodson JM, Groppo D, Halem S, Carpino E. Is obesity an oral bacterial disease? *J Dent Res*. 2009;88(6):519–523. doi:10.1177/0022034509338353
55. DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. *Mayo Clin Proc*. 2008;83(4):460–469. doi:10.4065/83.4.460
56. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol*. 2010;314(1):1–16. doi:10.1016/j.mce.2009.07.031
57. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol*. 2011;11(2):85–97. doi:10.1038/nri2921
58. Wilson JA, Clark JJ. Obesity: Impediment to wound healing. *Crit Care Nurs Q*. 2003;26(2):119–132. doi:10.1097/00002727-200304000-00006
59. Doyle SL, Lysaght J, Reynolds JV. Obesity and post-operative complications in patients undergoing non-bariatric surgery. *Obes Rev*. 2010;11(12):875–886. doi:10.1111/j.1467-789X.2009.00700.x
60. Chaffee BW, Weston SJ. Association between chronic periodontal disease and obesity: A systematic review and meta-analysis. *J Periodontol*. 2010;81(12):1708–1724. doi:10.1902/jop.2010.100321
61. De Castilhos ED, Horta BL, Gigante DP, Demarco FF, Peres KG, Peres MA. Association between obesity and periodontal disease in young adults: A population-based birth cohort. *J Clin Periodontol*. 2012;39(8):717–724. doi:10.1111/j.1600-051X.2012.01906.x
62. Suvan J, Petrie A, Moles DR, et al. Body mass index as a predictive factor of periodontal therapy outcomes. *J Dent Res*. 2014;93(1):49–54. doi:10.1177/0022034513511084
63. Scorzett L, Marcattili D, Pasini M, Mattei A, Marchetti E, Marzo G. Association between obesity and periodontal disease in children. *Eur J Paediatr Dent*. 2013;14(3):181–184. PMID:24295000.
64. Franchini R, Petri A, Migliario M, Rimondini L. Poor oral hygiene and gingivitis are associated with obesity and overweight status in paediatric subjects. *J Clin Periodontol*. 2011;38(11):1021–1028. doi:10.1111/j.1600-051X.2011.01770.x
65. Zuza EP, Machado Nascimento LA, Caetano SL, et al. Periodontal disease and body weight assessment in children. *J Dent Child (Chic)*. 2017;84(1):3–8. PMID:28387183.
66. Vallogini G, Nobili V, Rongo R, et al. Evaluation of the relationship between obesity, dental caries and periodontal disease in adolescents. *Eur J Paediatr Dent*. 2017;18(4):268–272. doi:10.23804/ejpd.2017.18.04.02
67. Kim EJ, Jin BH, Bae KH. Periodontitis and obesity: A study of the Fourth Korean National Health and Nutrition Examination Survey. *J Periodontol*. 2011;82(4):533–542. doi:10.1902/jop.2010.100274

68. Andriankaja OM, Sreenivasa S, Dunford R, DeNardin E. Association between metabolic syndrome and periodontal disease. *Aust Dent J*. 2010;55(3):252–259. doi:10.1111/j.1834-7819.2010.01231.x
69. Benguigui C, Bongard V, Ruidavets JB, et al. Metabolic syndrome, insulin resistance, and periodontitis: A cross-sectional study in a middle-aged French population. *J Clin Periodontol*. 2010;37(7):601–608. doi:10.1111/j.1600-051X.2010.01571.x
70. Timonen P, Niskanen M, Suominen-Taipale L, Jula A, Knuuttila M, Ylöstalo P. Metabolic syndrome, periodontal infection, and dental caries. *J Dent Res*. 2010;89(10):1068–1073. doi:10.1177/0022034510376542
71. Reeves AF, Rees JM, Schiff M, Hujoel P. Total body weight and waist circumference associated with chronic periodontitis among adolescents in the United States. *Arch Pediatr Adolesc Med*. 2006;160(9):894–899. doi:10.1001/archpedi.160.9.894
72. Trottni M, Bossù M, Corridore D, et al. Assessing risk factors for dental caries: A statistical modeling approach. *Caries Res*. 2015;49(3):226–235. doi:10.1159/000369831
73. Kesim S, Çiçek B, Aral CA, Öztürk A, Mazicioğlu MM, Kurtoğlu S. Oral health, obesity status and nutritional habits in Turkish children and adolescents: An epidemiological study. *Balkan Med J*. 2016;33(2):164–172. doi:10.5152/balkanmedj.2016.16699
74. Goyal S, Gupta G, Thomas B, Bhat KM, Bhat GS. Stress and periodontal disease: The link and logic! *Ind Psychiatry J*. 2013;22(1):4–11. doi:10.4103/0972-6748.123585
75. Deinzer R, Granrath N, Spahl M, Linz S, Waschul B, Herforth A. Stress, oral health behaviour and clinical outcome. *Br J Health Psychol*. 2005;10(Pt 2):269–283. doi:10.1348/135910705X26858
76. Deinzer R, Hilpert D, Bach K, Schawacht M, Herforth A. Effects of academic stress on oral hygiene – a potential link between stress and plaque-associated disease? *J Clin Periodontol*. 2001;28(5):459–464. doi:10.1034/j.1600-051x.2001.028005459.x
77. Hildebrand HC, Epstein J, Larjava H. The influence of psychological stress on periodontal disease. *J West Soc Periodontol Periodontol Abstr*. 2000;48(3):69–77. PMID:11381953.
78. Gupta OP. Psychosomatic factors in periodontal disease. *Dent Clin North Am*. 1966;10(1):11–19. doi:10.1016/S0011-8532(22)03408-5
79. Rai K, Hegde AM, Shetty S, Shetty S. Estimation of salivary cortisol in children with rampant caries. *J Clin Pediatr Dent*. 2010;34(3):249–252. doi:10.17796/jcpd.34.3.l858480k80031jn2
80. Caruso S, Gatto R, Cinque B, Cifone MG, Mattei A. Association between salivary cortisol level and caries in early childhood. *Eur J Paediatr Dent*. 2018;19(1):10–15. doi:10.23804/ejpd.2018.19.01.02
81. Tikhonova S, Booij L, D'Souza V, Crosara KT, Siqueira WL, Emami E. Investigating the association between stress, saliva and dental caries: A scoping review. *BMC Oral Health*. 2018;18(1):41. doi:10.1186/s12903-018-0500-z
82. Martínez M, Montero E, Carasol M, et al. Association between caries and periodontal diseases in a sample of employed adults in Spain: A cross-sectional study. *Clin Oral Investig*. 2021;25(6):3957–3966. doi:10.1007/s00784-020-03726-2
83. Cakmak O, Tasdemir Z, Aral CA, Dundar S, Koca HB. Gingival crevicular fluid and saliva stress hormone levels in patients with chronic and aggressive periodontitis. *J Clin Periodontol*. 2016;43(12):1024–1031. doi:10.1111/jcpe.12614
84. Haririan H, Andrukhov O, Böttcher M, et al. Salivary neuropeptides, stress, and periodontitis. *J Periodontol*. 2018;89(1):9–18. doi:10.1902/jop.2017.170249
85. Schmidt J, Strecker P, Kreuz M, et al. Stress-related hormones in association with periodontal condition in adolescents – results of the epidemiologic LIFE Child study. *Clin Oral Investig*. 2019;23(4):1793–1802. doi:10.1007/s00784-018-2599-3
86. Panagiotou E, Agouropoulos A, Vadiakas G, Pervanidou P, Chouliaras G, Kanaka-Gantenbein C. Oral health of overweight and obese children and adolescents: A comparative study with a multivariate analysis of risk indicators. *Eur Arch Paediatr Dent*. 2021;22(5):861–868. doi:10.1007/s40368-021-00643-0

Three-year follow-up of the additive effect of concentrated growth factor to the coronally advanced flap in Miller's class I and class II recession defects: A randomized controlled clinical trial

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Abstract

Background. The fundamental goals of traditional periodontal therapy have been the eradication of disease and the preservation of a healthy, functional dentition and its supporting tissues. However, in recent years, the emphasis has shifted increasingly toward esthetic outcomes, extending beyond dental prostheses and tooth color to encompass the soft tissues surrounding the dentition.

Objectives. The main aim of this research was to compare the impact of the coronally advanced flap (CAF) in combination with concentrated growth factor (CGF) with that of CAF alone in the management of gingival recession.

Material and methods. This randomized controlled trial (RCT) included 90 patients with Miller's class I or class II gingival recession, who were categorized into 2 groups: CAF; and CAF with CGF. Follow-up assessments were conducted at 6 months, 1 year and 3 years. Periodontal clinical parameters, along with buccal recession depth (BRD) and the width of attached gingiva (AGW), were measured. Postoperative pain was assessed using a visual analog scale (VAS) 10 days after the intervention in both groups. Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 23.

Results. The CAF+CGF group showed better results in terms of mean BRD, AGW and clinical attachment level (CAL), as well as reduced postoperative pain ($p < 0.05$).

Conclusions. With a 3-year long-term follow-up, the present trial supports the additive effect of CGF in combination with CAF for maxillary gingival augmentation coronal to the recession.

Keywords: gingival recession, root coverage, coronally advanced flap, concentrated growth factor

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Highlights

- The addition of concentrated growth factor (CGF) to the coronally advanced flap (CAF) improved gingival recession outcomes compared with CAF alone.
- CAF+CGF resulted in a greater reduction in buccal recession depth (BRD), an increased width of attached gingiva (AGW) and an improved clinical attachment level (CAL) over the 3-year follow-up.
- Postoperative pain and patient discomfort were significantly lower with CAF+CGF than with CAF alone.
- The findings support the additive benefit of CGF as an adjunct to CAF for long-term gingival augmentation and recession coverage.

Introduction

The fundamental goals of traditional periodontal therapy have been the eradication of disease and the preservation of a healthy, functional dentition and its supporting tissues. However, in recent years, the emphasis has shifted increasingly toward esthetic outcomes, extending beyond dental prostheses and tooth color to also include the soft tissues surrounding the dentition. One of the most common esthetic concerns involving periodontal tissues is gingival recession. Gingival recession is characterized by the apical displacement of the free gingival margin from the cemento-enamel junction (CEJ), which may contribute to increased root hypersensitivity, caries and attachment loss. Its onset has frequently been associated with dental malposition, traumatic toothbrushing, the frenal pull, and periodontal disease.¹

A myriad of studies have shown that various periodontal plastic surgical techniques can effectively treat gingival recession. Many techniques, including free gingival grafts, pedicle flaps with and without grafts, coronally advanced flaps (CAFs), and others, have been studied.^{2,3} Numerous graft materials, such as connective tissue grafts (CTGs), xenogeneic collagen matrices, dermal matrices, guided tissue regeneration membranes, allografts, and platelet concentrates, have been used.^{4,5} Among these techniques and graft materials, the combination of CAF and CTG has the greatest potential for achieving complete root coverage (CRC).^{6,7}

Numerous clinical and histological studies have shown that different types of platelet concentrates may be effective in promoting soft tissue repair and periodontal regeneration.^{8–10} Concentrated growth factor (CGF) is one of the cutting-edge platelet concentrates. The centrifuge rotation speed fluctuates between 2,400 and 3,000 rpm during the preparation of CGF.¹¹ As a result of this variation in rotation speed, the fibrin matrix produced by centrifugation is larger, more concentrated, and contains higher levels of growth factors than platelet-rich fibrin (PRF).¹² Compared with other platelet preparations, CGF has greater viscosity, tensile strength and adhesive properties. In addition to growth factors, CGF contains platelets, leukocytes and CD34+ stem cells, which contribute to the regenerative process.¹³ Being an autologous fibrin-rich matrix, CGF also contains immunological cells, which may effectively control the inflammation processes.¹⁴

Investigations have been conducted to evaluate the effect of CGF on recession coverage, with follow-up periods ranging from 3 months to 1 year.^{15–17} Nevertheless, there are no long-term studies evaluating the potential benefits of using CGF in addition to CAF compared with the conventional CAF approach for the treatment of gingival recession. Hence, comparing the impact of CGF in combination with CAF treatment with that of CAF alone in terms of recession coverage was the primary aim of this randomized clinical trial (RCT). Patient-centered outcomes were assessed as secondary objectives.

Material and methods

The Consolidated Standards of Reporting Trials (CONSORT) statement was followed in reporting the present study. This clinical trial was designed as a parallel, randomized, single-center study. Two alternative therapeutic approaches were evaluated in this trial: CAF; and CAF with CGF.

Study setting and population

Between January 2020 and November 2020, patients with Miller's class I or class II gingival recession in the maxilla who reported to the Department of Periodontology, Saveetha Dental College and Hospitals, Chennai, India, were enrolled in this trial. The study protocol was approved by the institutional ethics committee (IHEC/SDC/PERIO-2001/19/056). Written informed consent was obtained from all participants. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki for research involving human participants.

Eligibility criteria

The inclusion criteria were as follows: individuals aged between 18 and 45 years; systemically healthy individuals; full-mouth plaque score (FMPS) and bleeding score of less than 15%; teeth with a probing depth (PD) ≤ 3 mm and no evidence of periodontal disease; presence of a clearly identifiable CEJ; and Miller's class I or class II gingival recession in the maxilla.

The exclusion criteria were as follows: patients with systemic diseases; patients with a history of long-term medication use; smokers; pregnant or lactating women; patients who had undergone periodontal therapy or any mucogingival surgery within the previous 3 months; patients with non-caries cervical lesions.

Randomization

This clinical trial was a monocentric, parallel, randomized, controlled, single-blinded study. Simple randomization was performed by asking each patient to select a chit bearing the type of intervention. The study was conducted in 4 phases: enrollment; allocation; follow-up; and analysis. The sample size was calculated using the G*Power software (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>), based on data from a previous study.¹⁶

Intervention

All surgical procedures were performed by a single operator (C.K.). The experimental procedures were carried out in the Department of Periodontology, Saveetha Dental College and Hospitals, by an experienced operator with extensive expertise in periodontal plastic surgery, including root coverage techniques. All procedures were performed under sterile and adequately equipped conditions, following the predefined surgical protocol.

Groups

The patients were assigned to the groups as follows: group I (control group) – CAF ($n = 44$); and group II (test group) – CAF+CGF ($n = 46$).

Surgical technique

All patients were instructed to rinse their mouth with 2% chlorhexidine for 1 min. Prior to the procedure, local infiltrative anesthesia was administered using 2% lidocaine with epinephrine (1:100,000). Following anesthesia, 2 oblique incisions extending beyond the mucogingival junction (MGJ) were made along the line angles of the adjacent teeth. Intra-sulcular incisions were then made on the buccal aspect of the teeth with recession. Following subpapillary incisions, the flap was raised in the split-full-split thickness pattern. The flap was then passively displaced coronally to achieve tension-free closure. The surgical site was thoroughly irrigated with saline. Subsequently, the papillae adjacent to the teeth with recession were de-epithelialized. Root debridement was performed using a Gracey curette (Hu-Friedy Manufacturing, Chicago, USA) on the buccal and interdental exposed root surfaces. Root biomodification was then performed using 24% ethylenediaminetetraacetic acid (EDTA) for 1 min, followed by thorough rinsing with saline.

In the test group, approx. 9 mL of the patient's venous blood was collected into a sterile Vacuette tube without the use of anticoagulants. The tubes were then centrifuged using a one-step centrifugation process (Medifuge; Silfradent, Santa Sofia, Italy) consisting of acceleration for 30 s, followed by centrifugation at 2,700 rpm for 2 min, 2,400 rpm for 4 min and 3,000 rpm for 3 min, followed by deceleration and stopping for 36 s. This process separated the blood into 4 layers: the 1st layer consisting of serum; the 2nd layer consisting of CGF; the 3rd layer consisting of the fibrin buffy coat; and the 4th layer consisting of red blood cells (RBCs). The CGF layer was separated using sterile scissors. The CGF membrane was then obtained using a compressor and immediately placed over the recession area.

The flap was then coronally displaced by 1–2 mm beyond CEJ in both the test and control groups. A sling suture was used to position the flap coronally, using 5-0 Vicryl sutures. Subsequently, interrupted sutures were placed to approximate the releasing incisions.

Data collection

Clinical outcome measurements were recorded at baseline, 6 months, 1 year, and 3 years. All clinical parameters were assessed by a single examiner (A.R.), using a UNC-15 periodontal probe. Clinical outcome measures included buccal recession depth (BRD), the width of attached gingiva (AGW), probing depth (PD), and the clinical attachment level (CAL).

Postoperative pain and potential complications or adverse effects were assessed 10 days after the intervention. Patient discomfort was evaluated using a visual analog scale (VAS).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 23 (IBM Corp, Armonk, USA). The normality of the data was assessed using the Shapiro–Wilk test. Based on the results, the data followed a parametric distribution. Frequencies and percentages were used to describe qualitative variables, whereas means and standard deviations ($M \pm SD$) were used for quantitative variables. The analysis of variance (ANOVA) was performed to compare differences in the outcome parameters between the groups at different time points.

Results

Baseline features of the patients and the defects

A total of 90 patients received treatment for gingival recession. Forty-four patients received CAF alone, while

46 patients received CAF+CGF. Of the 90 patients, 4 patients in the control group and 6 patients in the test group discontinued the study during the follow-up period (Fig. 1).

The mean age of the control group was 42.1 ± 11.4 years, with 23 of the 40 participants (57.5%) being female and the remaining 17 (42.5%) being male. Among the 71 maxillary teeth treated with CAF alone, 11 were incisors (15.5%), 19 were canines (26.8%), 36 were premolars (50.7%), and 5 were first molars (7.0%). In the test group, 25 of the 40 participants (62.5%) were female and 15 (37.5%) were male, with a mean age of 45.4 ± 7.0 years. A total of 70 maxillary teeth were treated with CAF+CGF, including 12 incisors (17.1%), 22 canines (31.4%), 28 premolars (40.0%), and 8 first molars (11.4%). The demographic characteristics of the study population, including age and gender, are presented in Table 1. There was no statistically significant difference between the 2 groups.

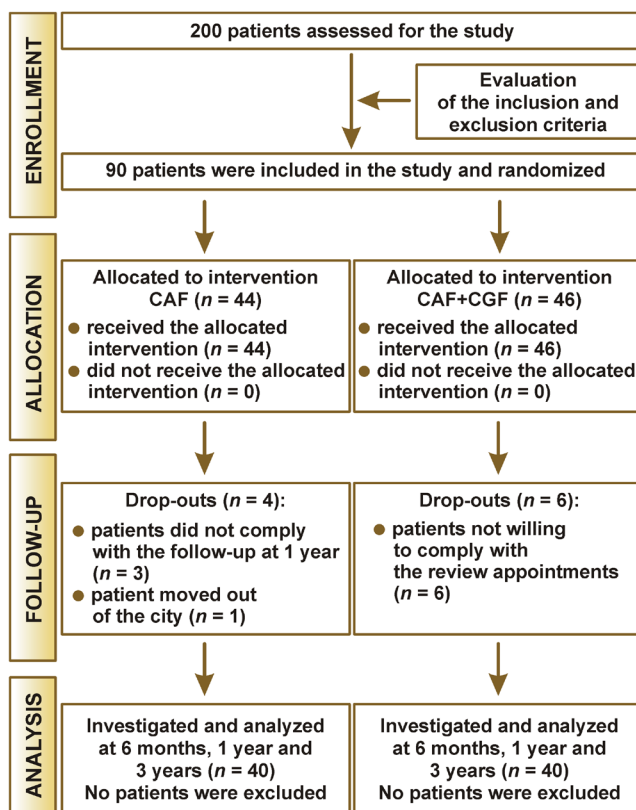


Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart of the study

CAF – coronally advanced flap; CGF – concentrated growth factor.

Table 1. Characteristics of the study groups in terms of age and gender

Variable	Group I (n = 40)	Group II (n = 40)	p-value (χ^2 test)
Age [years]	42.1 ± 11.4	45.4 ± 7.0	0.893
Gender			
M	17 (42.5)	15 (37.5)	0.769
F	23 (57.5)	25 (62.5)	

Data presented as mean standard $\pm$ deviation ($M \pm SD$) or as number (percentage) (n (%)).

Groups: I – CAF (control); II – CAF+CGF (test). M – male; F – female.

Assessment of the surgical technique and postoperative pain (at 10 days)

The mean duration of the surgical procedure was 69.4 ± 6.6 min in the test group and 64.7 ± 4.2 min in the control group, with no statistically significant difference between the groups ($p = 0.128$). The comparison of the VAS scores showed that patients in the control group reported significantly greater postoperative pain than those in the test group (41.2 ± 9.4 vs. 32.0 ± 1.9 ; $p = 0.032$).

Clinical outcomes (at baseline, 6 months, 1 year, and 3 years)

Table 2 depicts the clinical characteristics of the participants in both groups at baseline, 6 months, 1 year, and 3 years in terms of BRD, AGW, PD, and CAL. At baseline, there was no statistically significant difference between the groups for any of the parameters. At 6 months, 1 year, and 3 years of follow-up, there was a statistically significant reduction in BRD, an increase in AGW and improvement in CAL in the test group ($p = 0.000$). At all time points, there was no statistically significant difference in the PD values between the 2 groups ($p > 0.05$). Recession coverage was significantly improved as compared to baseline at each follow-up interval in both groups. However, the test group demonstrated better results at all time points (Fig. 2).

Table 2. Clinical outcome measures at baseline, 6 months, 1 year, and 3 years

Time point	Parameter	Group I	Group II	p-value (ANOVA)
Baseline	BRD [mm]	4.8 ± 0.9	5.1 ± 0.8	0.138
	AGW [mm]	3.9 ± 0.6	3.9 ± 1.2	0.415
	PD [mm]	2.3 ± 1.9	2.1 ± 0.9	0.142
	CAL [mm]	7.0 ± 2.3	7.1 ± 5.2	0.651
6 months	BRD [mm]	1.9 ± 0.7	1.0 ± 0.3	0.000*
	AGW [mm]	5.8 ± 0.5	6.9 ± 0.2	0.000*
	PD [mm]	2.2 ± 1.3	1.9 ± 0.3	0.173
	CAL [mm]	4.2 ± 2.0	2.8 ± 1.2	0.000*
1 year	BRD [mm]	2.1 ± 1.2	1.1 ± 1.9	0.000*
	AGW [mm]	5.5 ± 3.2	6.7 ± 0.7	0.000*
	PD [mm]	2.0 ± 2.4	2.2 ± 0.9	0.765
	CAL [mm]	4.1 ± 0.6	3.1 ± 0.4	0.000*
3 years	BRD [mm]	3.8 ± 0.9	2.0 ± 0.2	0.000*
	AGW [mm]	4.7 ± 1.2	6.1 ± 0.3	0.000*
	PD [mm]	2.1 ± 1.9	2.2 ± 0.7	0.755
	CAL [mm]	6.0 ± 1.2	3.5 ± 2.1	0.000*

Data presented as $M \pm SD$.

Groups: I – CAF (control); II – CAF+CGF (test). BRD – buccal recession depth; AGW – width of attached gingiva; PD – probing depth; CAL – clinical attachment level; *statistically significant.

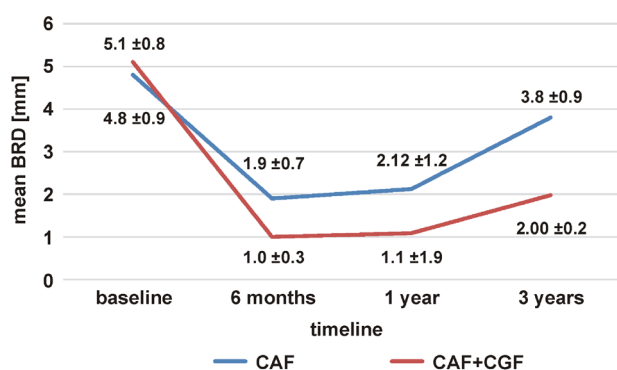


Fig. 2. Difference in buccal recession depth (BRD) after the treatment of gingival recession with the coronally advanced flap (CAF) alone and in combination with concentrated growth factor (CAF+CGF) at different time points

Discussion

For the treatment of gingival recession, the present RCT compared the use of CGF in combination with CAF with CAF alone. The study evaluated changes in BRD and AGW, and subsequent root coverage, in addition to periodontal clinical parameters, such as PD and CAL.

The literature remains equivocal regarding the impact of platelet concentrates on the outcomes of root coverage procedures. Although recent analyses have reported that platelet concentrates may reduce PD, CAL and recession depth, as well as patient morbidity,^{18,19} earlier reviews found that platelet concentrate therapies did not improve root coverage, the width of keratinized tissue or CAL.^{20,21} Recent advances in platelet concentrate centrifugation techniques and preparation protocols have raised concerns that these variations may alter the characteristics of the fibrin structure and, consequently, influence therapeutic outcomes.²² However, the literature still lacks a sufficient number of clinical studies evaluating the effect of platelet-associated biomaterials on root coverage outcomes. To the best of our knowledge, this is the first investigation to report the efficacy of CGF in recession coverage with a 3-year follow-up.

In the present trial, with no significant difference in PD and lower VAS scores, the CAF+CGF group outperformed the CAF group in terms of recession coverage, AGW and CAL. Doğan et al. employed CGF for the first time to treat gingival root defects in the maxilla.²³ After 6 months, they reported a mean root coverage of 86.67% in the CAF+CGF group. According to their study results, CGF treatment had no additive effect on root coverage. However, it was suggested that the use of CGF with CAF may improve long-term stability by increasing the width and thickness of keratinized tissue and improving CAL.²³ The findings of the present study, which are in agreement with those of Doğan et al.,²³ demonstrated enhanced AGW and CAL.

Probing depth is considered a direct indicator of periodontal health,²⁴ and was therefore included as an outcome

measure in the present study. At baseline, the mean PD was 2.3 ± 1.9 mm in the control group and 2.1 ± 0.9 mm in the test group. At 6 months, 1 year and 3 years, there was no statistically significant change in PD in either group. These findings are consistent with previous studies that evaluated the use of platelet concentrates, such as CGF and PRE, in the treatment of recession defects.¹⁵ When CAL was compared between the 2 groups, the test group demonstrated better results than the control group. This finding is in agreement with previous studies supporting the potential benefit of platelet concentrates in improving CAL following recession coverage procedures.^{25,26}

Apart from a few limitations, such as not considering buccal recession width (BRW), the height of the interproximal papilla, the gingival biotype, and the thickness of keratinized gingiva, several factors were considered in the present study to determine the effect of CGF on recession coverage and its long-term stability. Non-carious cervical lesions were excluded from the study, as they are known to have a negative impact on root coverage over time. Studies have also emphasized the influence of the tooth position on the outcomes of root coverage procedures.^{27,28} More recently, Zucchelli et al. highlighted that the tooth position was crucial in determining the amount of root coverage that could be achieved, as sites in the mandibular arch demonstrated significantly lower mean and complete recession coverage than the treated maxillary recession defects.²⁹ In the present study, only maxillary teeth were included. Collectively, the findings of this long-term trial support the additive effect of CGF in gingival augmentation coronal to the recession.

Conclusions

Within the limitations of the present study, the following conclusion can be drawn: With a 3-year long-term follow-up, the CAF+CGF group showed better results in terms of root coverage and AGW, as well as lower pain scores and reduced patient discomfort, compared with the CAF group.

Trial registration

The trial was registered with the Clinical Trials Registry–India (CTRI) (2019/02/079317).

Ethics approval and consent to participate

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki for research involving human participants. The study protocol was approved by the institutional ethics committee at the Department of Periodontology, Saveetha Dental College and Hospitals, Chennai, India (IHEC/SDC/PERIO-2001/19/056). 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

- Pradeep K, Rajababu P, Satyanarayana D, Sagar V. Gingival recession: Review and strategies in treatment of recession. *Case Rep Dent*. 2012;2012:563421. doi:10.1155/2012/563421
- Kassab MM, Cohen RE. Treatment of gingival recession. *J Am Dent Assoc*. 2002;133(11):1499–1506. doi:10.14219/jada.archive.2002.0080
- Evginer MS, Olgun E, Parlak HM, Dolgun AB, Keceli HG. Comparison of two techniques in gingival recession treatment: A randomized one-year clinical follow-up study. *Dent Med Probl*. 2022;59(1):121–130. doi:10.17219/dmp/137621
- Moraschini V, Calasans-Maia MD, Dias AT, et al. Effectiveness of connective tissue graft substitutes for the treatment of gingival recessions compared with coronally advanced flap: A network meta-analysis. *Clin Oral Investig*. 2020;24(10):3395–3406. doi:10.1007/s00784-020-03547-3
- 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
- Tatakis DN, Trombelli L. Gingival recession treatment: Guided tissue regeneration with bioabsorbable membrane versus connective tissue graft. *J Periodontol*. 2000;71(2):299–307. doi:10.1902/jop.2000.71.2.299
- Suchetha A, Lakshmi P, Bhat D, Mundinamane DB, Soorya KV, Bharwani GA. Platelet concentration in platelet concentrates and periodontal regeneration – unscrambling the ambiguity. *Contemp Clin Dent*. 2015;6(4):510–516. doi:10.4103/0976-237X.169850
- Del Fabbro M, Bortolin M, Taschieri S, Weinstein R. Is platelet concentrate advantageous for the surgical treatment of periodontal diseases? A systematic review and meta-analysis. *J Periodontol*. 2011;82(8):1100–1111. doi:10.1902/jop.2010.100605
- Tabatabaei F, Aghamohammadi Z, Tayebi L. In vitro and in vivo effects of concentrated growth factor on cells and tissues. *J Biomed Mater Res A*. 2020;108(6):1338–1350. doi:10.1002/jbm.a.36906
- Bhattacharya HS, Gummaluri SS, Rani A, Verma S, Bhattacharya P, Rayashettyyura 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
- Bommala M, Koduganti RR, Panthula VR, et al. Efficacy of root coverage with the use of the conventional versus laser-assisted flap technique with platelet-rich fibrin in class I and class II gingival recession: A randomized clinical trial. *Dent Med Probl*. 2023;60(4):583–592. doi:10.17219/dmp/150887
- Chen J, Jiang H. A comprehensive review of concentrated growth factors and their novel applications in facial reconstructive and regenerative medicine. *Aesthetic Plast Surg*. 2020;44(3):1047–1057. doi:10.1007/s00266-020-01620-6
- Rodella LF, Favero G, Boninsegna R, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microsc Res Tech*. 2011;74(8):772–777. doi:10.1002/jemt.20968
- Luo H, Liu W, Zhou Y, et al. Concentrated growth factor regulates the macrophage-mediated immune response. *Regen Biomater*. 2021;8(6):rbab049. doi:10.1093/rb/rbab049
- Dede FÖ, Doğan ŞB, Çelen K, Çelen S, Devenci ET, Cezairli NS. Comparison of the clinical efficacy of concentrated growth factor and advanced platelet-rich fibrin in the treatment of type I multiple gingival recessions: A controlled randomized clinical trial. *Clin Oral Investig*. 2023;27(2):645–657. doi:10.1007/s00784-022-04775-5
- Korkmaz B, Balli U. Clinical evaluation of the treatment of multiple gingival recessions with connective tissue graft or concentrated growth factor using tunnel technique: A randomized controlled clinical trial. *Clin Oral Investig*. 2021;25(11):6347–6356. doi:10.1007/s00784-021-03935-3
- Akcan SK, Ünsal B. Gingival recession treatment with concentrated growth factor membrane: A comparative clinical trial. *J Appl Oral Sci*. 2020;28:e20190236. doi:10.1590/1678-7757-2019-0236
- Riquelme Rodas MA, de Paula BL, Cabrera Pazmiño VF, Dos Santos Lot Vieira FF, Santiago Junior JF, Varize Silveira EM. Platelet-rich fibrin in coverage of gingival recession: A systematic review and meta-analysis. *Eur J Dent*. 2020;14(2):315–326. doi:10.1055/s-0040-1701907
- Panda S, Satpathy A, Das AC, et al. Additive effect of platelet rich fibrin with coronally advanced flap procedure in root coverage of Miller's class I and II recession defects: A PRISMA compliant systematic review and meta-analysis. *Materials (Basel)*. 2020;13(19):4314. doi:10.3390/ma13194314
- Keceli HG, Sengun D, Berberoğlu A, Karabulut E. Use of platelet gel with connective tissue grafts for root coverage: A randomized-controlled trial. *J Clin Periodontol*. 2008;35(3):255–262. doi:10.1111/j.1600-051X.2007.01181.x
- Huang LH, Neiva RE, Soehren SE, Giannobile WV, Wang HL. The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: A pilot human trial. *J Periodontol*. 2005;76(10):1768–1777. doi:10.1902/jop.2005.76.10.1768
- Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. *J Cutan Aesthet Surg*. 2014;7(4):189–197. doi:10.4103/0974-2077.150734
- Doğan ŞB, Dede FÖ, Balli U, Atalay EN, Durmuşlar MC. Concentrated growth factor in the treatment of adjacent multiple gingival recessions: A split-mouth randomized clinical trial. *J Clin Periodontol*. 2015;42(9):868–875. doi:10.1111/jcpe.12444
- Lang NP, Bartold PM. Periodontal health. *J Periodontol*. 2018;89(Suppl 1):S9–S16. doi:10.1002/JPER.16-0517
- Padma R, Shilpa A, Kumar PA, Nagasri M, Kumar C, Sreedhar A. A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's class-I and II recession defects. *J Indian Soc Periodontol*. 2013;17(5):631–636. doi:10.4103/0972-124X.119281
- Dandekar SA, Deshpande NC, Dave DH. Comparative evaluation of human chorion membrane and platelet-rich fibrin membrane with coronally advanced flap in treatment of Miller's class I and II recession defects: A randomized controlled study. *J Indian Soc Periodontol*. 2019;23(2):152–157. doi:10.4103/jisp.jisp_408_18
- Zucchelli G, Tavelli L, Ravidà A, Stefanini M, Suárez-López Del Amo F, Wang HL. Influence of tooth location on coronally advanced flap procedures for root coverage. *J Periodontol*. 2018;89(12):1428–1441. doi:10.1002/JPER.18-0201
- César Neto JB, Cavalcanti MC, Sekiguchi RT, Pannuti CM, Romito GA, Tatakis DN. Root coverage for single deep gingival recessions: Outcomes based on a decision-making algorithm. *Int J Dent*. 2019;2019:1830765. doi:10.1155/2019/1830765
- Zucchelli G, Tavelli L, Barootchi S, et al. The influence of tooth location on the outcomes of multiple adjacent gingival recessions treated with coronally advanced flap: A multicenter re-analysis study. *J Periodontol*. 2019;90(11):1244–1251. doi:10.1002/JPER.18-0732

Impact of hypertension and antihypertensive medications on early implant failure following sinus augmentation

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Abstract

Background. Hypertension (HTN) is a prevalent condition affecting a significant proportion of the population. It requires careful management due to its potential systemic complications. While the overall effects of HTN are well documented, its specific impact on implant dentistry procedures, such as sinus augmentation, remains less clear.

Objectives. The present study aimed to investigate the influence of HTN and antihypertensive medications on early implant failure (EIF) following sinus augmentation.

Material and methods. A retrospective cohort study was conducted at a tertiary referral center between 2013 and 2021. Data from 425 implants in 152 patients were analyzed. The primary outcome was EIF. Univariate and multivariable analyses were performed to identify potential risk factors. A p -value <0.05 was considered statistically significant.

Results. Hypertension did not significantly affect EIF. However, beta-blocker therapy was identified as an independent risk factor at both the patient and implant levels (OR (odds ratio) = 4.05; $p = 0.018$ and $OR = 3.13$; $p = 0.045$, respectively). A higher number of implants per patient and tobacco smoking were also associated with an increased risk of EIF ($OR = 1.49$; $p = 0.039$ and $OR = 8.52$; $p < 0.001$, respectively). Other antihypertensive medications were not significantly associated with EIF.

Conclusions. Hypertension was not a significant risk factor for EIF following sinus augmentation. However, beta-blocker therapy was associated with an increased risk of failure, whereas other antihypertensive agents, including diuretics, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and calcium channel blockers (CCBs), were not significantly associated with EIF.

Keywords: risk factors, dental implants, medications, antihypertensive agents, maxillary sinus augmentation

Cite as

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Highlights

- In a retrospective cohort of 425 implants placed in 152 patients undergoing maxillary sinus augmentation, hypertension (HTN) itself was not associated with early implant failure (EIF) and should not, on its own, be regarded as a contraindication.
- Beta-blocker therapy was an independent risk factor for EIF at both the patient level ($OR = 4.05$) and the implant level ($OR = 3.13$).
- Diuretics, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and calcium channel blockers (CCBs) showed no significant association with EIF.
- Patients receiving beta blockers may warrant more detailed preoperative counselling and closer follow-up after sinus augmentation; prospective confirmation of this association is needed.

Introduction

Sinus augmentation is a common procedure aimed at increasing bone volume in the posterior maxilla to facilitate dental implant placement. First introduced by Boyne and James in the 1970s, the technique has undergone significant advancement, becoming a predictable and widely used procedure.¹ Although generally successful, implants placed following sinus augmentation may still fail, either at an early or late stage. Early implant failure (EIF) refers to implant failure occurring before osseointegration is achieved.^{2–4}

Numerous risk factors for EIF have been identified and extensively studied. These factors can be categorized as local or systemic.⁵ Local risk factors include anatomical factors, such as residual bone height, the quality of the peri-implant micro-environment, including the quality of the keratinized gingiva, and the specific surgical technique used.^{4–10} Systemic risk factors include various health conditions, medications and patient habits, including tobacco smoking, diabetes mellitus, and the patient's physical status as graded by the American Society of Anesthesiologists (ASA) classification.^{2–5,11–13}

Hypertension (HTN) is a condition characterized by persistently elevated arterial blood pressure. According to the American Heart Association (AHA), approx. 50% of American adults have HTN.¹⁴ In 2019, Eurostat data indicated that 22% of the European Union (EU) population aged 15 years and older reported having HTN, with the highest prevalence in Croatia (37%) and the lowest in Ireland (12%).¹⁵ Normal blood pressure is defined as a systolic blood pressure of less than 120 mmHg and a diastolic blood pressure of less than 80 mmHg.¹⁴ The AHA defines HTN as a systolic blood pressure of ≥ 130 mmHg or a diastolic blood pressure of ≥ 80 mmHg.¹⁶ In contrast, the European Society of Hypertension (ESH) defines HTN as an office systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg.¹⁷

There are 2 main types of HTN – primary and secondary. Primary HTN, also known as essential or idiopathic HTN, is characterized by elevated blood pressure without an identifiable underlying cause. It is a chronic and typically irreversible condition that is more common in older individuals. Secondary HTN, in contrast, refers to elevated blood pressure caused by an underlying medical condition,

such as obstructive sleep apnea (OSA) or kidney disease. Although potentially reversible when the underlying condition is addressed, secondary HTN can be more resistant to treatment and often affects younger individuals.¹⁸ Hypertension may lead to ischemic heart disease, heart failure, kidney disease, and sexual dysfunction.¹⁶

Treatment for HTN is initially based on lifestyle modifications, including weight loss, a diet low in saturated and total fat, low in sodium and high in potassium, regular aerobic exercise, and limiting or eliminating alcohol consumption. When these measures do not result in adequate blood pressure control, antihypertensive medication therapy is initiated.¹⁶ Antihypertensive medications comprise a diverse range of pharmacological agents, each targeting a distinct biological pathway to reduce blood pressure. Diuretics, such as thiazide and loop diuretics, promote the renal excretion of sodium and water, thereby reducing blood volume and cardiac output.¹⁹ Angiotensin-converting enzyme inhibitors (ACEIs), including lisinopril and enalapril, inhibit the conversion of angiotensin I to angiotensin II, thereby reducing peripheral vascular resistance.²⁰ Angiotensin II receptor blockers (ARBs), such as losartan and valsartan, antagonize angiotensin II receptors, preventing vasoconstriction and aldosterone release.²¹ Calcium channel blockers (CCBs), such as amlodipine and nifedipine, inhibit calcium influx into arterial smooth muscle cells, promoting vasodilation and reducing cardiac contractility.²² Finally, beta blockers, including metoprolol and atenolol, antagonize beta-adrenergic receptors, thereby reducing cardiac output and renin release.^{16,23}

First-line pharmacological treatment typically includes ACEIs or ARBs, such as enalapril or candesartan, CCBs, such as amlodipine, and diuretics, such as hydrochlorothiazide. Unless patients have a history of ischemic heart disease or heart failure, beta blockers are generally not prescribed as first-line agents, as their benefit in stroke prevention is lower than that of the aforementioned first-line therapies.^{16,24}

While the systemic effects of HTN are well established, its impact on implant dentistry, particularly sinus augmentation, remains less well understood. Recent systematic reviews by Mishra et al.²⁵ and Hamadé et al.²⁶ examined the relationship between HTN and implant failure, and concluded that HTN itself did not significantly increase

the failure rates, while patients receiving antihypertensive medications had comparable implant success rates to non-medicated patients. However, both reviews highlighted limitations in the existing literature, particularly the tendency to group different antihypertensive medications together, which limits a more nuanced understanding of their individual effects on implant outcomes.^{25,26}

To address this gap in knowledge, the present study aimed to investigate the influence of HTN, as well as specific medications used to manage the condition, on EIF following sinus augmentation. The null hypothesis was that neither HTN nor antihypertensive medications influence EIF following sinus floor elevation.

Material and methods

Following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines,²⁷ a retrospective cohort study was conducted at a tertiary referral center (Department of Oral and Maxillofacial Surgery, Rabin Medical Center, Petah Tiqwa, Israel) between January 2013 and December 2021. All study participants provided written informed consent prior to enrollment. The study adhered to the ethical standards outlined in the Declaration of Helsinki and was approved by the institutional review board at Rabin Medical Center (approval No. 0674-19).

The characteristics of this cohort reflect those of the medical center's patient population, which is referred to this department for the management of complex systemic and local conditions.

Surgical protocol

Patients with less than 5 mm of residual bone height underwent sinus floor elevation using the lateral window approach described by Boyne and James (1980). Individuals with 5–8 mm of residual bone height underwent the Summers technique for transcrestal sinus floor elevation to facilitate dental implant placement.^{1,28} Prior to the procedure, three-dimensional (3D) imaging, using either cone-beam computed tomography (CBCT) or multidetector computed tomography (MDCT), was performed to evaluate the bone structure.

The surgical procedure involved accessing the maxillary sinus through the creation of a surgical flap. Incisions were made along the midline of the alveolar crest and extended vertically, with additional incisions performed when necessary to facilitate adequate closure and ensure flap flexibility. A round diamond bur was used to create a window in the lateral sinus wall, through which the sinus membrane was carefully elevated. Any accidental perforations of the membrane were repaired using a collagen barrier (Ossix® Plus; Datum Dental, Lod, Israel). The resulting space was subsequently filled with a bovine-derived bone graft material (Bio-Oss®; Geistlich Pharma, Wolhusen, Switzerland).

Following augmentation, implants were placed according to the manufacturer's instructions, using the dedicated bur set, ensuring adequate primary stability. The surgical site was then meticulously closed with sutures to promote blood clot formation and facilitate healing.

Implants were either placed simultaneously with the augmentation procedure or using a delayed approach, in which implant placement was performed 4 months after the initial surgery. In the latter cases, a second minor surgical procedure was performed 5 months later to uncover the implants. Final tooth restorations were placed 9 months after the initial intervention, allowing sufficient time for healing and osseointegration.

The patients were discharged with a 7-day regimen of amoxicillin–clavulanate (875 mg twice daily). Patients with a penicillin allergy received clindamycin (300 mg 3 times daily) for 7 days. All patients were also prescribed xylometazoline nasal spray twice daily for 3 days.

Eligibility criteria

Patients were eligible for inclusion if they were either healthy (ASA I–II)²⁹ or had HTN, with the diagnosis of HTN based on the patients' medical records (see "Data collection" below for further details), were aged >18 years, underwent sinus augmentation using either a lateral or transcrestal approach, had complete radiographic records, and had complete follow-up data from the time of sinus augmentation until abutment connection.

Patients were excluded if they had incomplete documentation, underwent implant placement in the posterior maxilla without prior sinus augmentation, or had systemic conditions other than HTN.

Data collection

To minimize human and measurement errors, data were collected 3 times by two independent collectors (E.J. and D.M.). In cases of discrepancies between the two collectors, consensus was reached through discussion. The medical center's electronic medical record (EMR) systems provided access to data from other medical centers and community clinics, including prescribed and purchased medications, past and present diagnoses, imaging and pathology results, and complete blood count (CBC) data. The comprehensive nature of these medical records minimized the risk of non-disclosure bias, and enhanced the reliability and credibility of the database.

The data collected comprised:

- demographic data – age; gender;
- patient-level data – systemic conditions; medications (diuretics (furosemide, spironolactone, triamterene, hydrochlorothiazide, chlorthalidone), ACEIs (lisinopril, enalapril, ramipril, captopril), ARBs (losartan, valsartan, candesartan), CCBs (amlodipine, nifedipine, diltiazem, verapamil, felodipine), beta blockers (metoprolol, atenolol, propranolol)); tobacco smoking;

– surgical data – the sinus augmentation technique; residual alveolar ridge height; implant dimensions; and bone gain (calculated by subtracting residual alveolar ridge height from implant length).

Primary outcome variable

The primary outcome variable was EIF, defined as implant removal before temporary or permanent rehabilitation.

Statistical analysis

In this study, Python 3.12, Pandas 2.1.3 and Statsmodels 0.14.1 were used for comprehensive data analysis. Descriptive statistics were used to summarize the data, with continuous variables presented as means and standard deviations ($M \pm SD$) and categorical variables as frequencies and percentages. The normality of variable distribution was assessed using the Kolmogorov–Smirnov test.

Patient-level associations were explored using the χ^2 test for univariate analysis. Differences between independent groups were assessed using the Mann–Whitney U test for comparisons between two groups and the Kruskal–Wallis test for comparisons among more than two groups. To account for within-subject correlation at the implant level, generalized estimating equations (GEE) were employed. The GEE model accounted for subject-level clustering and was specified with a binomial distribution and an exchangeable correlation structure.

Multicollinearity was assessed using the variance inflation factor (VIF). Variables with a p -value < 0.10 and no evidence of multicollinearity were included in the multivariable regression analysis. Generalized estimating equations were applied at the implant level, whereas generalized linear models (GLM) were used at the patient level.

All statistical analyses were performed using a significance level of $p < 0.05$.

Results

A total of 425 implants were placed in 152 individuals. The mean patient age was 61.72 ± 14.27 years, and 101 patients (66.45%) were female. Nine patients were tobacco smokers, while 114 (75.00%) had HTN and the remainder were healthy (ASA I). Regarding antihypertensive medications, 25 patients (16.45%) were treated with diuretics, 46 (30.26%) with ACEIs, 31 (20.39%) with ARBs, 34 (22.37%) with CCBs, and 72 (47.37%) with beta blockers. On average, the patients took 1.37 ± 1.05 antihypertensive medications. Regarding the surgical technique, 58 patients (38.16%) underwent simultaneous implant placement and sinus augmentation, 54 (35.53%) underwent delayed implant placement, and the remaining patients underwent transcrestal sinus augmentation. Eighteen patients (11.84%) experienced at least one EIF (Table 1).

At the implant level, 25 implants (5.88%) were placed in smokers and 323 (76.00%) in patients with HTN. Additionally, 14.35% of implants were placed in patients taking diuretics, 30.59% in patients taking ACEIs, 21.65% in patients taking ARBs, 25.41% in patients taking CCBs, and 47.29% in those taking beta-blockers. The mean implant length was 12.40 ± 1.20 mm, the mean residual alveolar bone height was 4.87 ± 1.88 mm, and the mean bone gain was 7.52 ± 2.37 mm. Twenty-four implants (5.65%) were associated with at least one EIF (Table 2).

Univariate analysis at the patient level identified beta-blocker therapy ($p = 0.040$) and delayed implant placement ($p = 0.010$) as significant risk factors for EIF. In addition, the patients who experienced EIF received a significantly higher mean number of implants than those without EIF (3.78 ± 1.76 vs. 2.67 ± 1.30 , respectively; $p = 0.006$) (Table 3).

Table 1. Descriptive statistics at the patient level ($N = 152$)

Variable	Subgroup		<i>n</i> (%)	<i>M</i> ± <i>SD</i>
Age [years]	–		–	61.72 ±14.27
Gender	M		51 (33.55)	–
	F		101 (66.45)	–
Tobacco smoking	number of cigarettes per patient per day among smokers		–	16.67 ±17.75
	yes		9 (5.92)	–
	no		143 (94.08)	–
Medical condition	healthy		38 (25.00)	–
	HTN		114 (75.00)	–
Antihypertensive medications	diuretics	yes	25 (16.45)	–
		no	127 (83.55)	–
	ACEIs	yes	46 (30.26)	–
		no	106 (69.74)	–
	ARBs	yes	31 (20.39)	–
		no	121 (79.61)	–
	CCBs	yes	34 (22.37)	–
		no	118 (77.63)	–
	beta blockers	yes	72 (47.37)	–
		no	80 (52.63)	–
Number of medications	number of medications per patient per day		–	1.37 ±1.05
	0		38 (25.00)	–
	1		46 (30.26)	–
	2		44 (28.95)	–
	3		22 (14.47)	–
	4		2 (1.32)	–
Surgical technique	simultaneous		58 (38.16)	–
	delayed		54 (35.53)	–
	transcrestal		40 (26.32)	–
Number of implants per patient			–	2.79 ±1.40
EIF			18 (11.84)	–

M – male; F – female; ACEIs – angiotensin-converting enzyme inhibitors; ARBs – angiotensin II receptor blockers; CCBs – calcium channel blockers; EIF – early implant failure; HTN – hypertension; M – mean; SD – standard deviation.

Table 2. Descriptive statistics at the implant level ($N = 425$)

Variable	Subgroup	n (%)	$M \pm SD$
Age [years]	–	–	61.72 ± 14.27
Gender	M	155 (36.47)	–
	F	270 (63.53)	–
Tobacco smoking	number of cigarettes per implant among smokers	–	18.00 ± 16.95
	yes	25 (5.88)	–
	no	400 (94.12)	–
ASA classification	I	102 (24.00)	–
	II	132 (31.06)	–
	III	191 (44.94)	–
Medical condition	healthy	102 (24.00)	–
	HTN	323 (76.00)	–
Antihypertensive medications	diuretics	yes	61 (14.35)
		no	364 (85.65)
	ACEIs	yes	130 (30.59)
		no	295 (69.41)
	ARBs	yes	92 (21.65)
		no	333 (78.35)
	CCBs	yes	108 (25.41)
		no	317 (74.59)
	beta blockers	yes	201 (47.29)
		no	224 (52.71)

Variable	Subgroup	n (%)	$M \pm SD$
Number of medications	number of medications per implant	–	1.39 ± 1.03
	0	102 (24.00)	–
	1	124 (29.18)	–
	2	133 (31.29)	–
	3	62 (14.59)	–
Surgical technique	4	4 (0.94)	–
	simultaneous	161 (37.88)	–
	delayed	186 (43.76)	–
	transcrestal	78 (18.35)	–
Implant length [mm]	–	–	12.40 ± 1.20
Implant diameter [mm]	–	–	3.94 ± 0.40
Residual alveolar bone height [mm]	–	–	4.87 ± 1.88
Bone gain [mm]	–	–	7.52 ± 2.37
EIF	–	24 (5.65)	–

ASA – American Society of Anesthesiologists.

Table 3. Univariate statistical analysis at the patient level

Variable	Subgroup		Success N = 134	Failure N = 18	Test statistic	p-value
Categorical variables (χ^2 test) n (%)						
Gender	M		44 (32.84)	7 (38.89)	0.06	0.800
	F		90 (67.16)	11 (61.11)		
Tobacco smoking	yes		6 (4.48)	3 (16.67)	2.33	0.130
	no		128 (95.52)	15 (83.33)		
Medical condition	healthy		36 (26.87)	2 (11.11)	1.34	0.240
	HTN		98 (73.13)	16 (88.89)		
Antihypertensive medications	diuretics	yes	22 (16.42)	3 (16.67)	0.00	1.000
		no	112 (83.58)	15 (83.33)		
	ACEIs	yes	40 (29.85)	6 (33.33)	0.00	1.000
		no	94 (70.15)	12 (66.67)		
	ARBs	yes	28 (20.90)	3 (16.67)	0.01	0.910
		no	106 (79.10)	15 (83.33)		
	CCBs	yes	29 (21.64)	5 (27.78)	0.08	0.770
		no	105 (78.36)	13 (72.22)		
	beta blockers	yes	59 (44.03)	13 (72.22)	3.99	0.040**
		no	75 (55.97)	5 (27.78)		
Surgical technique	simultaneous		54 (40.30)	4 (22.22)	8.73	0.010***
	delayed		42 (31.34)	12 (66.67)		
	transcrestal		38 (28.36)	2 (11.11)		
Continuous variables (Mann–Whitney U test) M $\pm$ SD						
Age [years]			61.36 $\pm$ 14.81	64.40 $\pm$ 9.13	1,262	0.750
Number of medications			1.32 $\pm$ 1.04	1.67 $\pm$ 1.08	1,401	0.250
Number of implants per patient			2.67 $\pm$ 1.30	3.78 $\pm$ 1.76	1,669	0.006***

* $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$ (statistical significance).

At the implant level, univariate analysis showed that tobacco smoking significantly increased the odds of EIF by more than five-fold (OR (odds ratio) = 5.19; $p = 0.009$). Delayed sinus augmentation was also significantly associated with an increased risk of EIF ($OR = 3.69$; $p = 0.030$). Furthermore, each 1-millimeter increase in bone gain was associated with a 14% increase in the odds of EIF ($OR = 1.14$; $p = 0.030$). Beta-blocker use ($OR = 2.02$; $p = 0.080$), ARB use ($OR = 0.54$; $p = 0.090$) and residual alveolar bone height ($OR = 0.85$; $p = 0.070$) demonstrated borderline statistical significance ($0.05 < p < 0.10$) (Table 4).

Multivariable analysis at the patient level identified beta-blocker therapy ($OR = 4.05$; $p = 0.018$) and a higher number of implants ($OR = 1.49$ per additional implant; $p = 0.039$) as independent risk factors for EIF (Table 5). At the implant level, multivariable analysis using GEE demonstrated a 19.8% within-patient correlation among the implants placed in the same individual. Tobacco smoking ($OR = 8.52$; $p < 0.001$) and beta-blocker therapy ($OR = 3.13$; $p = 0.045$) were significant independent risk factors for EIF (Table 6).

Delayed sinus augmentation showed a consistent trend toward increased EIF in both multivariable models ($OR = 3.20$; $p = 0.068$ at the patient level; $OR = 3.67$; $p = 0.058$ at the implant level), but did not reach statistical significance.

Table 4. Univariate statistical analysis at the implant level

Variable	Subgroup		OR	95% CI	p-value
Categorical variables					
Gender	M		1	–	–
	F		0.91	0.33–2.54	0.860
Tobacco smoking	yes		5.19	1.49–18.01	0.009***
	no		1	–	–
Medical condition	healthy		1	–	–
	HTN		1.53	0.34–6.82	0.570
Antihypertensive medications	diuretics	yes	0.91	0.27–3.09	0.880
		no	1	–	–
	ACEIs	yes	0.84	0.30–2.37	0.750
		no	1	–	–
	ARBs	yes	0.54	0.16–1.86	0.090*
		no	1	–	–
	CCBs	yes	1.21	0.41–3.60	0.720
		no	1	–	–
	beta blockers	yes	2.02	0.68–6.02	0.080*
		no	1	–	–
Surgical technique	simultaneous		1	–	–
	delayed		3.69	1.10–12.33	0.030**
Continuous variables					
Age			1	0.99–1.03	0.400
Number of medications			1.07	0.67–1.71	0.760
Implant length			0.97	0.68–1.40	0.880
Implant diameter			1.57	0.58–4.25	0.380
Residual alveolar bone height			0.85	0.71–1.02	0.070*
Bone gain			1.14	1.01–1.29	0.030**

OR – odds ratio; CI – confidence interval; * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$ (statistical significance).

Table 5. Multivariable statistical analysis at the patient level

Risk factors for EIF	OR	95% CI	p-value
Surgical technique (delayed)	3.20	0.91–11.76	0.068
Surgical technique (transcrestal)	0.99	0.16–6.05	0.990
Beta-blocker therapy	4.05	1.27–12.93	0.018**
Number of implants	1.49	1.00–2.20	0.039**

Note: The reference group for “Surgical technique” is “simultaneous”.
* $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$ (statistical significance).

Table 6. Multivariable statistical analysis at the implant level

Risk factors for EIF	OR	95% CI	p-value
Smoking	8.52	2.68–27.08	<0.001***
Surgical technique (delayed)	3.67	0.96–14.07	0.058*
Surgical technique (transcrestal)	1.49	0.21–10.52	0.680
Beta-blocker therapy	3.13	1.00–9.57	0.045**
ARB therapy	0.46	0.14–1.59	0.220
Residual alveolar bone height	0.87	0.72–1.04	0.120

Notes: The reference group for “Surgical technique” is “simultaneous”; bone gain was removed from the analysis for multicollinearity with residual alveolar bone height. * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$ (statistical significance).

Discussion

Hypertension is a common condition, affecting approx. 50% of adults, and is frequently encountered in daily clinical practice.¹⁴ As the population ages, implant placement in medically compromised patients is becoming increasingly common, necessitating a comprehensive understanding of the effects of systemic chronic comorbidities and the medications used to manage them.⁵

The results of the present study showed that the patients diagnosed with HTN did not have significantly higher odds of EIF compared with healthy individuals, consistent with previous studies evaluating implants placed in patients with HTN.^{8,11,25,26,30–32} Hypertension has been associated with increased bone loss and impaired new bone formation, potentially due to its association with impaired angiogenesis.^{32,33} During HTN, endothelial function is compromised, resulting in the narrowing of the capillaries and reduced blood flow within the microvasculature, which may lead to vascular rarefaction, and potentially impair osteogenesis.³⁴ However, these findings do not necessarily indicate that HTN has no effect on the occurrence of EIF following sinus floor elevation. All patients with HTN included in this study were receiving antihypertensive treatment, and were therefore likely to have relatively well-controlled disease, which limits the generalizability of these findings. Consequently, we cannot conclude that uncontrolled HTN would not influence the occurrence of EIF or the success of sinus floor elevation procedures.

Studies investigating the effects of antihypertensive medications on bone metabolism have suggested that several classes, including diuretics, ACEIs, ARBs, and beta blockers, may have beneficial effects on bone metabolism, fracture resistance and calcium homeostasis, potentially promoting osseointegration.^{30,32,34} The beneficial effects of ACEIs, ARBs and CCBs on the small-artery structure may further contribute to improved osseointegration and a reduced EIF risk. These medications may inhibit adverse vascular remodeling, exert antioxidant effects, reduce vascular stiffness, and normalize collagen-to-elastin ratios in small arteries, potentially benefiting bone health and implant success.³²

Multivariable analyses at both the patient and implant levels revealed that the implants placed in the patients receiving beta-blocker therapy had significantly higher odds of failure (patient level: $OR = 4.05$; implant level: $OR = 3.13$). Beta blockers are commonly prescribed for persistent HTN or for patients with HTN and comorbid conditions, such as ischemic heart disease, cerebrovascular disease or heart failure.¹⁶ In the present study, however, these comorbidities were not significantly associated with EIF. Beta blockers have been reported to be less effective in promoting favorable vascular remodeling. Their lack of vasodilatory properties, together with their potential association with increased arterial stiffness, may impair microcirculation.³⁴ These changes could potentially reduce

blood flow to the implant site, thereby impairing osseointegration and increasing the risk of EIF.

It may therefore be hypothesized that the observed association between beta-blocker therapy and EIF reflects a more advanced stage of HTN among patients receiving these medications, which may adversely affect vascular remodeling and increase the risk of failure. The number of antihypertensive medications may serve as an indicator of disease severity.³⁵ Based on this hypothesis, the odds of failure would be expected to increase with a greater number of antihypertensive medications. However, in the present study, the number of antihypertensive medications was not significantly associated with EIF.

The influence of tobacco smoking on EIF and HTN has been extensively discussed in the literature.³⁶ Although cigarette smoking acutely increases blood pressure through the stimulation of the sympathetic nervous system, its long-term effects on blood pressure and the incidence of HTN remain equivocal.³⁷ Observational studies have reported a modest positive association between smoking and the risk of HTN.^{38,39} However, a definitive causal relationship between cigarette smoking and HTN has not been established, as smoking cessation does not consistently result in a reduction in blood pressure.³⁶

In the present study, tobacco smoking was associated with an 8.52-fold increase in the odds of EIF at the implant level. This finding is consistent with previous studies reporting a higher risk of EIF among smokers.^{4,5,8,9,13,40} Nicotine may adversely affect osseointegration and new bone formation by disrupting critical processes involved in microvascular function, angiogenesis and growth-factor migration. These effects may result from several interconnected mechanisms, including nicotine-induced vasoconstriction, increased catecholamine release and microvascular occlusion.⁴⁰

The number of implants per patient was a significant risk factor for EIF, with each additional implant increasing the odds of failure by 49%. This finding is consistent with previous studies.^{5,41} The increased risk may be attributed to the longer and more extensive surgical procedures associated with the placement of multiple implants, and larger augmentation procedures. These factors may prolong the healing period and compromise blood flow to the augmented sites, thereby increasing susceptibility to infection and impaired healing. Another potential explanation for the increased risk of failure is the association between residual alveolar bone height and the number of implants. In the present study, lower residual alveolar bone height was associated with a higher number of implants. Although the present analysis did not demonstrate a significant association between EIF and residual alveolar bone height, several studies have reported that reduced residual bone height is associated with lower success rates following sinus floor augmentation.^{5,42–44} It may therefore be hypothesized that, in addition to the increased surgical complexity associated with multiple implants, reduced residual bone height may

further compromise the biological processes involved in bone augmentation and osseointegration in these patients.

Limitations

The limitations of this study arise from its retrospective design, the involvement of multiple operators, and the characteristics of the medical center. The participation of multiple operators may have introduced variability in case selection, the surgical technique, and the management of intraoperative complications. In addition, the data were collected from a tertiary medical center that treats patients with complex systemic and local conditions, which may limit the external validity and generalizability of the study findings.

Conclusions

In conclusion, HTN was not significantly associated with EIF following sinus floor augmentation. However, beta-blocker therapy was identified as a significant risk factor, whereas other antihypertensive medications, including diuretics, ACEIs, ARBs, and CCBs, were not significantly associated with EIF. In addition, a higher number of implants per patient and tobacco smoking were identified as significant risk factors for EIF.

Ethics approval and consent to participate

All study participants provided written informed consent prior to enrollment. The study adhered to the ethical standards outlined in the Declaration of Helsinki and was approved by the institutional review board at Rabin Medical Center, Petah Tiqwa, Israel (approval No. 0674-19).

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

- Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg.* 1980;38(8):613–616. PMID:6993637.
- Chrcanovic BR, Kisch J, Albrektsson T, Wennerberg A. Factors influencing early dental implant failures. *J Dent Res.* 2016;95(9):995–1002. doi:10.1177/0022034516646098
- Basson AA, Mann J, Findler M, Chodick G. Correlates of early dental implant failure: A retrospective study. *Int J Oral Maxillofac Implants.* 2023;38(5):897–906. doi:10.11607/jomi.10199
- Yari A, Fasih P, Alborzi S, Nikzad H, Romozi E. Risk factors associated with early implant failure: A retrospective review. *J Stomatol Oral Maxillofac Surg.* 2024;125(4):101749. doi:10.1016/j.jormas.2023.101749
- Masri D, Jonas E, Avishai G, Rosenfeld E, Chaushu L, Chaushu G. Risk factors contributing to early implant failure following sinus augmentation: A study of a challenging cohort. *J Oral Rehabil.* 2023;50(11):1239–1252. doi:10.1111/joor.13560
- Guarnieri R, Reda R, Zanza A, Miccoli G, Di Nardo D, 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
- 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
- Chrcanovic BR, Kisch J, Albrektsson T, Wennerberg A. Analysis of risk factors for cluster behavior of dental implant failures. *Clin Implant Dent Relat Res.* 2017;19(4):632–642. doi:10.1111/cid.12485
- Atarchi AR, Miley DD, Omran MT, Abdulkareem AA. Early failure rate and associated risk factors for dental implants placed with and without maxillary sinus augmentation: A retrospective study. *Int J Oral Maxillofac Implants.* 2020;35(6):1187–1194. doi:10.11607/jomi.8447
- Jonas E, Masri D, Ghanaie O, Avishai G, Chaushu G, Chaushu L. The impact of sinus anatomy on early implant failure following sinus augmentation: A retrospective cohort study. *Int Dent J.* 2025;75(2):984–991. doi:10.1016/j.identj.2024.10.009
- Alsaadi G, Quirynen M, Michiles K, Teughels W, Komárek A, Van Steenberghe D. Impact of local and systemic factors on the incidence of failures up to abutment connection with modified surface oral implants. *J Clin Periodontol.* 2008;35(1):51–57. doi:10.1111/j.1600-051X.2007.01165.x
- Oates TW, Dowell S, Robinson M, McMahan CA. Glycemic control and implant stabilization in type 2 diabetes mellitus. *J Dent Res.* 2009;88(4):367–371. doi:10.1177/0022034509334203
- Fu M, Ye Y, Pu R, Zhu D, Yang G, Jiang Z. Patient and implant-related risk factors for implant failure of one-stage lateral sinus floor elevation: A 2- to 10-year retrospective study. *Clin Implant Dent Relat Res.* 2024;26(6):1221–1232. doi:10.1111/cid.13380
- American Heart Association. Understanding blood pressure readings. <https://www.heart.org/en/health-topics/high-blood-pressure/understanding-blood-pressure-readings>. Accessed July 27, 2024.
- Eurostat. 22% of people in the EU have high blood pressure. <https://ec.europa.eu/eurostat/web/products-eurostat-news/-/edn-20210929-1>. Accessed December 23, 2024.
- Carey RM, Moran AE, Whelton PK. Treatment of hypertension: A review. *JAMA.* 2022;328(18):1849–1861. doi:10.1001/jama.2022.19590
- Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. *J Hypertens.* 2018;36(10):1953–2041. doi:10.1097/HJH.0000000000001940
- Tandan N, Lavie CJ, Stewart MH, Milani RV, Ventura HO. Secondary hypertension: Evaluation and management. *Curr Opin Cardiol.* 2023;38(4):318–325. doi:10.1097/HCO.0000000000001059
- Ernst ME, Fravel MA. Thiazide and the thiazide-like diuretics: Review of hydrochlorothiazide, chlorthalidone, and indapamide. *Am J Hypertens.* 2022;35(7):573–586. doi:10.1093/ajh/hpac048
- Turner JM, Kodali R. Should angiotensin-converting enzyme inhibitors ever be used for the management of hypertension? *Curr Cardiol Rep.* 2020;22(9):95. doi:10.1007/s11886-020-01352-8

21. Gallo G, Volpe M, Rubattu S. Angiotensin receptor blockers in the management of hypertension: A real-world perspective and current recommendations. *Vasc Health Risk Manag.* 2022;18:507–515. doi:10.2147/VHRM.S337640
22. Shah K, Seeley S, Schulz C, Fisher J, Rao SG. Calcium channels in the heart: Disease states and drugs. *Cells.* 2022;11(6):943. doi:10.3390/cells11060943
23. Fumagalli C, Maurizi N, Marchionni N, Fornasari D. β -blockers: Their new life from hypertension to cancer and migraine. *Pharmacol Res.* 2020;151:104587. doi:10.1016/j.phrs.2019.104587
24. Lindholm LH, Carlberg B, Samuelsson O. Should beta blockers remain first choice in the treatment of primary hypertension? A meta-analysis. *Lancet.* 2005;366(9496):1545–1553. doi:10.1016/S0140-6736(05)67573-3
25. Mishra SK, Sonnahalli NK, Chowdhary R. Do antihypertensive medications have an effect on dental implants? A systematic review. *Oral Maxillofac Surg.* 2024;28(2):459–468. doi:10.1007/s10006-023-01167-1
26. Hamadé L, El-Disoki S, Chrcanovic BR. Hypertension and dental implants: A systematic review and meta-analysis. *J Clin Med.* 2024;13(2):499. doi:10.3390/jcm13020499
27. Cuschieri S. The STROBE guidelines. *Saudi J Anaesth.* 2019;13(Suppl 1):S31–S34. doi:10.4103/sja.SJA_543_18
28. Summers RB. A new concept in maxillary implant surgery: The osteotome technique. *Compendium.* 1994;15(2):152–162. PMID:8055503.
29. Maloney WJ, Weinberg MA. Implementation of the American Society of Anesthesiologists Physical Status classification system in periodontal practice. *J Periodontol.* 2008;79(7):1124–1126. doi:10.1902/jop.2008.070625
30. Masri D, Bar-Hai D, Masri-Iraqi H, Kahn A, Chaushu G, Chaushu L. Early implant failure in patients using antihypertensive medications: A retrospective cohort study. *Int Dent J.* 2025;75(2):1081–1087. doi:10.1016/j.identj.2024.05.003
31. Tonini KR, Hadad H, Egas LS, Sol I, Perri de Carvalho PS, Ponzoni D. Successful osseointegrated implants in hypertensive patients: Retrospective clinical study. *Int J Oral Maxillofac Implants.* 2022;37(3):501–507. doi:10.11607/jomi.9425
32. Wu X, Al-Abedalla K, Eimar H, et al. Antihypertensive medications and the survival rate of osseointegrated dental implants: A cohort study. *Clin Implant Dent Relat Res.* 2016;18(6):1171–1182. doi:10.1111/cid.12414
33. Humar R, Zimmerli L, Battegay E. Angiogenesis and hypertension: An update. *J Hum Hypertens.* 2009;23(12):773–782. doi:10.1038/jhh.2009.63
34. Rizzoni D, De Ciuceis C, Salvetti M, et al. Interactions between macro- and micro-circulation: Are they relevant? *High Blood Press Cardiovasc Prev.* 2015;22(2):119–128. doi:10.1007/s40292-015-0086-3
35. Levy PD, Willock RJ, Burla M, et al. Total antihypertensive therapeutic intensity score and its relationship to blood pressure reduction. *J Am Soc Hypertens.* 2016;10(12):906–916. doi:10.1016/j.jash.2016.10.005
36. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol.* 2020;16(4):223–237. doi:10.1038/s41581-019-0244-2
37. Virdis A, Giannarelli C, Neves MF, Taddei S, Ghiadoni L. Cigarette smoking and hypertension. *Curr Pharm Des.* 2010;16(23):2518–2525. doi:10.2174/138161210792062920
38. Halperin RO, Gaziano JM, Sesso HD. Smoking and the risk of incident hypertension in middle-aged and older men. *Am J Hypertens.* 2008;21(2):148–152. doi:10.1038/ajh.2007.36
39. Bowman TS, Gaziano JM, Buring JE, Sesso HD. A prospective study of cigarette smoking and risk of incident hypertension in women. *J Am Coll Cardiol.* 2007;50(21):2085–2092. doi:10.1016/j.jacc.2007.08.017
40. Kasat V, Ladda R. Smoking and dental implants. *J Int Soc Prev Community Dent.* 2012;2(2):38–41. doi:10.4103/2231-0762.109358
41. Naert I, Koutsikakis G, Duyck J, Quirynen M, Jacobs R, Van Steenberghe D. Biologic outcome of implant-supported restorations in the treatment of partial edentulism. Part I: A longitudinal clinical evaluation. *Clin Oral Implants Res.* 2002;13(4):381–389. doi:10.1034/j.1600-0501.2002.130406.x
42. Pesce P, Menini M, Canullo L, et al. Radiographic and histomorphometric evaluation of biomaterials used for lateral sinus augmentation: A systematic review on the effect of residual bone height and vertical graft size on new bone formation and graft shrinkage. *J Clin Med.* 2021;10(21):4996. doi:10.3390/jcm10214996
43. Zinser MJ, Randelzhofer P, Kuiper L, Zöller JE, De Lange GL. The predictors of implant failure after maxillary sinus floor augmentation and reconstruction: A retrospective study of 1045 consecutive implants. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2013;115(5):571–582. doi:10.1016/j.oooo.2012.06.015
44. Felice P, Pistilli R, Piattelli M, Soardi E, Barausse C, Esposito M. 1-stage versus 2-stage lateral sinus lift procedures: 1-year post-loading results of a multicentre randomised controlled trial. *Eur J Oral Implantol.* 2014;7(1):65–75. PMID:24892114.

Endodontic status of patients with coronary atherosclerosis: A cross-sectional study

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Abstract

Background. There are no studies in the Colombian population assessing the prevalence and severity of apical lesions (ALs), or the relationship between the frequency and quality of endodontic treatment (ET) obturation and the presence of ALs in patients with coronary atherosclerosis (CA).

Objectives. The present study aimed to estimate the frequency of ALs and ET in Colombian patients with CA, and to identify a possible association between the frequency and severity of ALs and CA.

Material and methods. The study had a cross-sectional design. We included patients with the evidence of CA diagnosed by coronary angiography. The main outcome variables were the number of compromised coronary arteries and their degree of obstruction, as well as ET, including ALs and previously treated teeth (PTT). The periapical index (PAI) was assessed using periapical radiographs.

Results. A total of 1,394 teeth from 69 patients were evaluated ($\delta = 20.2$ teeth/patient). The frequency of ALs and ET was 5.7% and 10.1%, respectively, considering all teeth evaluated, with a greater presence in the maxilla (50.6% and 51.4%, respectively). Apical lesions were most frequently observed in premolars and molars (35.4%), whereas ET was more frequent in molars (38.6%). Among teeth with ALs, PAI 3 was the most frequent score. Apical lesions were present in 36.6% of endodontically treated teeth; 72.9% of these teeth had inadequate obturation, of which 40 (39.2%) had ALs ($p = 0.021$).

Conclusions. In patients with CA, a high incidence of ALs and ET was observed, with no evidence of an association between the frequency of ALs, the severity of coronary vessel occlusion, and the number of coronary arteries affected. More ALs were observed in teeth with inadequate ET. However, as we did not have a control group for comparison, these findings should be interpreted with caution.

Keywords: atherosclerosis, coronary artery disease, coronary disease, periapical periodontitis, root canal obturation

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Highlights

- This study reveals high rates of apical lesions (ALs) and endodontic treatment (ET) in patients with coronary atherosclerosis (CA), highlighting potential oral health implications in cardiovascular care.
- A greater presence of ALs was observed in males, which was partially related to a higher prevalence of CA in these patients.
- Teeth with inadequate ET had more ALs.

Introduction

Coronary atherosclerosis (CA) is the underlying cause of several cardiovascular diseases (CVDs). It is characterized by the narrowing of the arterial lumen due to the accumulation of fatty deposits and the loss of elasticity of the arterial walls, resulting in vessel stenosis and reduced blood flow.^{1,2} Coronary atherosclerosis has been defined as a lesion of the intimal layer of blood vessels, comprising a fibrous cap and an atheromatous core. The plaque is composed of smooth muscle cells, the extracellular matrix (ECM), inflammatory cells, calcifications, lipids, and necrotic debris.³ Cardiovascular diseases are among the leading causes of disability and mortality worldwide.⁴ According to Joseph et al., in 2015, deaths attributable to CVDs accounted for approx. 1/3 of all deaths globally.⁵ In Colombia, CVDs are a leading cause of death. According to the Pan American Health Organization (PAHO), in 2021, 190.2 deaths from CVDs were reported in Colombia per 100,000 inhabitants.⁶

Apical periodontitis (AP) is an acute or chronic inflammatory lesion around the apex of a tooth caused by a polymicrobial infection of the root canal system,^{7,8} characterized by periapical bone resorption, referred to as an apical lesion (AL).⁹ This condition is one of the persistent inflammatory disorders of the oral cavity and may contribute to systemic inflammation.^{10,11} It has been associated with platelet aggregation and hypercoagulability.^{8,12} The etiological factors of AP include various forms of dental pulp damage, such as caries, trauma, iatrogenic injury, and the failure of endodontic treatment (ET).¹³ Endodontic treatment can prevent AP or restore dental health by eliminating intraradicular infection,¹⁴ promoting bone repair¹⁵ and maintaining proper tooth function.¹⁶ Adequate root canal filling plays an important role in successful ET by preventing bacterial reinfection through the reduction of coronal leakage and, consequently, halting disease progression.¹⁷ The impact of ET quality has been evaluated based on radiographic criteria, and root canal fillings can be classified as adequate or inadequate.¹⁸

The reported prevalence of AP and ET varies worldwide.^{19–21} However, there are no studies in the Colombian population evaluating the frequency and severity of ALs, their relationship with the frequency and quality of ET obturation, or their association with CA. Therefore, this

study aimed to estimate the frequency of ALs and ET in Colombian patients with CA, and to identify a possible association between the frequency and severity of ALs and CA.

Material and methods

This was an observational, descriptive, cross-sectional study that included patients with the angiographic evidence of CA, recruited between 2017 and 2020 at the Fundación Valle del Lili, Cali, Colombia.

The study was approved in November 2017 by the Biomedical Research Ethics Committee of the Fundación Valle del Lili (approval No. 730-217). All patients provided written informed consent prior to enrollment in the study. The study was conducted in accordance with ethical considerations and the Declaration of Helsinki.

Coronary angiography was performed using the Innova 3100 IQ system (GE HealthCare, Waukesha, USA). The procedure began with arterial puncture (femoral or radial), followed by the insertion of a specialized catheter into the left chambers of the heart. A contrast medium was then injected through the catheter, allowing the visualization of the coronary artery lumen with the use of specialized imaging techniques, and enabling the presence or absence of atherosclerosis to be determined.

Convenience sampling was used to recruit patients during the study period. We included patients over 18 years of age who had at least one tooth in the mouth, a confirmed diagnosis of CA, and who agreed to participate in the study and signed the informed consent form. Further details of the study population are presented in Fig. 1. Patients with contraindications to X-ray exposure, pregnant women, and patients who had received dental treatment within the previous 3 months were excluded.

Sociodemographic data and medical information related to the cardiovascular condition were collected, including the number of compromised coronary arteries and their degree of occlusion, classified as non-significant ($\leq 50\%$ occlusion), significant (51–70%), severe (71–90%), or critical ($>90\%$). Information regarding ET was also collected, including the presence of ALs and previously treated teeth (PTT). The periapical index (PAI) was assessed using periapical radiographs²²; this index allows the evaluation of the

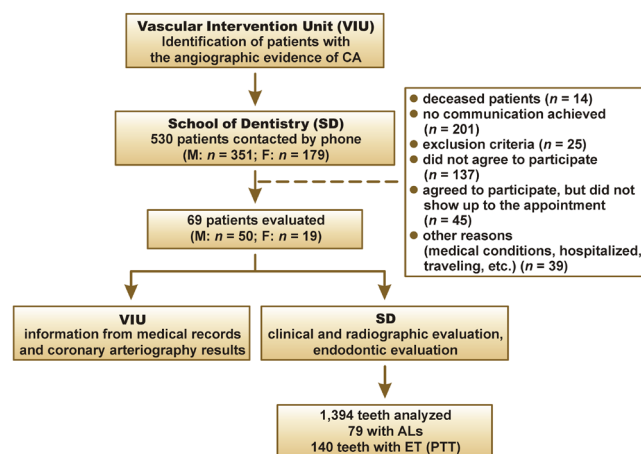


Fig. 1. Study design – selection of patients according to the inclusion and exclusion criteria

CA – coronary atherosclerosis; M – male; F – female; AL – apical lesion; ET – endodontic treatment; PTT – previously treated teeth.

condition of the periradicular tissues. For this study, PAI scores of 1 and 2 were considered indicative of periapical health, whereas PAI scores ≥ 3 were considered indicative of AP.^{23–25} According to the American Association of Endodontists (AAE), teeth that had undergone ET were defined as PTT.²⁶ The quality of root canal obturation was classified as adequate or inadequate.¹⁸

Statistical analysis

For statistical analysis, absolute and relative frequencies were calculated for categorical variables. The measures of central tendency and dispersion were calculated for clinical, radiographic, and clinical history variables. Associations between the categorical variables related to PAI

and endodontic diagnoses were assessed using the χ^2 test. The Shapiro–Wilk and Levene tests were used to assess the distribution and homogeneity of variance, respectively, when comparing the mean numbers of teeth according to endodontic diagnoses and PAI. The severity of coronary artery occlusion in patients with CA was compared using the Kruskal–Wallis test. The confidence level was set at 95%, and statistical significance was defined as $p < 0.05$.

Results

A total of 530 patients with CA were identified through coronary angiography. Of these, 114 met the inclusion criteria, 69 agreed to participate and attended the scheduled assessment, and 1,394 teeth were evaluated ($\delta = 20.2$ teeth/patient) (Fig. 1).

Among the 69 included patients, the mean age was 65.7 ± 8.6 years, and 50 (72.5%) were men. At the patient level, ALs and PTTs were present in 35 (50.7%) and 41 (59.4%) patients, respectively (Table 1). At the tooth level, ALs and PTT were observed in 79 (5.7%) and 140 (10.1%) teeth, respectively (Fig. 2A), with a greater proportion located in the

Table 1. Presence of apical lesions (ALs) and previously treated teeth (PTT) according to sex

Presence of ALs and PTT		M	F	Total
ALs	yes	25 (71.4)	10 (28.6)	35 (50.7)
	no	25 (73.5)	9 (26.5)	34 (49.3)
PTT	yes	29 (70.7)	12 (29.3)	41 (59.4)
	no	21 (75.0)	7 (25.0)	28 (40.6)
Total		50 (72.5)	19 (27.5)	69 (100.0)

Data presented as number (percentage) (n (%)). $p > 0.05$.

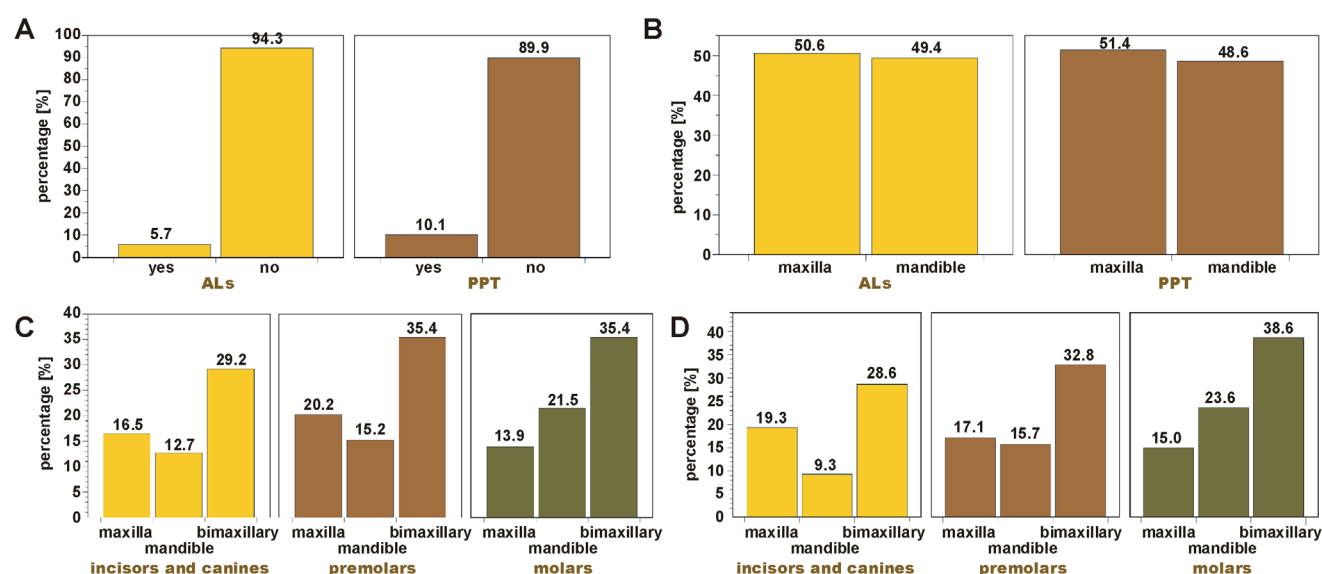


Fig. 2. Presence of apical lesions (ALs) and previously treated teeth (PTT)

A – ALs and PTT according to the total number of teeth evaluated; B – ALs and PTT according to the type of jaw; C – ALs in both jaws according to the type of tooth; D – PTT in both jaws according to the type of tooth.

maxilla, accounting for 40 (50.6%) ALs and 72 (51.4%) PTT (Fig. 2B). In both jaws, ALs were most frequently observed in premolars and molars, accounting for 28 (35.4%) cases (Fig. 2C), whereas PTT were more frequently observed in molars, accounting for 54 (38.6%) cases (Fig. 2D).

Among patients with ALs, 10 (28.6%) had at least one coronary artery with a significant atherosclerotic lesion, and the same proportion had at least one artery with a severe lesion. Additionally, 8 (22.9%) patients had at least one artery with a non-significant lesion, and the same proportion had at least one artery with a critical lesion (Supplementary Table 1, available on request from the corresponding author). Severe and critical atherosclerotic lesions were more frequently observed (in 34 and 25 patients, respectively), with PAI 3 being the most frequent score in both groups (0.68 ± 1.32 and 0.80 ± 1.04 teeth, respectively) ($p > 0.05$) (Supplementary Table 2).

Among teeth with ALs, PAI 3 was the most frequent score, with no significant difference between endodontic diagnoses with regard to PAI (Table 2). Of the 140 PTT, 47 (36.6%) presented with ALs. Inadequate obturation was observed in 102 (72.9%) PTT, of which 40 (39.2%) had ALs ($p = 0.021$). Most patients did not recall the timing of ET or the type of operator who performed ET (Table 3).

Discussion

The prevalence of AP in patients with CA reported in the literature ranges from 50.8% to 85.3%,^{23,27} similar to the findings of this study. An AP frequency of 39.2% in males and 44.2% in females has been reported in previous studies.²⁸ In this study, a greater presence of ALs was observed in males, which may be partially related to the higher prevalence of CA among male patients.²⁹ The presence of ALs in teeth was 5.7%, with asymptomatic AP being the most prevalent periapical diagnosis (5.5%). These results are similar to those reported by Tibúrcio-Machado et al., who found a global prevalence of AP of 52% in patients without systemic compromise and 5% in teeth.³⁰ Likewise, de Oliveira et al. reported a prevalence of AP of 42.6% in patients with CA and 40.1% in endodontically compromised teeth,²⁸ which is similar to the findings of this study. The prevalence of ET reported in previous studies ranges from 41.0% to 60.8% in patients, and from 2% to 9% in teeth,^{31,32} which is also similar to our findings. Moreover, the prevalence of AP in teeth with ET found in this study is consistent with the previously reported global prevalence of 41%.³³

De Oliveira et al. reported a greater presence of AP in the maxilla, upper incisors and lower premolars (40.3%,

Table 2. Endodontic diagnoses according to the periapical index (PAI)

Endodontic diagnoses		ALs			Total	p-value
		PAI 3	PAI 4	PAI 5		
Pulp diagnoses	pulp necrosis	16 (50.0)	12 (37.5)	4 (12.5)	32 (100.0)	0.296
	PTT	32 (68.1)	11 (23.4)	4 (8.5)	47 (100.0)	
Periapical diagnoses	asymptomatic AP	48 (62.3)	22 (28.6)	7 (9.1)	77 (100.0)	0.092
	chronic apical abscess	0 (0.0)	1 (50.0)	1 (50.0)	2 (100.0)	

Data presented as *n* (%).

AP – apical periodontitis.

Table 3. Distribution of endodontic variables according to the presence of apical lesions (ALs)

Variables		Presence of ALs		Total	p-value
		yes	no		
PTT		47 (33.6)	93 (66.4)	140 (100.0)	–
Radiographic quality of the obturation	adequate	7 (18.4)	31 (81.6)	38 (100.0)	0.021*
	inadequate	40 (39.2)	62 (60.8)	102 (100.0)	
Timing of ET	do not remember	34 (35.1)	63 (64.9)	97 (100.0)	0.316
	1 year	1 (50.0)	1 (50.0)	2 (100.0)	
	1–2 years	6 (46.2)	7 (53.8)	13 (100.0)	
	3–5 years	0 (0.0)	8 (100.0)	8 (100.0)	
	5 years	7 (35.0)	13 (65.0)	20 (100.0)	
Operator	do not remember	39 (37.5)	65 (62.5)	104 (100.0)	0.242
	general dentist	6 (21.4)	22 (78.6)	28 (100.0)	
	endodontic specialist	2 (25.0)	6 (75.0)	8 (100.0)	

Data presented as *n* (%). * statistically significant.

52.8% and 46.2%, respectively).²⁸ In the present study, the maxilla, upper premolars and lower molars had a higher frequency of ALs. Previous studies have reported a higher prevalence of ET in the maxilla,^{34–40} and a high prevalence of ET in molars.^{34,38} These findings are consistent with those of the present study.

Apical periodontitis and periodontal disease differ in terms of their etiology and pathogenesis; however, they share similar characteristics. Both are chronic oral infectious diseases, share a common Gram-negative microbiota and result in increased levels of inflammatory mediators.^{28,39} A potential relationship between periodontal disease and CVDs has been reported previously^{40–42}; therefore, it has been hypothesized that AP could also impact patients with cardiovascular compromise due to its close relationship with periodontal disease.²⁸ Several studies have demonstrated an increased risk associated with AP in patients with CVDs and CA, suggesting that an increase in the number of teeth with AP is associated with a higher risk of CVDs.^{23,24,27,28,43–46}

There are no reports in the literature on the relationship between the frequency and severity of ALs, the number of coronary vessels affected, and the severity of their occlusion. Virtanen et al. conducted a cross-sectional study in which medical information was collected from randomly selected subjects, who were classified as patients with or without AP, and found a greater tendency and severity of ALs in patients with CVDs.⁴⁷ They showed that AP was significantly associated with CVDs and suggested that the presence of AP in subjects who also regularly had periodontal disease might indicate a higher oral inflammatory load.⁴⁷ In contrast to the findings of Virtanen et al.,⁴⁷ but in line with those reported by Frisk et al.⁴⁸ and Frisk,⁴⁹ we found no association between the severity of ALs and CA. This may be explained by the fact that both AP and CVDs can occur independently without directly affecting each other.¹⁰ It is important to highlight that the quality of the current evidence is moderate to low, and a causal relationship between these two diseases could not be established.⁵⁰

Regarding the quality of ET, no studies exclusively evaluating populations with CA were found. Kielbassa et al. reported a prevalence of adequate ET of 9.9%, while 50.3% of ET was considered inadequate.³⁷ Virtanen et al. found that, among the 60.8% of patients with ET analyzed, 38.3% were considered to have satisfactory ET, whereas 51.7% were considered to have unsatisfactory ET.⁴⁷ Moreno et al. reported that 66.7% of the teeth evaluated had inadequate ET; of these, 54.0% presented ALs.³⁵ These results are similar to our findings, in which a statistically significant association was found. Different studies have reported a significant association between the quality of ET and the status of the periapical tissues,^{35–37,51} suggesting that inadequate ET may act as a risk factor for the development of ALs.^{33,52} This may indicate that ET has a protective effect on the periapical tissues when performed adequately.

Various studies have evaluated the success rate of ET, which is characterized by the restoration of dental health (i.e., no evidence of AP), the prevention of AP, the continued retention of the tooth, and the restoration of its function. Alley et al. reported a higher success rate for ET performed by endodontists (98.1%) than by general dentists (89.7%) over a 5-year period.⁵³ Lazarski et al. found no differences in the ET success rates according to the type of operator, reporting an overall success rate of 94.4% over a period of 3.5 years.⁵⁴ Burry et al. analyzed the survival of teeth with ET, and found a decrease in the survival rates at 1, 5 and 10 years.⁵⁵ They did not observe differences between operators at 1 and 5 years; however, at 10 years, they found better survival of the molars treated by endodontists.⁵⁵ These results are not directly comparable with our study, since most patients did not remember the type of operator who performed ET. In addition, an unequal proportion of treatment performed by general dentists and endodontists was observed.

Limitations

Among the limitations of this study is the use of a convenience sampling method, which may introduce sampling bias and limit the generalizability of the results. In addition, we did not conduct a power analysis to determine the required sample size, which may have influenced the results obtained. Moreover, we acknowledge that the lack of a control group is a major limitation; therefore, we suggest that future studies include a matched control group to strengthen the conclusions drawn. Although the objective of this study was to determine whether there was an association between the frequency and severity of ALs and CA, the statistical approach used cannot be interpreted as a correlation or used to establish impact, as other factors that could influence the results were not included in the analysis (e.g., oral hygiene and mechanical stress). A larger sample size, a more balanced representation of men and women, and a wider age range would also be desirable to further validate our results. Finally, we suggest that future studies incorporate cone-beam computed tomography (CBCT), as it provides a more objective assessment of ALs.

Conclusions

A high frequency of ALs and ET was found in patients with CA. No relationship was found between the frequency of ALs, the severity of coronary occlusion, and the number of coronary arteries affected. A greater presence of AL was found in teeth with inadequate ET. Since most patients could not recall the timing of their ET or the type of operator who performed ET, an association between the presence of ALs, the timing of ET and the type of operator could not be determined. However, as this study did not include a control group for comparison with patients with CA, our results should be interpreted with caution.

Ethics approval and consent to participate

This study was approved by the Biomedical Research Ethics Committee of the Fundación Valle del Lili, Cali, Colombia (approval No. 730-217). All patients provided written informed consent prior to enrollment in the study. The study was conducted in accordance with ethical considerations and the Declaration of Helsinki.

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

- Insull Jr. W. The pathology of atherosclerosis: Plaque development and plaque responses to medical treatment. *Am J Med.* 2009;122(Suppl 1):S3–S14. doi:10.1016/j.amjmed.2008.10.013
- Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. *Circ Res.* 2016;118(4):535–546. doi:10.1161/CIRCRESAHA.115.307611
- Kumar V, Abbas AK, Aster JC. Heart. In: Kumar V, Abbas AK, Aster JC, eds. *Robbins Basic Pathology*. 10th ed. Amsterdam, The Netherlands: Elsevier; 2018:399–440.
- Ye L, Cao L, Song W, Yang C, Tang Q, Yuan Z. Interaction between apical periodontitis and systemic disease (review). *Int J Mol Med.* 2023;52(1):60. doi:10.3892/ijmm.2023.5263
- Joseph P, Leong D, McKee M, et al. Reducing the global burden of cardiovascular disease, part 1: The epidemiology and risk factors. *Circ Res.* 2017;121(6):677–694. doi:10.1161/CIRCRESAHA.117.308903
- Pan American Health Organization (PAHO). Cardiovascular disease burden in the Region of the Americas, 2000–2021. ENLACE data portal. Washington, D.C.: PAHO; 2025. <https://www.paho.org/en/enlace/cardiovascular-disease-burden>. Accessed August 20, 2026.
- Eriksen HM. Epidemiology of apical periodontitis. In: Ørstavik D, Pitt Ford TR, eds. *Essential Endodontology: Prevention and Treatment of Apical Periodontitis*. Oxford, UK: Blackwell Science Ltd.; 1998:179–191.
- Caplan DJ, Chasen JB, Krall EA, et al. Lesions of endodontic origin and risk of coronary heart disease. *J Dent Res.* 2006;85(11):996–1000. doi:10.1177/154405910608501104
- Karamifar K, Tondari A, Saghir MA. Endodontic periapical lesion: An overview on the etiology, diagnosis and current treatment modalities. *Eur Endod J.* 2020;5(2):54–67. doi:10.14744/eej.2020.42714
- Jiménez-Sánchez MC, Cabanillas-Balsera D, Areal-Quecuty V, Velasco-Ortega E, Martín-González J, Segura-Egea JJ. Cardiovascular diseases and apical periodontitis: Association not always implies causality. *Med Oral Patol Oral Cir Bucal.* 2020;25(5):e652–e659. doi:10.4317/medoral.23665
- Gomes MS, Blattner TC, Sant'Ana Filho M, et al. Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. *J Endod.* 2013;39(10):1205–1217. doi:10.1016/j.joen.2013.06.014
- Cowan LT, Lakshminarayan K, Lutsey PL, Beck J, Offenbacher S, Pankow JS. Endodontic therapy and incident cardiovascular disease: The Atherosclerosis Risk in Communities (ARIC) study. *J Public Health Dent.* 2020;80(1):79–91. doi:10.1111/jphd.12353
- El Merini H, Amarir H, Lamzawaq A, Hamza M. Periapical status and quality of root canal fillings in a Moroccan subpopulation. *Int J Dent.* 2017;2017:1068982. doi:10.1155/2017/1068982
- Alghamdi F, Alsulaimani M. Regenerative endodontic treatment: A systematic review of successful clinical cases. *Dent Med Probl.* 2021;58(4):555–567. doi:10.17219/dmp/132181
- Wu MK, Shemesh H, Wesselink PR. Limitations of previously published systematic reviews evaluating the outcome of endodontic treatment. *Int Endod J.* 2009;42(8):656–666. doi:10.1111/j.1365-2591.2009.01600.x
- Marquis VL, Dao T, Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto Study. Phase III: Initial treatment. *J Endod.* 2006;32(4):299–306. doi:10.1016/j.joen.2005.10.050
- 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
- Tronstad L, Asbjørnsen K, Døving L, Pedersen I, Eriksen HM. Influence of coronal restorations on the periapical health of endodontically treated teeth. *Endod Dent Traumatol.* 2000;16(5):218–221. doi:10.1034/j.1600-9657.2000.016005218.x
- Segura-Egea JJ, Jiménez-Pinzón A, Poyato-Ferrera M, Velasco-Ortega E, Ríos-Santos JV. Periapical status and quality of root fillings and coronal restorations in an adult Spanish population. *Int Endod J.* 2004;37(8):525–530. doi:10.1111/j.1365-2591.2004.00826.x
- Rincon Forero LM. *Characterization of patients attending the Endodontics Postgraduate Clinic of the Faculty of Dentistry, National University of Colombia, 2012–2015* [in Spanish]. Bogotá, Colombia: National University of Colombia; 2016 [thesis].
- Luna Jaramillo NA, Santacruz Insuasty AX, Palacio Córdoba BD, Mafla Chamorro AC. Prevalence of chronic apical periodontitis in endodontically treated teeth in the academic community at Universidad Cooperativa de Colombia, Pasto, 2008 [in Spanish]. *Rev Fac Odontol Univ Antioq.* 2009;21(1):42–49.
- Orstavik D, Kerekes K, Eriksen HM. The periapical index: A scoring system for radiographic assessment of apical periodontitis. *Endod Dent Traumatol.* 1986;2(1):20–34. doi:10.1111/j.1600-9657.1986.tb00119.x
- Rodrigues Costa TH, de Figueiredo Neto JA, Figueiredo de Oliveira AE, de Figueiredo Lopes e Maia M, de Almeida AL. Association between chronic apical periodontitis and coronary artery disease. *J Endod.* 2014;40(2):164–167. doi:10.1016/j.joen.2013.10.026
- Liljestrand JM, Mäntylä P, Paju S, et al. Association of endodontic lesions with coronary artery disease. *J Dent Res.* 2016;95(12):1358–1365. doi:10.1177/0022034516660509
- Segura-Egea JJ, Jimenez-Moreno E, Calvo-Monroy C, et al. Hypertension and dental periapical condition. *J Endod.* 2010;36(11):1800–1804. doi:10.1016/j.joen.2010.08.004
- Glickman GN. AAE Consensus Conference on Diagnostic Terminology: Background and perspectives. *J Endod.* 2009;35(12):1619–1620. doi:10.1016/j.joen.2009.09.029
- Pasqualini D, Bergandi L, Palumbo L, et al. Association among oral health, apical periodontitis, CD14 polymorphisms, and coronary heart disease in middle-aged adults. *J Endod.* 2012;38(12):1570–1577. doi:10.1016/j.joen.2012.08.013
- de Oliveira BP, Câmara AC, Aguiar CM. Prevalence of asymptomatic apical periodontitis and its association with coronary artery disease in a Brazilian subpopulation. *Act Stomatol Croatia.* 2017;51(2):106–112. doi:10.15644/asc51/2/3

29. Ferreira-González I. The epidemiology of coronary heart disease. *Rev Esp Cardiol (Engl Ed)*. 2014;67(2):139–144. doi:10.1016/j.rec.2013.10.002
30. Tibúrcio-Machado CS, Michelon C, Zanatta FB, Gomes MS, Marin JA, Bier CA. The global prevalence of apical periodontitis: A systematic review and meta-analysis. *Int Endod J*. 2021;54(5):712–735. doi:10.1111/iej.13467
31. López-López J, Jané-Salas E, Estrugo-Devesa A, et al. Frequency and distribution of root-filled teeth and apical periodontitis in an adult population of Barcelona, Spain. *Int Dent J*. 2012;62(1):40–46. doi:10.1111/j.1875-595X.2011.00087.x
32. Jiménez-Pinzón A, Segura-Egea JJ, Poyato-Ferrera M, Velasco-Ortega E, Ríos-Santos JV. Prevalence of apical periodontitis and frequency of root-filled teeth in an adult Spanish population. *Int Endod J*. 2004;37(3):167–173. doi:10.1111/j.0143-2885.2004.00759.x
33. Jakovljevic A, Nikolic N, Jacimovic J, et al. Prevalence of apical periodontitis and conventional nonsurgical root canal treatment in general adult population: An updated systematic review and meta-analysis of cross-sectional studies published between 2012 and 2020. *J Endod*. 2020;46(10):1371–1386.e8. doi:10.1016/j.joen.2020.07.007
34. Peters LB, Lindeboom JA, Elst ME, Wesselink PR. Prevalence of apical periodontitis relative to endodontic treatment in an adult Dutch population: A repeated cross-sectional study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;111(4):523–528. doi:10.1016/j.tripleo.2010.10.035
35. Moreno JO, Alves FR, Gonçalves LS, Martinez AM, Rôças IN, Siqueira Jr. JF. Periradicular status and quality of root canal fillings and coronal restorations in an urban Colombian population. *J Endod*. 2013;39(5):600–604. doi:10.1016/j.joen.2012.12.020
36. Archana D, Gopikrishna V, Gutmann JL, Savadamoorthi KS, Pradeep Kumar AR, Narayanan LL. Prevalence of periradicular radiolucencies and its association with the quality of root canal procedures and coronal restorations in an adult urban Indian population. *J Conserv Dent*. 2015;18(1):34–38. doi:10.4103/0972-0707.148888
37. Kielbassa AM, Frank W, Madaus T. Radiologic assessment of quality of root canal fillings and periapical status in an Austrian subpopulation – an observational study. *PLoS One*. 2017;12(5):e0176724. doi:10.1371/journal.pone.0176724
38. Connert T, Truckenmüller M, ElAyouti A, et al. Changes in periapical status, quality of root fillings and estimated endodontic treatment need in a similar urban German population 20 years later. *Clin Oral Investig*. 2019;23(3):1373–1382. doi:10.1007/s00784-018-2566-z
39. Berlin-Broner Y, Febbraio M, Levin L. Apical periodontitis and atherosclerosis: Is there a link? Review of the literature and potential mechanism of linkage. *Quintessence Int*. 2017;48(7):527–534. doi:10.3290/j.qi.a38162
40. Lockhart PB, Bolger AF, Papapanou PN, et al.; American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young Council on Epidemiology and Prevention, Council on Peripheral Vascular Disease, and Council on Clinical Cardiology. Periodontal disease and atherosclerotic vascular disease: Does the evidence support an independent association? A scientific statement from the American Heart Association. *Circulation*. 2012;125(20):2520–2544. doi:10.1161/CIR.0b013e31825719f3
41. Tang K, Wu Y, Zheng Q, Chen X. Bibliometric research on analysis of links between periodontitis and cardiovascular diseases. *Front Cardiovasc Med*. 2023;10:1255722. doi:10.3389/fcvm.2023.1255722
42. Men B, Li Y, Jiang S. Updates on the role of periodontitis-related epigenetics, inflammation, oral microbiome, and treatment in cardiovascular risk. *J Inflamm Res*. 2024;17:837–851. doi:10.2147/JIR.S449661
43. An GK, Morse DE, Kunin M, Goldberger RS, Psoter WJ. Association of radiographically diagnosed apical periodontitis and cardiovascular disease: A hospital records-based study. *J Endod*. 2016;42(6):916–920. doi:10.1016/j.joen.2016.03.011
44. Caplan DJ. Chronic apical periodontitis is more common in subjects with coronary artery disease. *J Evid Based Dent Pract*. 2014;14(3):149–150. doi:10.1016/j.jebdp.2014.07.003
45. Hardan L, Matta A, Bourgi R, et al. Association between dental and cardiovascular diseases: A systematic review. *Rev Cardiovasc Med*. 2023;24(6):159. doi:10.31083/j.rcm2406159
46. Malvicini G, Marruganti C, Leil MA, et al. Association between apical periodontitis and secondary outcomes of atherosclerotic cardiovascular disease: A case–control study. *Int Endod J*. 2024;57(3):281–296. doi:10.1111/iej.14018
47. Virtanen E, Nurmi T, Söder PÖ, Airila-Månsson S, Söder B, Meurman JH. Apical periodontitis associated with cardiovascular diseases: A cross-sectional study from Sweden. *BMC Oral Health*. 2017;17(1):107. doi:10.1186/s12903-017-0401-6
48. Frisk F, Hakeberg M, Ahlqvist M, Bengtsson C. Endodontic variables and coronary heart disease. *Acta Odontol Scand*. 2003;61(5):257–262. doi:10.1080/00016350310005510
49. Frisk F. Epidemiological aspects on apical periodontitis. Studies based on the Prospective Population Study of Women in Göteborg and the Population Study on Oral Health in Jönköping, Sweden. *Swed Dent J Suppl*. 2007(189):11–78. PMID:18047231.
50. Berlin-Broner Y, Febbraio M, Levin L. Association between apical periodontitis and cardiovascular diseases: A systematic review of the literature. *Int Endod J*. 2017;50(9):847–859. doi:10.1111/iej.12710
51. Lemagner F, Maret D, Peters OA, et al. Prevalence of apical bone defects and evaluation of associated factors detected with cone-beam computed tomographic images. *J Endod*. 2015;41(7):1043–1047. doi:10.1016/j.joen.2015.03.011
52. Hussein FE, Cheen Liew AK, Ramlee RA, Abdullah D, Chong BS. Factors associated with apical periodontitis: A multilevel analysis. *J Endod*. 2016;42(10):1441–1445. doi:10.1016/j.joen.2016.07.009
53. Alley BS, Kitchens GG, Alley LW, Eleazer PD. A comparison of survival of teeth following endodontic treatment performed by general dentists or by specialists. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;98(1):115–118. doi:10.1016/j.tripleo.2004.01.004
54. Lazarski MP, Walker 3rd WA, Flores CM, Schindler WG, Hargreaves KM. Epidemiological evaluation of the outcomes of nonsurgical root canal treatment in a large cohort of insured dental patients. *J Endod*. 2001;27(12):791–796. doi:10.1097/00004770-200112000-00021
55. Burry JC, Stover S, Eichmiller F, Bhagavatula P. Outcomes of primary endodontic therapy provided by endodontic specialists compared with other providers. *J Endod*. 2016;42(5):702–705. doi:10.1016/j.joen.2016.02.008

Impact of clear aligner (Invisalign®) treatment and occlusal changes on the masticatory function and temporomandibular disorders: A cross-sectional study

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Abstract

Background. Clear aligner treatment (CAT) may induce masticatory muscle tenderness and trigger temporomandibular disorders (TMD). Considering that CAT may interfere with the masticatory system, it is important to understand the relationship between CAT and occlusal changes.

Objectives. This study aimed to analyze occlusal changes during CAT and assess the influence of post-orthodontic occlusion on masticatory performance. A secondary objective was to determine whether the case complexity, the facial biotype and malocclusion were associated with occlusal alterations, masticatory performance, or the onset of TMD signs and symptoms during CAT.

Material and methods. Forty-two individuals who underwent and completed CAT were evaluated at 3 time points: before treatment (T0); at the end of treatment (T1); and 3 months after treatment, during which time clear aligners (CAs) were used only at night (T2). Temporomandibular disorders were assessed using Axis I of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) at T0, T1 and T2. Masticatory performance was evaluated at T1 and T2 using a two-colored chewing gum test and colorimetric analysis. Occlusal area and anterior and posterior occlusal contacts were evaluated digitally. Non-parametric statistical tests were used, with the significance level set at $p < 0.05$.

Results. The occlusal area decreased significantly from T0 to T1 ($p = 0.016$). At T1, normodivergent individuals had significantly more anterior contacts than hyperdivergent patients ($p = 0.013$). Masticatory performance increased with the number of posterior occlusal contacts ($p < 0.05$), and individuals with Class I molar relationship had more anterior contacts than Class II patients ($p = 0.004$). At T2, Class III individuals showed higher values than Class II patients ($p = 0.011$).

Conclusions. The number of occlusal contacts and occlusal contact area decreased during treatment. Nighttime use of CAs slightly enhanced occlusal contact recovery. Complex cases presented more posterior contacts at T0; simple cases presented a greater occlusal area at the end of CAT. Class I individuals presented more anterior contacts at T0. These movements increased the anterior contacts in Class III molar cases at the end of treatment and 3 months later. No significant differences in masticatory performance were found according to the molar relationship, the facial biotype or the case complexity; no TMD signs and symptoms were found during or after CAT.

Keywords: TMD, masticatory function, clear aligners, masticatory performance, occlusal contacts

Highlights

- Clear aligner treatment (CAT) temporarily reduced occlusal contacts and contact area, with slight recovery after 3 months of nighttime aligner use.
- Masticatory performance was associated with the number of posterior occlusal contacts but was not significantly influenced by molar relationship, facial biotype or case complexity.
- No signs or symptoms of temporomandibular disorders developed during or after treatment, suggesting that CAT did not adversely affect temporomandibular disorder status in this sample.

Introduction

Within the twenty-first century's digital era, the merging of new technology gave rise to modern orthodontic treatments (OTs). Aside from the conventional OT with fixed appliances, options such as clear aligners (CAs) have emerged.¹ Orthodontic treatment ensures the proper alignment of teeth and improves the occlusal and jaw relationships.² For the orthodontist, the esthetic characteristics of malocclusions are an important factor in determining the demand for OT^{3,4} and achieving a stable and functional occlusion. Appropriate masticatory function at the end of OT is another critical goal that cannot be overlooked.

Equally distributed contacts that are softer anteriorly and stronger posteriorly are among the occlusal goals. In order to achieve proper occlusion at the end of OT, the clinician needs to create heavy contact on the splint during treatment.⁵ Respecting the abovementioned objectives is a key factor in ensuring the long-term stability of OT. Orthodontic pain and occlusal changes during treatment are the main factors influencing how the masticatory muscles adapt to OT.⁶ During treatment, the CA covers the entire occlusal surfaces of the teeth, promoting a bite-block effect for approx. 22 h per day.^{7,8} This duration does not allow the occlusion to settle as intended, creating artificial interference within the occlusal space.⁹

The failure to obtain stable and solid occlusal contacts during treatment has been identified as the main limitation of CA treatment (CAT).⁸ This lack of posterior contacts can be resolved during the settling of the occlusion. Previous studies have documented that complete settling requires time and that the number of occlusal contacts increases during the post-treatment period.¹⁰ However, occlusal disharmonies are present during CAT, and its relationship with potential issues such as temporomandibular disorders (TMD), periodontal disease, cervical abfraction, or less effective mastication remains controversial.^{11,12}

Temporomandibular disorders are an umbrella term that involves dysfunction and pain in the masticatory muscles and/or temporomandibular joint (TMJ), causing damage to the masticatory function.^{13,14} The potential impact of OT on the onset and evolution of TMD has been controversially discussed over the years.¹⁵ Several studies suggest that OT increases the likelihood of the onset of symptoms

and signs of TMD.^{16,17} In contrast, others indicate positive effects on the resolution of TMD or, most crucially, on reducing the patient's risk of their development.¹⁸

Correction of malocclusions, often accompanied by mechanical transformation as well as skeletal changes, may alter the muscular and articular stability of the craniomandibular structures. Clear aligner interference has been reported to increase the frequency of wake-time tooth clenching episodes, trigger masticatory muscle tenderness, produce wear facets on aligner trays, and even induce the development of TMD.^{19,20} Individuals undergoing CAT may have temporary TMD due to repetitive clenching on their trays to relieve orthodontic pain.²¹ One potential drawback is that wake-time tooth clenching could contribute to masticatory muscle pain and TMD.²¹

It has been well established that masticatory performance is related to occlusion and occlusal contact areas.⁶ Individuals with malocclusion present lower masticatory performance than those with normal occlusion, as the occlusal areas of contact and near-contact are positively correlated with masticatory function. A critical issue of functional connectivity in the masticatory system is the influence of occlusal conditions on masticatory function.⁶

Considering that CAT may interfere with the masticatory structures, it is essential to understand the correlation between OT-related occlusal changes and their influence on mandibular function. Thus, this study aimed to analyze occlusal changes during CAT and assess the influence of post-orthodontic occlusion on masticatory performance (primary outcome); moreover, it assessed whether the complexity of CAT, the facial biotype, and the malocclusion may be related to possible alterations and the onset of TMD signs and symptoms during the use of CAs (secondary outcomes). The positive hypothesis is that CAT achieves the desired result without causing any TMD; the null hypothesis is that even if the result was reached, TMD were detected. The clinical significance of the study lies in understanding the functional outcomes of CA (Invisalign®) treatment by evaluating its effects on (1) chewing efficiency; (2) occlusal changes, to determine whether it achieved a stable occlusion, promoting optimal function; (3) TMD, which may be influenced by changes in occlusion and muscle activity; and (4) long-term stability and patient comfort. These findings may guide clinical

practice and treatment protocols, as well as help dentists and, specifically, orthodontists tailor treatments to prevent masticatory dysfunction and TMD-related complications. Its relevance is supported by the limited number of studies in the literature that have investigated CAs while evaluating 4 variables: occlusal changes; the facial biotype; chewing performance; and their possible correlation with TMD.

Material and methods

This quantitative, comparative, and observational longitudinal cohort study was conducted in accordance with the recommendations of the Declaration of Helsinki (2024) and the World Health Organization (WHO)²² regarding research involving human participants and followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.²³ Ethics approval was obtained from the Ethics Committee of the University Institute of Health Sciences (IUCS), Gandra, Portugal (approval No. 26/CE-IUCS/2020). All participants provided informed consent before the commencement of the study.

Study sample

A non-probability convenience sample was recruited from individuals aged between 16 and 45 years with complete permanent dentition (excluding third molars) undergoing CAT. The individuals were recruited from 2 independent private orthodontic clinics, Clínica Médico Dentária de São João da Madeira (São João da Madeira, Portugal) and Clínica de Medicina Dentária Dr. Manuel Neves (Porto, Portugal), from October 2019 to December 2022. All individuals were treated under the supervision of a specialist in both Orthodontics and Pediatric Dentistry who was also an Invisalign® Diamond Provider. The study comprised a sample of 42 individuals (28 female and 14 male) undergoing CAT; the sample was limited due to practical constraints. The participants were evaluated at 3 different time points: before starting treatment (T0); at the end of treatment with CAs without misfits (T1); and 3 months after the end of treatment, during which time CAs were only used at night (T2). The patients were invited to participate in the study in order to evaluate occlusal changes during CAT and assess the influence of post-orthodontic occlusion on masticatory performance. All invited individuals agreed to participate in the proposed analysis.

Sample size calculation

Considering the study design, a prospective cohort study comparing pre-treatment and post-treatment outcomes, the following parameters were adopted: a significance level (α) of 0.05, power ($1-\beta$) of 80% (0.80), and effect sizes (Cohen's d for the analysis of continuous variables) of 0.5 (medium effect) for occlusal changes, 0.4 (small-to-medium

effect) for temporomandibular dysfunction, and 0.5 (medium effect) for chewing performance. The standard deviation (σ) was assumed to represent 10% variation. The following formula was used for the calculation of the sample size:

$$N = (((Z\alpha/2) + Z\beta) \times \sigma) / \Delta)^2$$

where:

$Z\alpha/2 = 1.96$ (for 95% confidence);

$Z\beta = 0.84$ (for 80% power);

$\sigma = 10\%$; and

$\Delta = 5\%$ (expected mean difference).

Assuming an effect size of $d = 0.5$, the sample size was calculated as follows: $N = ((1.96 + 0.84)^2 \times 10^2) / 5^2$; $N = 31.36$. Therefore, the minimum sample size required for this study was 32 patients. Because of the elevated risk of dropout, 30% more patients were included, resulting in a total sample of 42 patients.

Eligibility criteria

Two professionals (VM and TP) conducted the evaluation of patients. The inclusion criteria were as follows: individuals who started and completed CAT; individuals whose masticatory performance was assessed at the end of treatment and 3 months after treatment; individuals who used nighttime aligners without maladjustments between the end of treatment and 3 months after treatment; and individuals with available and complete cephalometric analyses.

The exclusion criteria encompassed individuals who did not have complete dentition, patients whose occlusal records were incomplete, individuals presenting TMD signs or symptoms before OT, patients requiring mandibular advancement with the use of special wings (Invisalign®), those with cognitive or neurological changes or identified syndromes, individuals with a history of trauma and/or tumors of the head and neck or metabolic diseases affecting the joints and/or muscles, patients who were taking anti-inflammatory, analgesic or psychiatric medications, and individuals who suffered from dental pain or periodontal problems during the previous year.

Data collection for TMD diagnosis

The diagnosis of TMD was made before starting CAT (T0), after completing OT (T1), and 3 months after treatment (T2), in accordance with international guidelines using Axis I of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).²⁴ Before starting the study, a pilot study was conducted to assess intra- and inter-examiner reliability in 30 participants with the same characteristics as the primary study sample. These individuals were not included in the main study sample because the goal of the pilot study was the intra- and inter-examiner calibration of the professionals who would collect the diagnostic data on TMD after OT. Three examiners (VM, MPG, GVOF) independently performed the evaluation

procedure on 2 occasions separated by a one-week interval, demonstrating excellent reliability, with an intraclass correlation coefficient (ICC) >0.90.

After appropriate calibration of the examiners who applied the DC/TMD, information on TMD signs and symptoms was collected at 3 time points (T0, T1 and T2), with the aligners removed during the assessment.

Data collection for masticatory performance

Masticatory function was evaluated using a masticatory performance test with two-colored chewing gum. Participants sat upright and chewed 2 pieces of gum (Hue-Check Gum®; Orophys GmbH, Muri bei Bern, Switzerland), one pink and one blue, for 20 chewing cycles.²⁵ The operator counted the chewing cycles silently. The gums were initially moistened in water and bonded together with moderate force. The chewing gum was placed in the patient's mouth, with the blue side facing the tongue. Participants were instructed to chew normally, and no further instructions were given. After 20 chewing cycles, the operator asked the patient to stop chewing, and the gums were removed from the oral cavity, placed in a new clear plastic bag, and subsequently flattened using a 1-mm thick plate. Both sides of the specimen were scanned immediately after collection to minimize enzymatic degradation, and the images were saved in JPEG format.

The composite images were then evaluated using a specifically developed program that is freely available (Viewgum software; dHAL Software, Kifissia, Greece). The software first transformed the images into the HSI color space and then semi-automatically calculated the hue value for each pixel in the segmented images. The hue variation (VOH) was the measure of mixing calculated by the software. The VOH is also considered a measure of masticatory performance because it has a logarithmic association with the number of chewing cycles.²⁶ A high VOH results from poorly mixed colors due to deficient chewing, whereas adequate chewing leads to well-mixed colors and a low VOH. The hue variation was considered a measure of mixing, with values closer to 0 indicating better color mixing and, consequently, better masticatory performance. The Hue-Check Gum® method was used.²⁷ Information was collected at T1 and T2 and recorded.

Digital measurements

After analyzing the clinical records, the characteristics of each participant were collected and evaluated, including gender and age at the beginning of CAT. Cephalometric tracing, overbite, overjet, and facial biotype were measured by 1 professional (VM). The facial biotype classification was performed using the Frankfort-mandibular plane angle (FMA). Facial biotypes were classified as hypodivergent with values equal to or below 22 degrees, normodivergent

with values between 23 and 27 degrees, and hyperdivergent with values equal to or above 28 degrees.²⁸ Intraoral photographs and images of the occlusal contacts planned in the ClinCheck® software (Align Technology, Tempe, USA) for CAT were collected for each participant.

Subsequently, intraoral photographs and images obtained using the intraoral scanner at T0, T1 and T2 were compared using the iTero® software (Align Technology). The files in PLY format obtained using the same software were downloaded and analyzed. The PLY file of each participant had the following characteristics selected in the iTero® software: "open Shell", "arches combined (arches locked in bite relation)", and "PLY (color)". Cephalometric measurements, the number of anterior and posterior occlusal contacts (T0, T1 and T2) and occlusal areas were organized in a Microsoft Excel (Microsoft Corp., Redmond, USA) file. The collected data was later used for statistical analysis.

To obtain the area and the number of occlusal contacts at T0, T1 and T2, the files in PLY format were processed using the MeshLab® software (<https://www.meshlab.net>). The ClinCheck® software was used to quantify the number of occlusal contacts. Any opposing pair of maxillary and mandibular teeth with the same tooth number was counted as 1 occlusal contact, and the counting was performed on the lower arch. The maximum expected number of occlusal contacts in a 28-tooth dentition was 14. The maximum number of posterior occlusal contacts was 8, and the maximum number of anterior occlusal contacts was 6. Third molars were excluded from the occlusal area and contact measurements.

Orthodontic intervention

The individuals were instructed to use each CA for as many hours as possible (20–22 h/day), as instructed by the provider, and to remove the aligners only when eating and performing oral hygiene. The aligners were changed every 7 days from the beginning of treatment, as recommended by Align® protocols. Compliance with aligner use was verified with an aligner wear monitor based on fading compliance indicators and was confirmed by asking the patient about their use. Follow-up visits were conducted every 2 months. Additional aligners were required at the end of the study to serve as a retention method and were used only at night to improve the occlusal contacts.

For the present investigation, only 1 type of aligner system was used for all individuals to reduce confounding factors linked to the mechanical properties of the material. It has already been demonstrated that differences in thermoplastic material composition and thickness result in different flexibilities, force activation, and consequent stress-relaxation behavior.²⁹ After completing the treatment, the additional aligners were used without mismatch between the upper and lower arches and were worn only at night.

Clinical assessment and complexity of the case

Based on the clinical data (clinical photographs, radiographs and dental casts), the complexity levels of cases receiving CAT were assessed independently by 2 professionals (VM and TP). In cases of discrepancy, a third researcher was consulted (TP). For this purpose, an online assessment tool, Invisalign® Doctor Site, was used.³⁰ The orthodontic malocclusion traits were classified as requiring simple, moderate or complex correction based on Align® recommendations and the Invisalign® evaluation tool.³¹ According to the Invisalign® criteria, patients need to present only 1 complex problem for their case to be classified as complex. This classification of complexity considers different parameters, namely the type of dentition, the need for surgery, the amount of spacing and crowding, rotations, narrow arches, posterior crossbite, anteroposterior correction, anterior crossbite, anterior open bite, deep bite, and the need for extraction. Each of these parameters has sub-parameters, which were also evaluated.

Statistical analysis

Data was analyzed using the IBM SPSS Statistics for Windows software, v. 29 (IBM Corp., Armonk, USA). Descriptive statistics were calculated to estimate frequencies, percentages, means, medians, standard deviations, and minimum and maximum values. The Shapiro–Wilk test was used to assess the normality of the variables under study.

Due to the non-normal distribution of the data, Friedman's two-way analysis of variance (ANOVA) was used to compare the occlusal contact areas and the number of anterior and posterior occlusal contacts among T0, T1 and T2. This is a non-parametric statistical test used to determine whether 3 related groups differ from one another. The comparison of masticatory performance between the end of the first series of aligners and 3 months after nighttime use was performed using the Wilcoxon signed-rank test (a non-parametric test used to compare 2 periods within the same sample to assess whether their population mean ranks differ). The contact area and the number of anterior and posterior occlusal contacts at T0, T1 and T2, as well as masticatory performance at T1 and T2, were compared among the different groups according to the facial biotype, the case complexity, the molar class, and the vertical bite pattern using the Kruskal–Wallis test (a non-parametrical test), followed by Dunn's test with Bonferroni correction to determine which groups differed significantly from one another. The Bonferroni correction adjusts the alpha level to account for the number of pairwise comparisons performed. To evaluate the relationship between occlusal area, anterior and posterior contacts, and masticatory performance at T1, as well as

the corresponding relationships at T2, Spearman's correlation coefficient was used (a non-parametric measure of the association between 2 variables). The significance level was set at 0.05.

The effect size (Cohen's *d*, with bias correction using Hedges' method) was calculated to compare groups and examine the correlation between variables. A Cohen's *d* of 0.0–0.2 was considered a small effect size; *d* of 0.5 was considered a medium effect size; and *d* ≥ 0.8 was considered a large effect size. Therefore, if the means of the 2 groups did not differ by more than 0.2 standard deviations, the difference was considered trivial, even if it was statistically significant.

Results

Sample characteristics

Participants were aged between 16 and 45 years (mean age of 22.19 ± 10.51 years), with 28 females (66.7%) and 14 males (33.3%). None of the patients presented TMD signs or symptoms. Regarding the facial biotype, 20 participants (47.6%) had a normodivergent biotype, 12 (28.6%) were hyperdivergent and 10 (23.8%) were hypodivergent. Most participants had a normal overbite (57.1%) and a normal overjet (59.5%). The clinical assessment of the sample revealed 17 individuals (40.5%) with a deep bite, 15 (35.7%) with a normal bite, and 10 (23.8%) with an open bite. Concerning the complexity of the cases, 27 cases (64.3%) were of moderate complexity, 9 cases (21.4%) were considered complex, and 6 (14.3%) were simple.

Changes in occlusal contacts and areas throughout CAT

The changes in occlusal area and posterior and anterior contacts throughout CAT are presented in Table 1. The comparison of the occlusal area throughout treatment showed that the initial occlusal area (T0) decreased significantly by the end of CAT (T1) ($p = 0.002$), with a large effect size ($d = 0.94$).

Comparison of masticatory performance at the end of CAT and 3 months after nighttime use only

When comparing the median values of masticatory performance at the end of CAT (T1) and 3 months later, when CAs were used only at night (T2), a reduction in the median values was observed, indicating better masticatory performance. However, no statistically significant differences were found between the 2 assessment time points, and the effect size was considered small ($d = 0.23$) (Table 2).

Comparison of occlusal area, occlusal contacts and masticatory performance throughout CAT

Case complexity

At T1, individuals with simple cases presented higher occlusal area values than those with moderate or complex cases ($p = 0.027$). A statistically significant difference in occlusal area was found between moderate and simple cases ($p = 0.023$). Significant differences were also found in posterior contacts at T0, with complex cases showing

lower median values than moderate and simple cases ($p = 0.046$) (Table 3).

Facial biotype

Table 4 compares occlusal area, anterior and posterior contacts, and masticatory performance according to the facial biotype of individuals undergoing CAT at the 3 assessment time points. Regarding anterior contacts, statistically significant differences were found at T1, with normodivergent individuals presenting a significantly higher number of contacts than hyperdivergent individuals ($p = 0.013$), with a large effect size ($d = 1.45$).

Table 1. Comparison of occlusal area and posterior and anterior occlusal contacts throughout clear aligner treatment (CAT) ($N = 42$)

Variable	Time point	Me (IQR)	χ^2	p-value	Bias-corrected effect size (Hedges)		
					T0-T1	T1-T2	T0-T2
Occlusal area	T0	47.37 (28.85–88.50)*	7.87	0.002	0.94	0.44	0.71
	T1	25.20 (15.48–43.57)*					
	T2	31.07 (21.19–45.90)					
Posterior contacts	T0	8.00 (5.00–8.00)	1.54	0.462	0.66	0.78	0.00
	T1	7.00 (5.00–8.00)					
	T2	8.00 (6.00–8.00)					
Anterior contacts	T0	4.00 (2.00–6.00)	0.60	0.743	0.00	0.00	0.00
	T1	4.00 (3.00–5.00)					
	T2	4.00 (2.75–6.00)					

* significant difference between T0 and T1 ($p = 0.016$); IQR – interquartile range; Me – median; T0 – before treatment; T1 – at the end of treatment; T2 – 3 months after the end of treatment, during which time clear aligners were only used at night.

Table 2. Comparison of masticatory performance at the end of clear aligner treatment (CAT) (T1) and 3 months later, when clear aligners were used only at night (T2) ($N = 42$)

Variable	Time point	Me (IQR)	z	p-value	Bias-corrected effect size (Hedges)
Masticatory performance	T1	0.133 (0.079–0.231)	-0.631	0.528	0.23
	T2	0.115 (0.055–0.207)			

Table 3. Comparison of occlusal area, occlusal contacts and masticatory performance according to case complexity

Variable	Time point	Complex cases ($n = 9$)	Moderate cases ($n = 27$)	Simple cases ($n = 6$)	H	p-value	Bias-corrected effect size (Hedges)		
							complex–moderate	complex–simple	moderate–simple
Occlusal area	T0	32.36 (15.17–47.41)	51.19 (25.04–89.93)	84.13 (21.28–134.21)	3.81	0.149	0.63	1.36	0.87
	T1	15.66 (8.40–31.48)	25.12 (16.02–50.39)*	41.02 (34.48–48.07)*	7.22	0.027	0.58	2.48	0.98
	T2	33.59 (27.63–45.38)	31.96 (20.01–45.20)	30.09 (16.02–57.78)	0.11	0.949	0.13	0.23	0.13
Anterior contacts	T0	2.00 (0.50–5.50)	4.00 (2.00–6.00)	5.00 (2.00–6.00)	2.15	0.341	0.92	1.22	0.49
	T1	4.00 (2.50–5.00)	3.00 (3.00–6.00)	5.00 (3.5–5.00)	0.81	0.669	0.68	0.87	1.39
	T2	4.00 (3.00–6.00)	5.00 (3.00–6.00)	2.00 (0.00–6.00)	1.73	0.421	0.65	0.85	1.60
Posterior contacts	T0	5.00 (3.50–7.50)	8.00 (6.00–8.00)*	8.00 (7.25–8.00)*	6.18	0.046	2.25	1.78	0.00
	T1	6.00 (4.50–7.00)	7.00 (5.00–8.00)	7.5 (5.75–8.00)	2.35	0.310	0.68	1.17	0.34
	T2	8.00 (6.00–8.00)	8.00 (6.00–8.00)	7.00 (5.50–8.00)	0.17	0.917	0.00	0.85	0.93
Masticatory performance	T1	0.11 (0.03–0.14)	0.14 (0.08–0.23)	0.13 (0.05–0.18)	1.37	0.505	0.11	0.04	0.13
	T2	0.13 (0.08–0.22)	0.10 (0.05–0.20)	0.19 (0.13–0.47)	3.75	0.154	0.08	0.10	0.91

Data presented as Me (IQR); * significant difference between the moderate and simple groups ($p = 0.023$).

Molar class relationship

At T0, individuals with Class I molar relationship had significantly more anterior contacts than those with Classes II and III. Dunn's test showed that these differences were significant between the Class I and Class II groups ($p = 0.004$). At T1, individuals with Class III molar relationship had a significantly higher number of anterior

contacts than those with Class II molar relationships, with the effect size of $d = 1.51$ ($p = 0.011$). At T2, individuals with Class III molar relationship presented higher numbers of anterior contacts than those with Classes I and II. These differences were statistically significant between the Class I and Class II groups ($p = 0.024$) and between the Class II and Class III groups ($p = 0.011$) (Table 5).

Table 4. Comparison of occlusal area, occlusal contacts and masticatory performance according to the facial biotype

Variable	Time point	Facial biotype			<i>H</i>	<i>p</i> -value	Bias-corrected effect size (Hedges)		
		normodivergent (<i>n</i> = 20)	hyperdivergent (<i>n</i> = 10)	hypodivergent (<i>n</i> = 12)			normodivergent– hyperdivergent	normodivergent– hypodivergent	hyperdivergent– hypodivergent
Occlusal area	T0	52.53 (35.69–80.50)	38.07 (16.36–91.65)	28.67 (14.57–89.57)	1.46	0.481	0.47	0.81	0.23
	T1	34.77 (13.20–34.77)	19.96 (15.47–43.09)	19.93 (18.10–34.47)	1.69	0.431	1.22	1.46	0.00
	T2	34.90 (20.79–48.54)	25.40 (14.18–38.50)	32.77 (24.77–43.30)	1.78	0.413	0.69	0.17	0.66
Anterior contacts	T0	5.00 (2.25–6.00)	2.00 (0.75–6.00)	3.50 (1.00–6.00)	2.32	0.313	1.36	0.69	0.56
	T1	4.50 (3.00–6.00)*	2.00 (0.00–4.00)*	3.50 (3.00–5.00)	8.16	0.013	1.45	0.73	0.94
	T2	5.00 (4.00–6.00)	2.50 (0.75–6.00)	4.00 (2.25–5.75)	2.70	0.259	1.43	0.74	0.66
Posterior contacts	T0	8.00 (5.00–8.00)	8.00 (4.75–8.00)	7.50 (5.50–8.00)	0.30	0.862	0.00	0.31	0.28
	T1	6.50 (5.00–8.00)	6.50 (5.75–8.00)	7.50 (5.25–8.00)	0.41	0.814	0.00	0.52	0.48
	T2	7.00 (5.25–8.00)	8.00 (6.25–8.00)	8.00 (6.25–8.00)	1.77	0.412	0.79	0.80	0.00
Masticatory performance	T1	0.15 (0.10–0.28)	0.11 (0.09–0.18)	0.08 (0.06–0.13)	5.72	0.057	0.51	0.91	0.82
	T2	0.12 (0.08–0.21)	0.10 (0.05–0.26)	0.11 (0.05–0.16)	0.45	0.798	0.15	0.16	0.07

Data presented as *Me* (*IQR*); * significant difference between the normodivergent and hyperdivergent groups ($p = 0.013$).

Table 5. Comparison of occlusal area, occlusal contacts and masticatory performance according to the initial molar class relationship

Variable	Time point	Molar relationship			<i>H</i>	<i>p</i> -value	Bias-corrected effect size (Hedges)		
		Class I (<i>n</i> = 25)	Class II (<i>n</i> = 10)	Class III (<i>n</i> = 7)			I–II	I–III	II–III
Occlusal area	T0	56.79 (27.45–95.02)	38.62 (19.33–62.55)	39.50 (9.86–51.19)	3.21	0.201	0.57	0.53	0.04
	T1	34.48 (18.23–48.59)	18.55 (15.47–24.16)	20.85 (9.38–35.01)	3.59	0.166	1.18	0.90	0.25
	T2	30.00 (17.97–42.94)	32.77 (19.74–48.42)	37.50 (28.15–58.07)	1.51	0.468	0.21	0.56	0.31
Anterior contacts	T0	6.00 (3.50–6.00)*	2.00 (0.75–2.25)*	2.00 (1.00–5.00)	12.06	0.002	3.44	2.72	0.00
	T1	4.00 (3.00–5.50)	2.50 (0.00–3.25)**	5.00 (3.00–6.00)**	10.59	0.004	1.08	0.75	1.51
	T2	5.00 (3.00–6.00)***	1.50 (0.75–3.25)***	5.00 (4.00–6.00)***	10.13	0.006	2.38	0.00	2.87
Posterior contacts	T0	8.00 (5.50–8.00)	7.50 (5.00–8.00)	7.00 (2.00–8.00)	2.98	0.225	0.37	0.56	0.21
	T1	6.00 (5.00–8.00)	7.00 (5.75–8.00)	6.00 (3.00–8.00)	0.62	0.735	0.69	0.00	0.53
	T2	7.00 (6.00–8.00)	8.00 (5.50–8.00)	8.00 (7.00–8.00)	1.55	0.460	0.91	1.06	0.00
Masticatory performance	T1	0.14 (0.08–0.31)	0.09 (0.06–0.12)	0.14 (0.08–0.19)	5.67	0.059	0.54	0.00	1.13
	T2	0.12 (0.07–0.20)	0.10 (0.05–0.27)	0.08 (0.05–0.24)	0.68	0.710	0.24	0.54	0.18

Data presented as *Me* (*IQR*); * significant difference between the Class I and Class II groups ($p = 0.004$); ** significant difference between the Class II and Class III groups ($p = 0.011$); *** significant difference between the Class I and Class II groups ($p = 0.024$) and between the Class II and Class III groups ($p = 0.011$).

Table 6. Comparison of occlusal area, occlusal contacts and masticatory performance according to the vertical bite trend

Variable	Time point	Vertical bite trend			<i>H</i>	<i>p</i> -value	Bias-corrected effect size (Hedges)		
		deep (<i>n</i> = 17)	normal (<i>n</i> = 15)	open (<i>n</i> = 10)			deep–normal	deep–open	normal–open
Occlusal area	T0	48.86 (25.91–76.46)	32.30 (24.58–56.79)	80.26 (19.20–103.88)	1.72	0.423	0.75	0.94	1.58
	T1	18.07 (12.33–41.02)	25.40 (19.02–45.21)	34.77 (17.36–62.08)	2.03	0.361	0.52	0.92	0.52
	T2	33.59 (21.51–42.82)	30.18 (23.70–48.72)	28.19 (13.80–42.29)	0.99	0.609	0.27	0.43	0.14
Anterior contacts	T0	4.00 (2.00–6.00)	3.00 (1.00–6.00)	4.50 (1.75–6.00)	0.72	0.696	0.50	0.24	0.73
	T1	4.00 (3.00–5.50)	4.00 (3.00–5.00)	3.00 (1.00–5.25)	1.23	0.542	0.00	0.52	0.46
	T2	5.00 (3.00–6.00)	5.00 (2.00–6.00)	3.50 (0.75–5.00)	2.17	0.338	0.00	0.83	0.68
Posterior contacts	T0	8.00 (6.00–8.00)	6.00 (5.00–8.00)	8.00 (7.25–8.00)	3.96	0.138	2.52	0.00	5.16
	T1	6.00 (5.50–8.00)	7.00 (5.00–8.00)	6.50 (5.00–8.00)	0.23	0.892	0.71	0.36	0.32
	T2	7.00 (6.00–8.00)	8.00 (7.00–8.00)	8.00 (5.75–8.00)	3.90	0.143	0.92	0.93	0.00
Masticatory performance	T1	0.09 (0.06–0.25)	0.14 (0.08–0.23)	0.14 (0.09–0.16)	0.64	0.725	0.66	0.62	0.00
	T2	0.12 (0.10–0.23)	0.11 (0.05–0.25)	0.08 (0.04–0.14)	3.19	0.203	0.17	0.65	0.58

Data presented as *Me* (*IQR*).

Table 7. Spearman's correlation analysis of the relationships between occlusal area, occlusal contacts and masticatory performance (*N* = 42)

Variable	Masticatory performance (T1)	Masticatory performance (T2)
Occlusal area (T1)	–0.229	–
Anterior contacts (T1)	–0.072	–
Posterior contacts (T1)	–0.378*	–
Occlusal area (T2)	–	0.049
Anterior contacts (T2)	–	–0.200
Posterior contacts (T2)	–	–0.161

* statistically significant (*p* < 0.05).

Vertical bite trend

According to Table 6 and with regard to the vertical bite trend, no statistically significant differences were found in occlusal area, masticatory performance, or anterior and posterior contacts among the deep-, normal- and open-bite groups. Small effect sizes were observed for the occlusal area at T2 between deep and normal bites (*d* = 0.27) and normal and open bites (*d* = 0.14); for anterior contacts at T0 between deep and open bites (*d* = 0.24), at T1 between deep and normal bites (*d* = 0.00), and at T2 between deep and normal bites (*d* = 0.00); for posterior contacts at T0 between deep and open bites (*d* = 0.00), and at T2 between normal and open bites (*d* = 0.00); and for masticatory performance at T1 between normal and open bites (*d* = 0.00), and at T2 between deep and normal bites (*d* = 0.17).

Relationship between changes in occlusal area, occlusal contacts and masticatory performance

Table 7 shows the relationships between occlusal area and occlusal contacts with masticatory performance. The number of posterior contacts at T1 showed a weak, negative

and statistically significant correlation with masticatory performance at T1 (*r* = –0.378, *p* < 0.05). Increases in occlusal area and in the number of anterior and posterior contacts were associated with lower masticatory performance values, which represented better masticatory performance.

Discussion

Esthetics is a key consideration for orthodontists. However, it is crucial to keep in mind that masticatory function and orthopedic principles should also be considered. Orthopedic stability in the masticatory structures should focus on reducing the risk factors associated with the development of TMD and promoting good masticatory function.³² Thus, awareness of adequate occlusion is important for OT success since the two are directly related. Also, occlusion is strongly connected with the health of the TMJ and masticatory muscles.³³

A statistically significant decrease in occlusal area was recorded between T0 and T1. These results are in line with the study by Horton et al., which reported a similar occurrence in the interocclusal contact area after the use of an occlusal-covering appliance (Essix) compared with the use of a Hawley splint (without occlusal coverage).³⁴ Following CAT, a lack of posterior contacts is common and is expected to be resolved during occlusal settling. As suggested by Sultana et al., this could be explained by the fact that during the retention period, functional accommodation of the occlusion occurs, leading to an increase in the number of contact points.³⁵ According to the results obtained, although the differences were not statistically significant, the use of a CA only at night for 3 months after the completion of treatment appeared to enhance the restitution of occlusal contacts, including both the occlusal area and posterior occlusal contacts. This agrees with other studies that reported similar results despite using other retaining devices.³⁶

In this sense, the idealized static occlusal objectives can be accomplished using CAs with careful planning, knowledge of the limits of this OT system, and, most importantly, knowledge of how to overcome them. Restoring physiologically normal masticatory function after OT is an important goal that should not be overlooked. Reducing foods to smaller particles is a key component of mastication and occurs at the interface between occlusal areas of antagonistic teeth. As described in the literature, mechanical masticatory performance is closely related to several factors, such as the areas of interocclusal contact and near-contact between post-canine teeth during maximum intercuspation.^{37,38} The outcome variable of the technique used in this study to assess masticatory function was the degree of color mixing of the chewing gum, expressed by VOH and measured by electronic colorimetric analysis. Poorly mixed colors resulting from insufficient chewing produce a high VOH, whereas well-mixed colors resulting from sufficient chewing produce a low VOH. In the present study, no improvement in masticatory performance was observed between T1 and T2, contrary to what was expected and described in the literature.³⁹ A systematic review highlighted that OT significantly improves oral health-related quality of life, with a noted enhancement in masticatory performance after treatment. This improvement was attributed to the regulation of muscle activity, which promotes the symmetrical function of the masticatory muscles.⁴⁰ Similarly, other authors have emphasized that orthodontic interventions lead to measurable changes in occlusion and masticatory function, reinforcing the concept that orthodontic appliances positively affect masticatory efficiency.³⁷ Furthermore, Gameiro et al. conducted a longitudinal study that objectively assessed masticatory and swallowing performance, revealing that OT provides functional benefits beyond mere esthetic improvement.³ Their findings suggest that the primary goals of mastication were achieved after treatment, as evidenced by improved performance metrics. This aligns with the observations of Lee et al., who reported that the degree of improvement in masticatory performance was positively correlated with the degree of improvement in occlusal area,³⁸ and with Alshammari et al.,⁴ who noted that orthodontic interventions can improve problems related to jaw motor function and chewing, particularly in children with malocclusion.

The relationship between occlusal area or occlusal contacts and masticatory performance has garnered considerable attention, with studies consistently indicating that a larger occlusal contact area is positively correlated with enhanced masticatory efficiency. Lee et al. found a significant relationship between occlusal contact area and masticatory performance, particularly highlighting the posterior occlusal area, which demonstrated a stronger correlation with masticatory outcomes than the anterior area.⁴¹ This notion is further supported by Bunpu and Changsiripun, who concluded that a greater occlusal

contact area consistently facilitates improved chewing efficiency among patients.⁴² Miura et al. also highlighted that an increased occlusal contact area was pivotal in enhancing masticatory efficacy, particularly in individuals with pathological conditions such as cleft lip and palate, noting that low occlusal force and abnormal jaw movement can negatively impact masticatory efficiency.⁴³ Additionally, Gameiro et al. demonstrated that a reduced occlusal contact area in individuals with malocclusions could lead to diminished masticatory performance due to decreased muscle activity and stabilization during mastication.³ These findings underscore the critical role of occlusal area in determining masticatory performance, suggesting that maintaining or enhancing occlusal contacts should be a focal point in OT and functional restorative dentistry.

Kawai et al. reported that masticatory efficiency and maximal occlusal force can significantly improve with continued masticatory exercises, such as gum chewing, which enhance blood flow and reduce discomfort in the periodontal ligament.⁴⁴ This is further supported by the findings of Jang et al., who noted that eliminating unfavorable occlusal conditions post-extraction leads to smoother chewing cycles and improved masticatory performance.²⁷ Moreover, studies have shown that while masticatory performance improves, it may not reach the levels observed in individuals with normal occlusion. Magalhães et al. pointed out that although OT generally enhances masticatory function, some impairments may persist compared with those observed in individuals without malocclusion.⁴⁵ This suggests that while OT is beneficial, further interventions or exercises may be necessary to fully optimize masticatory performance. This may be one of the reasons for the lack of statistically significant results observed in the present study between T1 and T2. The findings may also indicate stabilization of the occlusion, which was achieved after treatment and maintained after 3 months of nighttime CA use.

Complex cases had fewer posterior occlusal contacts at the beginning and end of CAT. This may be explained by the fact that this type of case generally presents a greater number of anatomical and functional parameters that determine this classification, making its correction more difficult. Cassinelli et al. demonstrated a significant correlation between case complexity and treatment difficulty, which is consistent with the present results.³⁹

Strong relationships exist between the degree of skeletal divergence (hypodivergent, normodivergent or hyperdivergent), the number of occlusal contacts and masticatory function. Skeletal divergence has also been shown to be negatively correlated with maximum bite force. This means that a hypodivergent patient tends to present higher bite forces. The opposite is also true for hyperdivergent individuals. In addition to the bite-block effect theory,⁴⁶ the amount of force and the pattern of masticatory muscle activation, as well as changes in masticatory function, are important variables. Therefore, various effects can

be expected in individuals with different degrees of skeletal divergence, since different bite forces are observed in these facial types. It would be expected that individuals with a hypodivergent biotype would tend to have a higher bite force, leading to the development of stronger occlusal contacts and, consequently, better mechanical masticatory performance compared with hyperdivergent individuals, who have lower facial muscle strength.^{47,48}

Contrary to expectations, the results showed no statistically significant relationship between facial biotypes and masticatory performance.⁴⁸ However, even in the absence of statistical significance, together with the small improvement observed in masticatory performance, slight increases between occlusal area and posterior contacts were observed between T1 and T2 for normodivergent and hyperdivergent biotypes. These slight increases align with findings reported by other authors, showing that normodivergent individuals normally present a higher number of anterior occlusal contacts than hyperdivergent individuals at the end of treatment. This may be explained by the fact that the hyperdivergent individuals often present a tendency toward an open bite.⁴⁹ Moreover, the literature indicates only limited improvement in masticatory performance between the immediate post-OT period and 3 months thereafter, highlighting the complexity of neuromuscular adaptation processes. While some studies report initial increases in masticatory efficiency, long-term gains may not be as pronounced due to individual variations in neuromuscular adaptation and the potential need for a more extended adaptation period beyond 3 months.⁴⁰ The authors noted that improvements in masticatory performance can be inconsistent and dependent on multiple factors, including the patient's age and pre-existing conditions. Additionally, the findings of Cohen-Lévy et al. suggest that re-establishing normal function after treatment may involve considerable adaptation, with marked increases in bite force occurring initially and subsequently tapering off after several months.⁵⁰ Furthermore, Magalhães et al. emphasized that although OT often ameliorates certain aspects of mastication, overall performance may still be suboptimal compared with that of individuals without occlusal discrepancies, suggesting that observed changes after treatment may reflect a settling period rather than lasting improvement.⁴⁵ Collectively, these findings highlight the necessity of comprehensive longitudinal studies to clarify the time course of recovery in masticatory performance following OT.

According to Angle's malocclusion classification, Bae et al. reported that the masticatory performance may differ among malocclusion classes.⁴⁹ These authors concluded that masticatory performance was higher in individuals with Angle's Class I malocclusion,⁵¹ followed by those with Angle's Class II and, finally, Angle's Class III malocclusion. The results obtained in the present study are consistent with the contrasting findings of Watanabe-Kanno and Abrão, who stated that occlusal contacts cannot

be standardized according to malocclusion type.⁵² However, although Angle's classification system is the most universally accepted, it has several shortcomings. Malocclusion is a three-dimensional (sagittal, transverse and vertical) condition, whereas Angle's system considers only anteroposterior deviation. Since the present study evaluated masticatory performance according to Angle's classification, the applicability of its results is limited because this classification does not consider transverse and vertical problems,^{49,51} which may also influence masticatory performance.

The results show that at the beginning of treatment, individuals with Class I molar relationship presented significantly higher numbers of anterior contacts than those with Class II. The fact that individuals with Class II frequently present overjet may help explain these results, since the anterior teeth are not in direct contact with one another, resulting in fewer anterior contacts.⁵³ Consistent with these findings, at the end of CAT, the present study showed that individuals with Class III molar relationship had a significantly higher number of anterior contacts compared to Class II individuals.

The thickness of the CA within the interocclusal space promotes a bite-block effect, leading to anatomical changes and inherent difficulties in achieving posterior contacts.⁷ Fan et al. showed that intruding posterior teeth with CAs allowed anterior mandibular rotation.⁵⁴ Reducing facial height and improving pogonial projection with correct torque, inclination and maxillary molar distalization may exacerbate Class III malocclusion.^{55,56} However, throughout OT, the goal is to reposition the lower front teeth to a more posterior position relative to the upper teeth. As the teeth are moved into their new positions, it is common for patients to experience more anterior occlusal contacts at the end of OT. This is consistent with the results obtained. It is important to remember that the bite-block effect refers to the intrusion of posterior teeth due to the thickness of the aligners, which can influence occlusal contacts and vertical dimensions. Some patients using CAs experience transient open-bite tendencies after treatment due to insufficient occlusal settling and a reduction in posterior contacts.³² Therefore, brackets and wires do not act as a uniform bite block, although bite turbos or elastics can be used intentionally to manage deep or open bites. Hence, occlusal settling is generally better because the teeth can naturally come into contact throughout treatment, particularly after debonding.⁵⁷ Conversely, another study concluded that for the treatment of mild malocclusion in adolescents, CAs were as effective as fixed appliances, with significantly better outcomes with CAT in terms of tooth alignment, occlusal relationships and overjet.⁵⁸

The etiology of TMD is complex and multifactorial,^{59,60} a fact recognized by the scientific community. In addition, occlusion is known to play a role in TMD, but this relationship is still controversial.⁶¹ Studies have indicated that this role may be more important than generally accepted,

especially in internal derangements of the TMJ.²¹ The literature has already shown that occlusal changes resulting from CAT can alter the orthopedic stability of the craniofacial system and may create conditions conducive to the onset of TMD.⁵ However, our results showed that the use of CAs did not affect the onset of TMD signs and symptoms. This could be explained by the fact that a bio-mechanical environment tends to induce an adaptative response of the system, whether through remodeling of its structural components or changes in function, because the sensorimotor system is not static but plastic and has the capacity to adapt to the functional demands.^{62–64} It should also be remembered that cognitive, affective and emotional factors often determine whether and how a patient adjusts to dental treatment.^{65,66}

In this context, Hifny et al. suggested a direct association between TMD and occlusion, indicating that patients with a history of TMD and specific occlusal interferences may be more susceptible to maladaptive disorders following OT, potentially increasing the risk of developing complications if these factors are not adequately addressed during treatment.⁶⁷ A study by de Kanter et al. supported these results and also advocated for a strong focus on occlusal considerations during the diagnostic and treatment phases of orthodontics.⁶⁸ Nevertheless, the degree to which occlusion contributes to TMD remains debated, as discussed above. According to Kannan and Padmanabhan, a “stable occlusion” is an essential goal of OT; however, they emphasized that not attaining a “perfect occlusion” does not inherently lead to TMD signs and symptoms.⁶⁹ This notion is further reinforced by Manfredini et al., who describe orthodontics as generally neutral with respect to the risk of TMD, suggesting that while occlusal factors might play a role in some cases, they are not deterministic predictors of TMD outcomes.⁷⁰ The systematic review by Fernández-González et al. showed that the multifactorial nature of TMD cannot be overlooked; TMD may arise from various factors, such as trauma, emotional stress and parafunctional activities, alongside occlusal discrepancies.¹⁵ In this context, the relationship between occlusion and TMD symptoms may reflect a complex interplay rather than a clear causative chain. Moreover, some authors have suggested that while OT may indeed alter TMJ function, the clinical changes resulting from treatment may not significantly differ from those observed in untreated controls.⁷¹ Similarly, in a cohort study, Sim et al. indicated that although Class II malocclusion is associated with higher rates of TMD, the overarching conclusion was that OT does not definitively exacerbate or mitigate TMD symptoms.⁷² Additionally, it is essential to consider that not all types of malocclusion affect TMD signs equally. Kaku et al. reported that deep bites were significantly correlated with TMD symptoms, suggesting that specific occlusal patterns might warrant more focused investigation of their role as potential risk factors for TMD development.⁷³

With advances in healthcare technology, it is essential to discuss the types and properties of materials used in dentistry.^{74–76} A study comparing conventional resin and 3D-printed resin concluded that conventional heat-cured poly(methyl methacrylate) (PMMA) displayed superior flexural properties, while 3D-printed resin showed the highest hardness.⁷⁷ All tested materials were vulnerable to deterioration in their mechanical properties due to artificial aging, but conventional PMMA exhibited the greatest resistance to changes, making it the optimal choice for successful clinical performance. Another study⁷⁸ compared the mechanical properties of 3 different 3D-printed resins: IBT Resin (Formlabs Inc., Somerville, USA); BioMed Amber Resin (Formlabs Inc.); and Dental LT Clear Resin (Vertex-Dental, Soesterberg, the Netherlands). Tensile strength testing showed that BioMed Amber Resin yielded the most repeatable results, with similar force values required to fracture all resin specimens. The lowest tensile force was required to damage IBT Resin, which was the least stable and least rigid material, whereas Dental LT Clear Resin was the most resistant of the 3 resins. Complementing the previous study,⁷⁸ other authors⁷⁹ evaluated the properties of Dental LT Clear Resin and concluded that polishing had minimal influence on the properties of the resin but increased the resistance of the specimens to artificial aging, while artificial aging significantly affected the compressive modulus and tensile properties of the material. All these factors should be considered when using the aligners during treatment. Furthermore, another study⁸⁰ concluded that 3D-printed aligners can be successfully printed in-house and used in mildly crowded cases, with tooth movement accuracy comparable to that of other aligners.

The materials used in CA fabrication, specifically 3D-printed and thermoformed aligners, can significantly influence occlusal recovery outcomes during OT. Research indicates that thermoformed aligners, which are often thinner and may exhibit variable rigidity, can lose their fit against the teeth, particularly at the gingival edge. This loss of fit may impair effective tooth movement and occlusal adjustments.⁸¹ This loss of fit is linked to a reduction in the efficacy of the applied forces, as observed by Gaddam et al., who noted that variations in aligner thickness can lead to different amounts of torque being generated within the dental arch.⁸² Conversely, advancements in 3D printing technologies aim to produce aligners with improved fit and mechanical properties, potentially enhancing their ability to facilitate effective occlusal recovery.⁸³ Therefore, while traditional thermoformed aligners may adversely affect occlusal dynamics and recovery due to fit issues and stress distribution, 3D-printed aligners could provide a more precise and adaptable treatment modality, promoting better patient outcomes and post-treatment occlusal function.⁸⁴

In the context of ongoing technological developments and the importance of articulators in dental treatment planning, including systems such as CADIAX® compact

2 and MODJAW™,⁷⁹ one study compared mechanical and computerized registration methods using 2 selected kinematic facebows: the Gerber Dynamic Facebow; and the computerized ARCUSdigma II axiograph. The authors concluded that the use of a mechanical facebow was associated with a higher risk of manual measurement errors during the tracing procedure. Moreover, because of the substantial discrepancies between the results, the authors recommended using an articulator compatible with the facebow used for the measurements.

Limitations

One limitation of the present study was its small sample size resulting from the inclusion criteria, which limited the statistical power and reduced the reliability of the findings. A larger sample would provide better representation of the population and more accurate results, together with a longer follow-up period. Also, there was no control group (e.g., fixed appliance group), which should be considered and included in future studies for comparison with CAT. As the aim of the present study was to conduct a cohort analysis of CAT outcomes, specifically assessing occlusal changes and their influence on masticatory performance, a control group was not included at this stage. Such a comparison could improve understanding of the impact of different types of OT on occlusal outcomes. Moreover, no baseline (T0) data were recorded regarding the relationship between occlusal area, occlusal contact and masticatory performance, and these variables should be assessed in future studies. This may represent a potential confounding factor. Other potential confounding factors include variability in patient compliance with aligner use, dietary habits that may influence masticatory performance, and parafunctional habits such as bruxism.

Conclusions

The results demonstrated that during CAT, the number of occlusal contacts and the occlusal contact area decreased. It was also observed that using CAs only at night slightly enhanced the recovery of occlusal contacts. Regarding case complexity and occlusal factors, complex cases presented fewer posterior contacts at the beginning of CAT. In contrast, simple cases showed a greater occlusal area at the end of CAT. A higher number of anterior contacts was observed at the beginning of treatment in individuals with Class I molar relationship. These changes were associated with an increase in anterior contacts in Class III cases at the end of treatment and 3 months later. No significant differences in masticatory performance were found according to the molar relationship, the facial biotype or the case complexity. Furthermore, no TMD signs and symptoms were observed during or after CAT. Future studies should include a control group, better

account for potential confounding factors, increase the sample size, and conduct long-term follow-ups beyond 3 months to assess occlusal settling more thoroughly.

Ethics approval and consent to participate

Ethics approval was obtained from the Ethics Committee of the University Institute of Health Sciences (IUCS), Gandra, Portugal (approval No. 26/CE-IUCS/2020). All participants provided informed consent before the commencement of the study.

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. Tamer İ, Öztaş E, Marşan G. Orthodontic treatment with clear aligners and the scientific reality behind their marketing: A literature review. *Turk J Orthod.* 2019(4);32(4):241–246. doi:10.5152/TurkJOrthod.2019.18083
2. Partouche AJD, Castro F, Baptista AS, Costa LG, Fernandes JCH, Fernandes GVO. Effects of multibracket orthodontic treatment versus clear aligners on periodontal health: An integrative review. *Dent J (Basel).* 2022;10(10):177. doi:10.3390/dj10100177
3. Gameiro GH, Magalhães IB, Szymanski MM, Andrade AS. Is the main goal of mastication achieved after orthodontic treatment? A prospective longitudinal study. *Dental Press J Orthod.* 2017;22(3):72–78. doi:10.1590/2177-6709.22.3.072-078.oar
4. Alshammari A, Almotaury N, Kumar A, Grigoriadis A. Effect of malocclusion on jaw motor function and chewing in children: A systematic review. *Clin Oral Investig.* 2022;26(3):2335–2351. doi:10.1007/s00784-021-04356-y
5. Okeson JP. Evolution of occlusion and temporomandibular disorder in orthodontics: Past, present, and future. *Am J Orthod Dentofac Orthop.* 2015;147(5 Suppl):S216–S223. doi:10.1016/j.ajodo.2015.02.007
6. Bourdiol P, Hennequin M, Peyron MA, Woda A. Masticatory adaptation to occlusal changes. *Front Physiol.* 2020;11:263. doi:10.3389/fphys.2020.00263
7. Charalampakis O, Iliadi A, Ueno H, Oliver DR, Kim KB. Accuracy of clear aligners: A retrospective study of patients who needed refinement. *Am J Orthod Dentofac Orthop.* 2018;154(1):47–54. doi:10.1016/j.ajodo.2017.11.028
8. Ke Y, Zhu Y, Zhu M. A comparison of treatment effectiveness between clear aligner and fixed appliance therapies. *BMC Oral Health.* 2019;19(1):24. doi:10.1186/s12903-018-0695-z

9. Papadimitriou A, Mousoulela S, Gkantidis N, Kloukos D. Clinical effectiveness of Invisalign® orthodontic treatment: A systematic review. *Prog Orthod*. 2018;19(1):37. doi:10.1186/s40510-018-0235-z
10. Fritz F, Daratsianos N, Bourauel C, Papageorgiou SN, Jäger A. Changes in the distribution of occlusal forces in the course of the orthodontic retention phase: A prospective cohort study. *J Orofac Orthop*. 2025;86(2):1–13. doi:10.1007/s00056-023-00480-4
11. Hayakawa Y, Suita K, Ohnuki Y, et al. Vidarabine, an anti-herpes agent, prevents occlusal-disharmony-induced cardiac dysfunction in mice. *J Physiol Sci*. 2022;72(1):2. doi:10.1186/s12576-022-00826-4
12. Suhartini, Narmada IB, Hamzah Z, Joewarini E. NFkB and MMP-13 expression in condylar cartilage of temporomandibular joint with occlusal disharmony in vivo. *Dental J*. 2024;57(1):22–27. doi:10.20473/j.djmk.v57.i1.p22-27
13. Kapos FP, Exposto FG, Oyarzo JF, Durham J. Temporomandibular disorders: A review of current concepts in aetiology, diagnosis and management. *Oral Surg*. 2020;13(4):321–334. doi:10.1111/ors.12473
14. Zieliński G, Pająk-Zielińska B, Ginszt M. A meta-analysis of the global prevalence of temporomandibular disorders. *J Clin Med*. 2024;13(5):1365. doi:10.3390/jcm13051365
15. Fernández-González FJ, Cañigral A, López-Caballo JL, et al. Influence of orthodontic treatment on temporomandibular disorders. A systematic review. *J Clin Exp Dent*. 2015;7(2):e320–e327. doi:10.4317/jced.52037
16. Hwang SH, Park SG. Experience of orthodontic treatment and symptoms of temporomandibular joint in South Korean adults. *Iran J Public Health*. 2018;47(1):13–17. PMID:29318112.
17. Ugolini A, Garbarino F, Di Vecce L, Silvestrini-Biavati FS, Lanteri V. Determining risk factors for the development of temporomandibular disorders during orthodontic treatment. *Appl Sci*. 2020;10(22):8216. doi:10.3390/app10228216
18. Coronel-Zubiate FT, Marroquín-Soto C, Geraldo-Campos LAG, et al. Association between orthodontic treatment and the occurrence of temporomandibular disorders: A systematic review and meta-analysis. *J Clin Exp Dent*. 2022;14(12):e1032–e1043. doi:10.4317/jced.59970
19. Heleliwa-Feroli T, Vigo SC. Association between bruxism and the use of aligners in orthodontics. *J Clin Exp Dent*. 2024;16(10):e1247–e1255. doi:10.4317/jced.61951
20. Almalki SA, Al Jameel AH, Gowdar IM, et al. Impact of clear aligner therapy on masticatory musculature and stomatognathic system: A systematic review conducted according to PRISMA guidelines and the Cochrane handbook for systematic reviews of interventions. *BMC Oral Health*. 2024;24(1):350. doi:10.1186/s12903-024-04029-8
21. Tran J, Lou T, Nebiolo B, Castroflorio T, Tassi A, Cioffi I. Impact of clear aligner therapy on tooth pain and masticatory muscle soreness. *J Oral Rehabil*. 2020;47(12):1521–1529. doi:10.1111/joor.13088
22. World Health Organization. Ensuring ethical standards and procedures for research with human beings. <https://www.who.int/activities/ensuring-ethical-standards-and-procedures-for-research-with-human-beings>. Accessed March 1, 2025.
23. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344–349. doi:10.1016/j.jclinepi.2007.11.008
24. Marcelino V, Marcelino S, Baptista S, et al. Intra- and inter-examiner reliability for diagnostic criteria of temporomandibular disorders (DC/TMD) – Axis 1: Assessment in a sample of students. *Int J Sci Dent*. 2024;1:87–109. doi:10.22409/ijssd.v1i63.59145
25. Silva LC, Nogueira TE, Rios LF, Schimmel M, Leles CR. Reliability of a two-colour chewing gum test to assess masticatory performance in complete denture wearers. *J Oral Rehabil*. 2018;45(4):301–307. doi:10.1111/joor.12609
26. Schimmel M, Christou P, Miyazaki H, Halazonetis D, Herrmann FR, Müller F. A novel colourimetric technique to assess chewing function using two-coloured specimens: Validation and application. *J Dent*. 2015;43(8):955–964. doi:10.1016/j.jdent.2015.06.003
27. Jang I, Jang E, Choi DS, Cha BK. Chewing performance in orthodontic patients treated with extraction of premolars. *Oral Biol Res*. 2019;43(3):196–201. doi:10.21851/obr.43.03.201909.196
28. Jung CY, Park JH, Ku JH, Lee NK, Kim Y, Kook YA. Dental and skeletal effects after total arch distalization using modified C-palatal plate on hypo- and hyperdivergent Class II malocclusions in adolescents. *Angle Orthod*. 2021;91(1):22–29. doi:10.2319/031720-188.1
29. Putrino A, Barbato E, Galluccio G. Clear aligners: Between evolution and efficiency – a scoping review. *Int J Environ Res Public Health*. 2021;18(6):2870. doi:10.3390/ijerph18062870
30. Invisalign® Doctor Site. <https://learn.invisalign.com>. Accessed March 1, 2025.
31. Ragunathanan L, Maheshwari U, Vijayalakshmi D. Comparison of settling of occlusion in modified and full coverage thermoplastic retainers using t-scan. *APOS Trends Orthod*. 2022;12(2):115–124. doi:10.25259/APOS_107_2021
32. Marcelino V, Baptista S, Marcelino S, et al. Occlusal changes with clear aligners and the case complexity influence: A longitudinal cohort clinical study. *J Clin Med*. 2023;12(10):3435. doi:10.3390/jcm12103435
33. Lekaviciute R, Kriauciunas A. Relationship between occlusal factors and temporomandibular disorders: A systematic literature review. *Cureus*. 2024;16(2):e54130. doi:10.7759/cureus.54130
34. Horton JK, Buschang PH, Oliver DR, Behrents RG. Comparison of the effects of Hawley and perfector/spring aligner retainers on postorthodontic occlusion. *Am J Orthod Dentofac Orthop*. 2009;135(6):729–736. doi:10.1016/j.jado.2007.05.022
35. Sultana MH, Yamada K, Hanada K. Changes in occlusal force and occlusal contact area after active orthodontic treatment: A pilot study using pressure-sensitive sheets. *J Oral Rehabil*. 2002;29(5):484–491. doi:10.1046/j.1365-2842.2002.00849.x
36. Razdolsky Y, Sadowsky C, BeGole EA. Occlusal contacts following orthodontic treatment: A follow-up study. *Angle Orthod*. 1989;59(3):181–185. doi:10.1043/0003-3219(1989)059<0181:OCFOTA>2.0.CO;2
37. Zanon G, Contardo L, Reda B. The impact of orthodontic treatment on masticatory performance: A literature review. *Cureus*. 2022;14(10):e30453. doi:10.7759/cureus.30453
38. Lee SH, Fang ML, Choi YJ, et al. Changes in masticatory performance during the retention period following 4-premolar extraction and non-extraction orthodontic treatment. *Clin Oral Investig*. 2023;27(6):2609–2619. doi:10.1007/s00784-022-04817-y
39. Cassinelli AG, Firestone AR, Beck FM, Vig KWL. Factors associated with orthodontists' assessment of difficulty. *Am J Orthod Dentofac Orthop*. 2003;123(5):497–502. doi:10.1067/mod.2003.S088954060257013X
40. Sabzevari B, Fatemi A, Soleimani M, Sajedi SM, Babazadehkhoushrodi R. Masticatory performance and oral health related to quality of life before and after orthodontic treatment: A systematic review and meta-analysis. *Eur J Transl Myol*. 2024;34(1):12101. doi:10.4081/ejtm.2024.12101
41. Lee HS, Ko KH, Huh YH, Cho LR, Park CJ. Correlation between occlusal contact area at various levels of interocclusal thicknesses and masticatory performance. *J Oral Rehabil*. 2021;49(5):522–528. doi:10.1111/joor.13292
42. Bunpu P, Changsiripun C. Assessment of masticatory performance in patients undergoing orthognathic surgery: A systematic review and meta-analysis. *J Oral Rehabil*. 2023;50(7):596–616. doi:10.1111/joor.13447
43. Miura S, Ueda H, Iwai K, et al. Masticatory function assessment of adult patients with cleft lip and palate after orthodontic treatment. *Cleft Palate Craniofac J*. 2022;59(3):390–398. doi:10.1177/1055665621991733
44. Kawai N, Shibata M, Watanabe M, Horiuchi S, Fukushima K, Tanaka E. Effects of functional training after orthognathic surgery on masticatory function in patients with mandibular prognathism. *J Dent Sci*. 2020;15(4):419–425. doi:10.1016/j.jds.2020.01.006
45. Magalhães IB, Pereira LJ, Andrade A, Gouvêa DB, Gameiro GH. The influence of fixed orthodontic appliances on masticatory and swallowing threshold performances. *J Oral Rehab*. 2014;41(12):897–903. doi:10.1111/joor.12218
46. Lou T, Tran J, Castroflorio T, Tassi A, Cioffi I. Evaluation of masticatory muscle response to clear aligner therapy using ambulatory electromyographic recording. *Am J Orthod Dentofac Orthop*. 2021;159(1):e25–e33. doi:10.1016/j.jado.2020.08.012
47. Prema A, Vimala G, Rao U, Shameer A, Gayathri. Occlusal bite force changes during fixed orthodontic treatment in patients with different vertical facial morphology. *Saudi Dent J*. 2019;31(3):355–359. doi:10.1016/j.sdentj.2019.02.044
48. Del Santo Jr M. Treatment of adult patient with hyperdivergent retrognathic phenotype and anterior open bite: Report of a case with non-surgical orthodontic approach. *Dental Press J Orthod*. 2020;25(4):75–84. doi:10.1590/2177-6709.25.4.075-084.bbo

49. Bae J, Son WS, Kim SS, Park SB, Kim YI. Comparison of masticatory efficiency according to Angle's classification of malocclusion. *Korean J Orthod*. 2017;47(3):151–157. doi:10.4041/kjod.2017.47.3.151
50. Cohen-Lévy J, Boulos C, Rompré P, Montpetit A, Kerstein RB. Comparing occlusal contact quality after aligner and fixed appliance treatment using computerized occlusal analysis during 6 months of retention. *Research Square*. Preprint posted online October 12, 2021. doi:10.21203/rs.3.rs-955210/v1
51. Al-Rayes NZ, Hajeer MY. Evaluation of occlusal contacts among different groups of malocclusion using 3D digital models. *J Contemp Dent Pr*. 2014;15(1):46–55. doi:10.5005/jp-journals-10024-1486
52. Watanabe-Kanno GA, Abrão J. Study of the number of occlusal contacts in maximum intercuspation before orthodontic treatment in subjects with Angle Class I and Class II Division I malocclusion. *Dental Press J Orthod*. 2012;17(1):138–147. doi:10.1590/S2176-94512012000100017
53. Cao L, Li J, Yang C, Hu B, Zhang X, Sun J. High-efficiency treatment with the use of traditional anchorage control for a patient with Class II malocclusion and severe overjet. *Am J Orthod Dentofac Orthop*. 2019;155(3):411–420. doi:10.1016/j.ajodo.2017.08.030
54. Fan D, Liu H, Yuan CY, Wang SY, Wang PL. Effectiveness of the attachment position in molar intrusion with clear aligners: A finite element study. *BMC Oral Health*. 2022;22(1):474. doi:10.1186/s12903-022-02472-z
55. Pinho T, Santos M. Skeletal open bite treated with clear aligners and miniscrews. *Am J Orthod Dentofac Orthop*. 2021;159(2):224–233. doi:10.1016/j.ajodo.2019.07.020
56. Pinho T, Rocha D. Asymmetrical skeletal Class III camouflage treatment with clear aligners and miniscrew anchorage. *J Clin Orthod*. 2021;55(12):757–768. PMID:35358087.
57. Xia Q, Wang W, Wang C, et al. Comparative assessment of orthodontic clear aligner versus fixed appliance for anterior retraction: A finite element study. *BMC Oral Health*. 2024;24(1):80. doi:10.1186/s12903-023-03704-6
58. Borda AF, Garfinkle JS, Covell DA, Wang M, Doyle L, Sedgley CM. Outcome assessment of orthodontic clear aligner vs fixed appliance treatment in a teenage population with mild malocclusions. *Angle Orthod*. 2020;90(4):485–490. doi:10.2319/122919-844.1
59. 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
60. Chang CL, Wang DH, Yang MC, Hsu WE, Hsu ML. Functional disorders of the temporomandibular joints: Internal derangement of the temporomandibular joint. *Kaohsiung J Med Sci*. 2018;34(4):223–230. doi:10.1016/j.kjms.2018.01.004
61. Caldas W, Conti ACCF, Janson G, Conti PCR. Occlusal changes secondary to temporomandibular joint conditions: A critical review and implications for clinical practice. *J Appl Oral Sci*. 2016;24(4):411–419. doi:10.1590/1678-775720150295
62. Peck CC. Biomechanics of occlusion – implications for oral rehabilitation. *J Oral Rehabil*. 2016;43(3):205–214. doi:10.1111/joor.12345
63. Goodacre CJ, Roberts WE, Goldstein G, Wiens JP. Does the stomatognathic system adapt to changes in occlusion? Best evidence consensus statement. *J Prosthodont*. 2021;30(S1):5–11. doi:10.1111/jopr.13310
64. Moreno-Hay I, Okeson JP. Does altering the occlusal vertical dimension produce temporomandibular disorders? A literature review. *J Oral Rehabil*. 2015;42(11):875–882. doi:10.1111/joor.12326
65. Scandurra C, Gasparro R, Dolce P, et al. The role of cognitive and non-cognitive factors in dental anxiety: A mediation model. *Eur J Oral Sci*. 2021;129(4):e12793. doi:10.1111/eos.12793
66. Murad MH, Ingle NA, Assery MK. Evaluating factors associated with fear and anxiety to dental treatment – a systematic review. *J Family Med Prim Care*. 2020;9(9):4530–4535. doi:10.4103/jfmpc.jfmpc_607_20
67. Hifny SA, Alsaidi NM, AlKhater EA, et al. Temporomandibular disorders and occlusion effect on orthodontic treatment. *Int J Community Med Public Health*. 2021;8(11):5608–5613. doi:10.18203/2394-6040.ijcmph20214305
68. de Kanter RJAM, Battistuzzi PGFCM, Truin GJ. Temporomandibular disorders: "Occlusion" matters! *Pain Res Manag*. 2018;2018:874858. doi:10.1155/2018/8746858
69. Kannan A, Padmanabhan S. Orthodontic diagnosis and management of TMJ – a case report. *Sri Ramachandra J Health Sci*. 2022;2:69–72. doi:10.25259/srjhs_13_2022
70. Manfredini D, Stellini E, Gracco A, Lombardo L, Guarda Nardini L, Siciliani G. Orthodontics is temporomandibular disorder–neutral. *Angle Orthod*. 2015;86(4):649–654. doi:10.2319/051015-318.1
71. Jeelani W, Habib A, Ali B, Ahmed M. Effect of orthodontic treatment with straight-wire appliance on the incidence of temporomandibular disorders – a 3-year cohort study. *J Ind Orthod Soc*. 2019;53(4):249–255. doi:10.1177/0301574219868551
72. Sim HY, Kim HS, Jung DU, et al. Investigation of the association between orthodontic treatment and temporomandibular joint pain and dysfunction in the South Korean population. *Korean J Orthod*. 2019;49(3):181–187. doi:10.4041/kjod.2019.49.3.181
73. Kaku M, Koseki H, Kawazoe A, et al. Treatment of a case of skeletal class II malocclusion with temporomandibular joint disorder using miniscrew anchorage. *Cranio*. 2011;29(2):155–163. doi:10.1179/crn.2011.024
74. Fernandes GVO, Nassani LM. The implementation of CAD/CAM technology at schools of dentistry: A short communication. *Enviro Dent J*. 2023;5(1):6–8. doi:10.12944/EDJ.05.01.03
75. Nassani LM, Bencharit S, Schumacher F, Lu WE, Resende R, Fernandes GVO. 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
76. Nassani LM, Javed K, Amer RS, Pun MHJ, Abdelkarim AZ, Fernandes GVO. Technology readiness level of robotic technology and artificial intelligence in dentistry: A comprehensive review. *Surgeries*. 2024;5(2):273–287. doi:10.3390/surgeries5020025
77. Weźgowiec 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
78. 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
79. Manziuc MM, Dîrzu A, Almășan O, et al. Cadiax Compact 2 and MODJAW comparative analysis of condylar inclination: Innovative digital approaches in dentistry. *J Prosthet Dent*. 2024;133(2):482–488. doi:10.1016/j.prosdent.2024.05.014
80. Migliorati M, Drago S, Castroflorio T, et al. Accuracy of orthodontic movements with 3D printed aligners: A prospective observational pilot study. *Korean J Orthod*. 2024;54(3):160–170. doi:10.4041/kjod23.268
81. Mantovani E, Castroflorio E, Rossini G, et al. Scanning electron microscopy evaluation of aligner fit on teeth. *Angle Orthod*. 2018;88(5):596–601. doi:10.2319/120417-827.1
82. Gaddam R, Freer E, Kerr B, Weir T. Reliability of torque expression by the Invisalign® appliance: A retrospective study. *Aust Orthod J*. 2021;37(1):3–13. doi:10.21307/aoj-2021-001
83. Yu X, Li G, Zheng Y, et al. 'Invisible' orthodontics by polymeric 'clear' aligners molded on 3D-printed personalized dental models. *Regen Biomater*. 2022;9(2):rbac007. doi:10.1093/rb/rbac007
84. Machoy M, Tomasik M, Samulak R, Brzezińska-Zajac A, Woźniak K, Szyszka-Sommerfeld L. Clear aligner treatment as a safe method for treating occlusal trauma in elderly patients. *Appl Sci*. 2023;13(17):9506. doi:10.3390/app13179506

Temporary bite-raising onlays vs. stabilization splints for the management of pain-related temporomandibular disorders: A retrospective case–control study

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Abstract

Background. Management goals for patients with temporomandibular disorders (TMDs) include reducing pain, improving function and resuming normal daily activities.

Objectives. The present retrospective study compared the effectiveness and efficiency of 2 different gnathological treatment options – temporary bite-raising onlays (TBR) and stabilization splints (SS) – for the management of pain-related TMDs.

Material and methods. Medical records of 86 patients treated in the Department of Orthodontics of the Agostino Gemelli University Hospital IRCCS, Rome, Italy, were retrieved: 43 patients were treated with TBR; and 43 with SS. We included adult patients (aged 18–65 years) of both sexes, with a diagnosis of pain-related TMDs according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), treated with TBR or SS, with no systemic diseases or comorbidities, who provided written informed consent for the use of their clinical data. The outcomes assessed were the presence of pain and reduced mouth opening (<40 mm) at the end of treatment, the number of clinical check-ups and appliance adjustments, the overall treatment duration, self-reported patient discomfort, and the time frame within which patients experienced improvement.

Results. Temporary bite-raising onlays on molars achieved significantly higher pain recovery rates than SS in patients with pain-related TMDs (88.4% vs. 66.1%; $p < 0.05$). With regard to functional improvement in mouth opening, TBR and SS yielded comparable results, with no statistically significant difference between the groups (83.3% vs. 78.6%). Treatment with TBR was shorter than SS treatment, and required fewer clinical check-ups and repetitive occlusal adjustments. The initial self-reported discomfort was slightly more prevalent among TBR patients than among SS patients, while the initial improvement in pain symptomatology occurred earlier in the SS group. Overall, TBR was associated with a higher, although not statistically significant, prevalence of the initial self-reported discomfort, whereas SS was associated with statistically significantly earlier symptom relief.

Conclusions. Despite the initial discomfort, TBR could be considered an effective and time-efficient treatment option for pain management in patients with pain-related TMDs.

Keywords: mouth rehabilitation, treatment outcome, pain management, temporomandibular joint disorders, occlusal splints

Cite as

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Highlights

- Temporary bite-raising onlays (TBR) achieved significantly higher pain recovery rates than stabilization splints (SS) in pain-related temporomandibular disorders (TMDs) (88.4% vs. 66.1%; $p < 0.05$).
- With regard to functional improvement in mouth opening, TBR and SS yielded comparable results, with no statistically significant difference between the groups (83.3% vs. 78.6%).
- Treatment with TBR was associated with a reduced number of clinical check-ups, fewer occlusal adjustments and shorter overall treatment duration as compared to SS.
- The initial self-reported discomfort was more prevalent in TBR-treated patients, while SS provided earlier symptom relief.
- Despite initial discomfort, TBR could be considered an effective and time-efficient treatment option for pain management in patients with pain-related TMDs.

Introduction

The term “temporomandibular disorders” (TMDs) refers to a group of painful and/or dysfunctional conditions affecting the musculoskeletal and neuromuscular systems, specifically involving the temporomandibular joints (TMJs), the muscles of mastication, and other related craniofacial structures. A meta-analysis conducted by Valesan et al. reported that the prevalence of TMDs among adults and elderly people was approx. 38.8%, based on the globally recognized Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).¹ The prevalence was higher among females, with a female-to-male ratio of 1.5:1, and was highest among young and middle-aged adults, with a peak occurrence between 20 and 40 years of age.¹

Patients with TMDs commonly present with a range of symptoms, including pain, restricted or asymmetric mandibular movements, and TMJ sounds. Pain and discomfort are often localized to the jaw, TMJs, and associated masticatory muscles. Additional symptoms may include earache, a sensation of ear fullness, tinnitus, vertigo, cervicgia, and headaches. This painful condition can impair patients' ability to perform essential orofacial functions, such as mastication and speech, thereby negatively affecting their overall quality of life (QoL).²

Temporomandibular disorders frequently follow a recurrent, self-limiting or variable course over time. Current estimates suggest that only 3.6% to 7% of individuals diagnosed with TMDs require therapeutic intervention. Specifically, among patients with painful TMDs, the most recent data indicate that the prevalence of first-onset cases is approx. 3.9%.³

Conservative, reversible, and noninvasive therapies, including self-management instructions, behavioral modification, physical therapy, pharmacotherapy, and orthopedic appliances, are recommended for the management of nearly all TMDs. The therapeutic goals include pain reduction, the reduction of adverse loading, the restoration of function, and the resumption of normal daily activities. Most patients with TMDs experience significant symptom relief with conservative therapy. Long-term

studies indicate that 50% to more than 90% of patients report few or no symptoms following conservative treatment. Symptomatic stability typically occurs within 6–12 months of therapy.^{4,5}

Stabilization splints (SS) are removable orthopedic appliances made of acrylic resin that cover the dental arch (Fig. 1). Their indications include masticatory muscle pain and dysfunction, painful TMDs, and altered structural relationships of TMJ. Stabilization splints act by providing occlusal and joint stabilization, creating a smooth contact surface between the dental arches. The main therapeutic goal of SS is to eliminate occlusal prematurities that may generate nociceptive input and, over time, contribute to improper muscle activity. Additionally, SS have been associated with muscle relaxation by reducing parafunctional activity, the protection of the teeth and jaws, the normalization of periodontal proprioception, the modification of the joint space, and the redistribution of condylar shear forces.⁶

Over time, stabilization splints have emerged as one of the most extensively studied orthopedic therapies for the alleviation of pain in patients with TMDs. A recent systematic review conducted by Pfcifer et al. concluded that SS may play a significant role in the short-term management of TMDs, although their effectiveness appears to be



Fig. 1. Stabilization splint (SS) (bottom), and intraoral frontal and lateral views (top)

comparable to that of other therapeutic modalities during long-term follow-up (6–12 months).⁷

Temporary bite-raising onlays (TBR) are occlusal composite resin wedges bonded to the chewing surfaces of the posterior teeth (Fig. 2). The occlusal portion of TBR provides bilaterally balanced occlusal contacts in maximum intercuspation and promotes occlusal disclusion during eccentric jaw movements. The primary advantage of TBR over SSs is their ability to function 24 h a day without requiring patient compliance, while having minimal esthetic impact. Consequently, TBR may be recommended for the management of acute and chronic pain-related TMDs in patients for whom prolonged daily use of SS is required.

The therapeutic rationale of TBR is analogous to that of SS: The occlusal disengagement achieved through the mechanical interposition of a balanced thickness between the dental arches disrupts the neuromuscular pattern associated with the previous occlusion. The increased vertical dimension resulting from the thickness of the onlays promotes muscle relaxation and soft-tissue decompression (Fig. 3).⁸

In the short term, both SS and TBR can modify the neuromuscular pattern by inhibiting periodontal mechanoreceptors, thereby reducing abnormal muscular activity. In the long term, the uncontrolled tooth extrusion associated with TBR may be clinically advantageous for increasing the occlusal vertical dimension and reducing anterior tooth contact in patients with a deep bite.

To the best of our knowledge, no studies in the literature have directly compared the effectiveness and efficiency of TBR and SS in managing pain and functional symptoms in patients with pain-related TMDs.

This retrospective comparative study aimed to compare the effectiveness and efficiency of TBR and SS in managing pain and functional limitations in patients with pain-related TMDs. The primary objective was to evaluate pain reduction following treatment with TBR (the experimental group) or SS (the gold standard). The secondary objective was to assess improvement in functional limitations following treatment with either TBR or SS. The tertiary objective was to compare the efficiency of the 2 treatment



Fig. 2. Frontal and lateral views of temporary bite-raising onlays (TBR) in the oral cavity

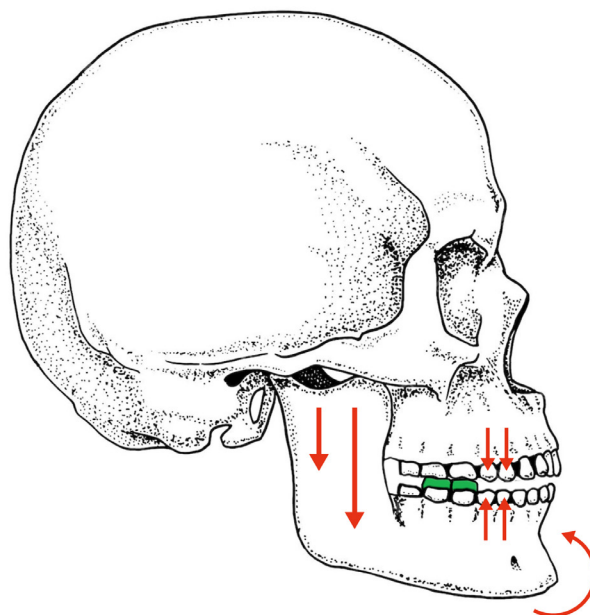


Fig. 3. Representation of the effects on the articular, muscular and dental structures, generated by the presence of temporary bite-raising onlays (TBR) in the oral cavity

modalities in terms of the number of clinical check-ups and appliance adjustments, overall treatment duration, patient discomfort, and time to the onset of pain relief.

Material and methods

The present single-center retrospective comparative study was conducted at the Department of Orthodontics of the Agostino Gemelli University Hospital IRCCS, Rome, Italy. The study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Supplementary material, available on request from the corresponding author.)

As no prior data were available for the primary outcome in the TBR group, the sample size was calculated based on the data published by Ekberg et al., which reported approx. 30% of patients as pain-free in the SS group.⁹ Assuming that approx. 60% of patients in the TBR group would be asymptomatic, a minimum sample size of 42 patients per group was calculated, with an allocation ratio of 1:1 (type I error = 0.05; type II error = 0.20; power = 80%).

Medical records from the database of the Department of Orthodontics of the Agostino Gemelli University Hospital IRCCS, Rome, Italy, were screened. Eligible subjects were treated consecutively between January 2008 and May 2018 by the same experienced clinician (R.U.). The electronic screening of the medical records was performed by a trained researcher (F.A.).

The eligibility criteria are outlined in Table 1. All patients included in this retrospective comparative study were diagnosed with pain-related TMDs according to the DC/TMD Axis I protocol.¹⁰

Table 1. Eligibility criteria for inclusion in the study

Inclusion criteria
age: 18–65 years
male and female patients
diagnosis of pain-related TMDs according to the DC/TMD (each subtype and severity)
treatment with TBR or SS by the same operator
absence of systematic diseases or comorbidities
complete medical records
written informed consent

TMDs – temporomandibular disorders (TMDs); DC/TMD – Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).

Clinical assessment was conducted according to the DC/TMD Axis I guidelines, which provide specific diagnostic algorithms for the identification of pain-related TMDs, including myalgia, local myalgia, myofascial pain with referral, arthralgia, and headache attributed to TMDs. Each patient underwent a structured clinical examination performed by trained examiners. The examination included a detailed assessment of TMJs and masticatory muscles through static and dynamic palpation, the measurement of the mandibular range of motion, provocation tests, and the evaluation of joint sounds. The masticatory muscles, including the masseter, temporalis, medial pterygoid, and lateral pterygoid muscles, were palpated bilaterally at predefined sites, using calibrated finger pressure, and pain responses were recorded according to the DC/TMD criteria. The TMJs were assessed for pain during mandibular movements and palpation, as well as for the presence of joint sounds, including clicking and crepitus, detected through auscultation and manual palpation during mandibular function.

The use of DC/TMD ensured a reliable and reproducible diagnostic process, enabling the precise identification of pain-related TMD phenotypes within the study population.

Electronic screening identified 86 patients who met the eligibility criteria (17 males and 69 females; mean age: 37.9 ± 13.3 years). Of these, 43 patients were treated with TBR (the experimental group) and 43 with SS (the gold standard).

Both kinds of treatment (SS and TBR) were performed by the same independent, experienced clinician (R.U.).

Stabilization splints were fabricated following an intraoral impression. The SS appliance was designed to provide simultaneous and uniform occlusal contacts between the arches, considered optimal for musculoskeletal stability during tooth contact. Patients were instructed to wear the occlusal splints for 22 h/day, removing them only during meals and oral hygiene procedures. They were advised to report any failure immediately, particularly breakage, loss, or episodes of acute pain, and to attend the clinic in case of emergencies. Every failure, whether identified by the clinician during the

scheduled visits or reported by the patient, was recorded in the patient's clinical file.

Temporary bite-raising onlays were occlusal composite resin wedges (Charisma®; Kulzer, Hanau, Germany) directly modeled in the mouth by the clinician and bonded to the occlusal surfaces of the posterior teeth. The occlusal plane was designed to be flat, allowing protrusive and lateral mandibular movements while ensuring evenly distributed interarch contacts to provide occlusal and orthopedic mandibular stability. The height of TBR was set at 2 mm, representing the minimum thickness required to prevent arch contact during closure and eccentric mandibular movements. Increases in the occlusal vertical dimension were calculated to maintain the interocclusal freeway space (2–5 mm on average). Temporary bite-raising onlays were applied to the upper or lower first and/or second molars, depending on the individual occlusal conditions, to ensure optimal orthopedic mandibular balance in centric occlusion.

At the end of active treatment in both groups, the TBR and SS appliances were removed, and all patients received verbal and written instructions to follow a personalized jaw home-exercise regimen for TMD management. This regimen included exercises for muscle contraction and body movements aimed at alleviating musculoskeletal pain and restoring normal function by reducing inflammation, decreasing and coordinating muscle activity, and promoting tissue repair and regeneration.^{10,11} The program for patients with TMD-related muscle pain and/or limited mouth opening included relaxation exercises with diaphragmatic breathing, the self-massage of the masticatory muscles, stretching, and coordination exercises, including proprioceptive training and postural exercises.^{10–12} There is a consensus among TMD experts that jaw exercises are effective for patients with myalgia, restricted mouth opening due to the hyperactivity of the jaw-closing muscles, and disc displacement without reduction.¹³

After SS delivery or TBR placement, patient check-ups were scheduled every 2 weeks during the first 2 months, followed by monthly visits, except in cases requiring emergency assessment.

The primary outcome was self-reported pain at baseline (T_0) and at the end of treatment (T_1). Pain was assessed using a unidimensional 10-point numeric rating scale (NRS). A score of 0 indicated the absence of pain, whereas scores from 1 to 10 indicated the presence of pain and its intensity. The percentage of patients reporting no pain at T_1 was calculated.

The secondary outcome – the functional limitation of mandibular movements – was assessed based on the maximum mouth opening (MMO). The percentage of patients with limited mouth opening at T_0 and T_1 was recorded. The maximum mouth opening was measured between the incisal edges. In this study, a physiological cut-off of 40 mm was adopted: $MMO \geq 40$ mm was considered optimal and indicative of the absence

of mandibular limitation, whereas values <40 mm indicated the presence of limitation. The percentage of patients with $\text{MMO} \geq 40$ mm at T_1 was also calculated. In patients presenting with mandibular limitation, the MMO values at both T_0 and T_1 were recorded.

The tertiary outcomes (efficiency) included:

- the number of repetitive occlusal adjustments and/or resurfacing procedures performed during treatment;
- the number of clinical check-ups;
- treatment duration (from SS delivery or TBR placement to removal, expressed in months);
- patients' discomfort experienced at the beginning of treatment (such as difficulty with chewing, swallowing or phonation, dental sensitivity, fatigue, and muscle pain); and
- the time to the onset of pain relief, expressed in weeks.

Data for the tertiary outcomes were reported using descriptive statistics.

Other clinical and demographic data, including age at the beginning of treatment, sex, diagnosis according to DC/TMD, the presence of parafunctional activities, and a history of previous gnathological treatment, were collected.

Statistical analysis

An Excel data collection form and data management system were used (Microsoft Excel 2011; Windows, v. 14.0.0; Microsoft Corp., Redmond, USA). All data were entered by a single blinded operator (F.A.). Before data entry, all records were evaluated for accuracy and completeness. Statistical analysis was performed using a superiority approach for all variables. The null hypothesis was that the experimental treatment (the TBR group) was superior to the standard treatment (the SS group) in terms of the absence of pain (a binary primary outcome) at the end of treatment.

For qualitative data, frequencies, proportions, and 95% confidence intervals (CIs) for proportions were calculated. In the bivariate analysis, the proportions were compared using appropriate statistical tests. The χ^2 test was used when no more than 20% of the cells in the contingency tables had expected frequencies ≤ 5 and no cell had an expected frequency <1 . When these assumptions were not met, Fisher's exact test was performed.

For each continuous variable, the mean and standard deviation ($M \pm SD$), and 95% CI were reported. The assumption of normality was assessed using the skewness/kurtosis test, with a normal distribution assumed when $p > 0.05$. The comparisons of means were performed to evaluate statistically significant differences using the t test, while the Wilcoxon rank-sum test or the Wilcoxon matched-pairs signed-rank test were used where appropriate. When a non-parametric test was used, the median (Me) was also reported. Statistical significance was set at $p < 0.05$ (5%). The statistician was blinded to group allocation and was

external to the research group. Data analysis was performed using the Stata/IC 16 software (StataCorp, College Station, USA).

Results

A total of 100 patient records were retrieved. Six patients were excluded from the sampled due to the systemic diseases and comorbidities reported at follow-up. The remaining 94 patients diagnosed with pain-related TMDs were randomly selected and invited to participate in the study. Among these, 8 patients dropped out (3 patients had moved away, and 5 did not attend the scheduled appointments). Written informed consent was obtained from each participant, resulting in a final sample of 86 patients (Fig. 4). Detailed demographic and clinical characteristics of the study population are summarized in Table 2.

Primary outcome – pain

At T_0 , 100% of patients reported pain symptoms. By the end of treatment, 23.3% of patients still reported pain, corresponding to an overall success rate of 76.7%. Complete pain resolution was achieved in 38 patients (88.4%) in the TBR group and 28 patients (66.1%) in the SS group. The difference between the groups was statistically significant ($p = 0.011$) (Table 3).

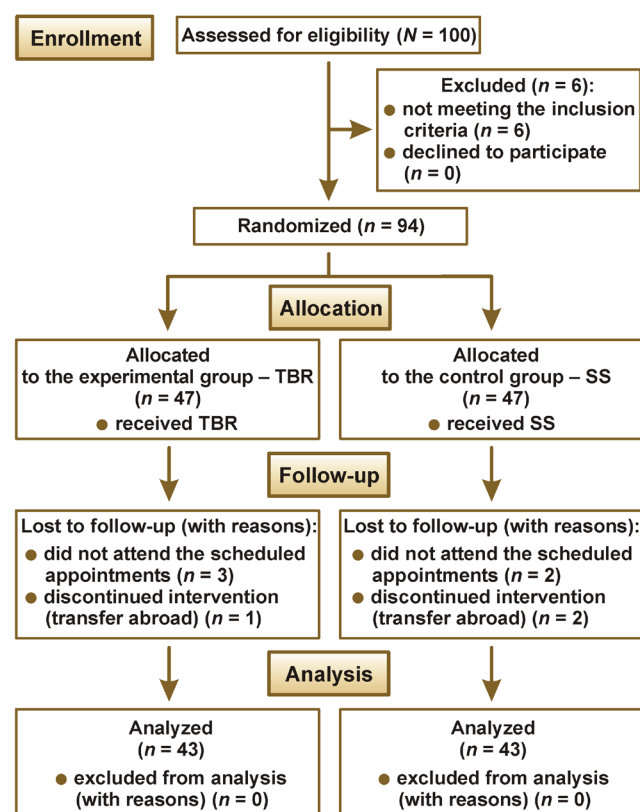


Fig. 4. CONSORT (Consolidated Standards of Reporting Trials) flow diagram of the study

Table 2. Demographic and clinical characteristics of the study groups

Variables		TBR group (n = 43)	SS group (n = 43)	p-value
Age [years]	<i>M</i> ± <i>SD</i>	35.5 ± 13.8	40.2 ± 12.5	0.107
	range	18–65	20–65	
	18–25 <i>n</i> (%)	15 (34.8)	7 (16.3)	0.060
	26–45 <i>n</i> (%)	15 (34.8)	17 (39.5)	0.590
	46–65 <i>n</i> (%)	13 (30.2)	19 (44.2)	0.179
Sex <i>n</i> (%)	M	10 (23.3)	7 (16.3)	0.417
	F	33 (76.7)	36 (83.7)	
Diagnosis (according to DC/TMD) <i>n</i> (%)	myalgia	20 (46.5)	24 (55.8)	0.388
	arthralgia	7 (16.3)	3 (7.0)	0.179
	myalgia and arthralgia	16 (37.2)	16 (37.2)	1.000
	headache attributed to TMDs	14 (32.6)	15 (34.8)	0.822
Parafunctional habits <i>n</i> (%)		20 (46.5)	19 (44.2)	0.822
Previous gnathological therapies <i>n</i> (%)		20 (46.5)	13 (30.2)	0.120

M – mean; *SD* – standard deviation; M – male; F – female.

Previous gnathological therapies refer to any prior conservative treatment for TMDs, e.g., splints, physical therapy, behavioral therapy.

Table 3. Primary outcome: pain at the end of treatment (the presence or absence of pain at baseline (*T*₀) and at the end of treatment (*T*₁) in both groups)

Variables		TBR group (n = 43)	SS group (n = 43)	p-value
Pain at <i>T</i> ₀ <i>n</i> (%)	absent	0 (0.0)	0 (0.0)	1.000
	present	43 (100.0)	43 (100.0)	
Pain at <i>T</i> ₁ <i>n</i> (%)	absent	38 (88.4)	28 (66.1)	0.011*
	present	5 (11.6)	15 (34.9)	

* statistically significant.

Secondary outcome – functional limitation and maximum mouth opening

At *T*₀, a total of 26 patients (30.2% of the study population) reported functional limitation, including 12 patients (27.9%) in the TBR group and 14 patients (32.6%) in the SS group. No statistically significant difference was observed between the groups at baseline (*p* = 0.639) (Table 4). At this time point, the overall mean MMO was 33.3 ± 3.3 mm, with no statistically significant difference between the TBR (32.8 ± 3.1 mm) and SS (33.8 ± 3.6 mm) groups (*p* = 0.477).

After treatment, only 2 patients reported functional limitation in the TBR group and 3 in the SS group. No statistically significant difference was observed between the groups post-treatment (*p* = 1.000) (Table 4). Maximum mouth opening increased at *T*₁ as compared to baseline in all patients. Differences in the change from *T*₀ to *T*₁ were statistically significant for the 2 groups.

Table 4. Secondary outcome: functional limitation (the presence or absence of functional limitation at baseline (*T*₀) and after treatment (*T*₁) in both groups)

Variables		TBR group (n = 43)	SS group (n = 43)	p-value
Functional limitation at <i>T</i> ₀ <i>n</i> (%)	absent	31 (72.1)	29 (67.4)	0.639
	present	12 (27.9)	14 (32.6)	
Functional limitation at <i>T</i> ₁ <i>n</i> (%)	absent	41 (95.3)	40 (93.0)	1.000
	present	2 (4.7)	3 (7.0)	

Tertiary outcome – treatment duration, number of clinical check-ups and number of occlusal adjustments

The overall treatment duration was 9.3 ± 3.8 months. Treatment duration was 8.3 ± 4.6 months in the TBR group and 10.3 ± 2.6 months in the SS group, with a statistically significant difference between the groups (*p* = 0.018). During treatment, an average of 7.1 ± 2.9 clinical check-ups were performed in the TBR group, compared with 9.2 ± 2.2 in the SS group (*p* = 0.000). A mean of 3.2 ± 2.1 occlusal adjustments and/or resurfacing procedures were performed overall, with the TBR group requiring 3.2 ± 2.1 adjustments and the SS group requiring 4.9 ± 1.1 adjustments (*p* = 0.000) (Table 5).

The number of clinical check-ups was positively correlated with treatment duration (Spearman's *ρ* = 0.4813) and negatively correlated with the time to improvement (Spearman's *ρ* = −0.3230).

Table 5. Tertiary outcomes (treatment duration, number of clinical check-ups, number of occlusal adjustments, discomfort, and time to the onset of pain relief)

Variables	TBR group (<i>n</i> = 43)	SS group (<i>n</i> = 43)	<i>p</i> -value
Number of occlusal adjustments	3.2 ±2.1	4.9 ±1.1	0.000*
Number of clinical check-ups	7.1 ±2.9	9.2 ±2.2	0.000*
Treatment duration [months]	8.3 ±4.6	10.3 ±2.6	0.018*
Discomfort (number of patients)	23 (53.5)	21 (48.8)	0.829
Onset of pain relief [weeks]	4.9 ±3.0	2.4 ±1.9	0.000*

Data presented as *M* ±*SD* or as number (percentage) (*n* (%)).* statistically significant.

Tertiary outcome – patient discomfort

Initial discomfort was reported shortly after treatment initiation by 21 patients (48.8%) in the SS group and 23 patients (53.5%) in the TBR group. However, this discomfort subsided within 7–10 days (Table 5).

Tertiary outcome – onset of pain relief

On average, pain relief occurred 3.6 ±2.8 months after treatment initiation. In the SS group, pain symptoms began to diminish after 2.4 ±1.9 months, whereas in the TBR group, pain relief occurred after 4.9 ±3.0 months. The difference in the time to the onset of pain relief between the TBR and SS groups was statistically significant (*p* = 0.000) (Table 5).

Discussion

The findings of this retrospective comparative study suggest a significant correlation between pain reduction and treatment with TBR. We have reason to believe that these results can be explained by the continuous use of a bonded appliance such as TBR, which ensures a constant therapeutic effect. In contrast, the success of SS depends heavily on patient compliance, as these are removable devices. Therefore, wear time significantly affects both the speed and efficacy of symptom resolution.

Approximately 30% of patients in each group initially presented with reduced mouth opening. While both treatment groups showed a statistically significant improvement from *T*₀ to *T*₁, the difference between TBR and SS did not reach statistical significance. However, the small sample size limits the generalizability of these findings. The observed, albeit nonsignificant, trend suggests that both treatment modalities may have the potential to improve functional limitation, but further studies with larger sample sizes are needed to confirm these results.

Treatment with TBR was found to be more efficient than SS, with shorter treatment duration. This efficiency is likely attributable to the constant presence of the bonded appliance. However, TBR initially cause greater discomfort,

which typically resolves within 7–10 days. Temporary bite-raising onlays also require fewer clinical check-ups and occlusal adjustments as compared to SS. The SS appliances, which are fabricated in the laboratory, require more frequent adjustments to achieve balanced occlusion. Nevertheless, the onset of pain relief occurred earlier with SS than with TBR. We have reason to believe that the occlusal contacts of TBR may initially act as occlusal prematurities, generating nociceptive input and inducing altered muscle activity, thereby delaying the resolution of symptoms.

Due to the limited evidence from other studies regarding TBR, it is challenging to correlate our findings with, or compare them to, the existing literature. However, studies investigating SS therapy provide some context. For instance, Zhang et al. recommended the use of splints for the management of TMDs, demonstrating their effectiveness in reducing pain and improving mouth opening in patients with restricted mandibular movement.¹⁴ Similarly, Ebrahim et al. demonstrated the efficacy of SS therapy in reducing pain, although the moderate quality of evidence highlights the need for further research.¹⁵ Ekberg et al., in a randomized clinical trial, concluded that SS therapy was effective in relieving TMD symptoms, particularly in patients with myogenic disorders.⁹ Moreover, Pficer et al. showed that SS therapy was more effective in the short term, while its long-term effects were comparable to those of other treatment modalities.⁷ Their meta-regression analysis emphasized that continuous splint use, rather than nocturnal use alone, yielded better outcomes.⁷ This finding is consistent with our observation that bonded appliances such as TBR, which provide continuous occlusal intervention, may stabilize the occlusion more effectively.

Alternative treatment options for pain-related TMDs have also been explored, including the use of botulinum toxin (BoNT). Although BoNT has gained popularity in the management of myofascial pain and bruxism due to its neuromuscular blocking effects, current evidence does not support its routine use as a first-line therapy. A recent narrative review by Delcanho et al. concludes that, although some randomized controlled trials have reported modest short-term reductions in pain, the heterogeneity of study designs, inconsistent diagnostic criteria, and the lack of long-term safety data significantly limit the generalizability of these findings.¹⁶ Furthermore, repeated injections may result in adverse effects, such as muscle atrophy and functional impairment. Therefore, BoNT should be reserved for selected cases that are refractory to conservative interventions, such as splint therapy or physical therapy.¹⁶ In light of these limitations, reversible approaches such as TBR and SS – particularly when they provide clinically significant symptom relief with minimal risk – remain appropriate first-line options in the management of pain-related TMDs.

The continuous wear time of TBR is crucial to its effectiveness, as it provides uninterrupted therapeutic action. The composite material used in TBR has minimal esthetic

impact, making the appliance more acceptable to patients. However, the long-term use of TBR may result in irreversible occlusal changes, such as the uncontrolled extrusion of premolars and the intrusion of the teeth to which the device is bonded. These changes may lead to unpredictable reductions in overbite, limiting the use of TBR to patients who have not responded to SS and who do not plan to undergo orthodontic or prosthetic rehabilitation following TMD therapy.

The long-term use of TBR in patients with bruxism may exacerbate occlusal instability. The molar intrusion resulting from constant occlusal contact may lead to the development of a posterior open bite, particularly in individuals with hyperactive masticatory muscles. Following TBR removal, the spontaneous re-eruption of molars may occur and partially restore the occlusal vertical dimension; however, this is often a slow and unpredictable process. During this transitional phase, patients may also develop functional side effects, such as cheek biting. The limitations of TBR treatment should therefore be carefully considered in the decision-making process, particularly in patients with bruxism; they represent an important clinical factor in patient selection and informed consent.

Despite these drawbacks, the uncontrolled extrusion of the uninvolved teeth may contribute to an increase in the occlusal vertical dimension and potentially promote occlusal stabilization over time. However, these changes should be carefully monitored, particularly when TBR is used for extended periods.

Recent findings further underscore the need for caution when using fixed occlusal appliances. An experimental study by Hutami et al. demonstrated that the continuous use of an anterior bite plane (ABP) in animal models resulted in significant morphological changes in TMJ, including the thinning of the articular cartilage and signs of condylar resorption.¹⁷ These structural alterations suggest that sustained occlusal disengagement may impose abnormal loading on TMJ, potentially triggering degenerative or adaptive remodeling responses. Although ABPs differ from posterior bite-raising devices such as TBR in terms of design and occlusal contact, the underlying biomechanical principles, particularly alterations in the vertical dimension and occlusal force distribution, may result in similar stress-related effects if not carefully monitored. These findings further emphasize the importance of limiting treatment duration and carefully selecting patients when prescribing fixed occlusal appliances.

Limitations and strengths

This study has limitations inherent to its retrospective design. Key variables that may have influenced the outcomes were not consistently available in the patient records.

Firstly, pain is a subjective experience that is perceived differently by each individual, as reflected by distinct

patterns of activation throughout the brain and within the brain regions involved in the processing of nociceptive stimuli.^{18,19} Differences in pain perception may be further influenced by patient characteristics, such as sex, anxiety, fear of pain, mood, personality traits, beliefs, sleep quality, attention, and empathy, all of which can shape the individual pain experience and contribute to temporal fluctuations in pain intensity.^{19,20} As highlighted by Orzeszek et al., clinical research on masticatory pain presents considerable challenges.²¹ In the present study, pain evaluation was based on subjective, self-reported experiences; however, the use of a validated questionnaire allowed standardized measurement and the subsequent data analysis. Pain intensity was assessed using a one-dimensional NRS. As all variables included in the analysis were routinely recorded in the medical records of the enrolled patients, the Graded Chronic Pain Scale (GCPS), v. 2, part of the DC/TMD criteria, was not employed for a more comprehensive assessment of pain etiology and severity.^{21,22} Furthermore, patients with painful symptoms of both muscular and joint origin were included in the analysis. However, as emphasized by Orzeszek et al., it may be beneficial to distinguish between dysfunctions of muscular origin and the conditions related to the articular disc or degenerative changes within TMJs.²¹

Secondly, the present study aimed to assess symptom and functional recovery, irrespective of anatomical changes in the joint structures. Baseline and follow-up magnetic resonance imaging (MRI) scans were not analyzed, as they were not available for all patients; therefore, it was not possible to evaluate the anatomical relationships between the TMJ structures at T₀ and T₁. However, a previous study by Ohnuki et al. reported that functional recovery and the reduction of acute pain symptoms, at least in the short term, were not dependent on changes in disc position.⁸

Thirdly, the study lacked a long-term comparison between TBR and SS. Future studies are needed to determine whether TBR provide more stable long-term outcomes as compared to SS, which have been shown to be effective, particularly in the short term.

Finally, the individualized jaw home-exercise regimen was prescribed only after the completion of gnathological therapy with TBR or SS, in order to avoid potential bias in the comparison between the 2 groups due to the therapeutic effects of the exercise regimen.

Despite these limitations, the study has several strengths. To the best of our knowledge, this is the first study to compare fixed (TBR) and removable (SS) therapies for the management of TMDs. The relatively large sample size and the study design helped minimize the potential for selection and information bias. All statistical analyses were performed in accordance with current best practices. Furthermore, occlusal characteristics were not considered as treatment determinants, in line with contemporary recommendations that advocate moving away from traditional gnathological paradigms in the management of TMDs.²³

Conclusions

This retrospective comparative study found a significant correlation between TBR treatment and pain reduction in patients with TMDs. The continuous therapeutic action of TBR as a bonded appliance, independent of patient compliance, may account for its clinical performance. Thus, TBR may represent a viable treatment alternative that does not rely on patient compliance for effective use. Its efficacy and efficiency warrant further prospective and experimental studies to validate these findings.

Ethics approval and consent to participate

The study protocol was approved by the Internal Review Board of Gemelli University Hospital, Rome, Italy (approval date: December 22, 2020; approval No. 0051819/20). All procedures were conducted in accordance with the Declaration of Helsinki of 1975 and its subsequent amendments. Written informed consent to participate in the study 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

- Valesan LF, Da-Cas CD, Réus JC, et al. Prevalence of temporomandibular joint disorders: A systematic review and meta-analysis. *Clin Oral Investig*. 2021;25(2):441–453. doi:10.1007/s00784-020-03710-w
- Emodi-Perlman A, Eli I. Temporomandibular disorders and bruxism – up-to-date assessment and screening tools the general dentist should be aware of. *Dent Med Probl*. 2024;61(2):169–171. doi:10.17219/dmp/175582
- De Leeuw R, Klasser GD, eds. *Orofacial Pain: Guidelines for Assessment, Diagnosis, and Management*. American Academy of Orofacial Pain. 6th ed. Hanover Park, IL: Quintessence Publishing; 2018.
- Chattratrat T, Thymi M, Su N, Lobbezoo F. Changes in self-reported sleep and awake bruxism in relation to the management of temporomandibular disorders ("care as usual") in a specialty clinic population. *Dent Med Probl*. 2024;61(5):697–704. doi:10.17219/dmp/193125
- Zielinski G, Pająk-Zielinska B, Pająk A, Wójcicki M, Litko-Rola M, Ginszt M. Global co-occurrence of bruxism and temporomandibular disorders: A meta-regression analysis. *Dent Med Probl*. 2025;62(2):309–321. doi:10.17219/dmp/201376
- Chen J, Ning R, Lu Y. Effects of occlusal splint and exercise therapy, respectively, for the painful temporomandibular disorder in patients seeking for orthodontic treatment: A retrospective study. *BMC Oral Health*. 2022;22(1):527. doi:10.1186/s12903-022-02538-y
- Pficer JK, 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
- Ohnuki T, Fukuda M, Nakata A, et al. Evaluation of the position, mobility, and morphology of the disc by MRI before and after four different treatments for temporomandibular joint disorders. *Dentomaxillofac Radiol*. 2006;35(2):103–109. doi:10.1259/dmfr/25020275
- Ekberg E, Vallon D, Nilner M. The efficacy of appliance therapy in patients with temporomandibular disorders of mainly myogenous origin. A randomized, controlled, short-term trial. *J Orofac Pain*. 2003;17(2):133–139. PMID:12836501.
- Schiffman E, Ohrbach R, Truelove E, et al. 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
- Michelotti A, de Wijer A, Steenks M, Farella M. Home-exercise regimes for the management of non-specific temporomandibular disorders. *J Oral Rehabil*. 2005;32(11):779–785. doi:10.1111/j.1365-2842.2005.01513.x
- Tarallo F, Chimenti C, Paiella G, Cordaro M, Tepedino M. Biomarkers in the gingival crevicular fluid used to detect root resorption in patients undergoing orthodontic treatment: A systematic review. *Orthod Craniofac Res*. 2019;22(4):236–247. doi:10.1111/ocr.12329
- Lindfors E, Arima T, Baad-Hansen L, et al. Jaw exercises in the treatment of temporomandibular disorders – an international modified Delphi study. *J Oral Facial Pain Headache*. 2019;33(4):389–398. doi:10.11607/ofph.2359
- Zhang C, Wu JY, Deng DL, et al. Efficacy of splint therapy for the management of temporomandibular disorders: A meta-analysis. *Oncotarget*. 2016;7(51):84043–84053. doi:10.18632/oncotarget.13059
- Ebrahim S, Montoya L, Busse JW, Carrasco-Labra A, Guyatt GH; Medically Unexplained Syndromes Research Group. The effectiveness of splint therapy in patients with temporomandibular disorders: A systematic review and meta-analysis. *J Am Dent Assoc*. 2012;143(8):847–857. doi:10.14219/jada.archive.2012.0289
- 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
- Hutami IR, Novianty SI, Indrawati SV, et al. The effects of anterior bite plane on temporomandibular joint and mandibular morphology. *Saudi Dent J*. 2023;35(6):720–726. doi:10.1016/j.sdentj.2023.06.002
- Zhao R, Su Q, Song Y, et al. Brain-activation-based individual identification reveals individually unique activation patterns elicited by pain and touch. *Neuroimage*. 2022;260:119436. doi:10.1016/j.neuroimage.2022.119436
- 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
- Kleykamp BA, Ferguson MC, McNicol E, et al. The prevalence of comorbid chronic pain conditions among patients with temporomandibular disorders: A systematic review. *J Am Dent Assoc*. 2022;153(3):241–250.e10. doi:10.1016/j.adaj.2021.08.008
- 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
- Azzi L, Maurino V, Vinci R, et al. ADULT syndrome: Dental features of a very rare condition. *J Biol Regul Homeost Agents*. 2017;31(2 Suppl 1):61–65. PMID:28691455.
- 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

Effects of injectable platelet-rich fibrin and Hank's balanced salt solution as rejuvenating media for the periodontal ligament cells of avulsed teeth

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Conflict of interest

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Abstract

Background. The problem of avulsed teeth often being brought to the clinic in a dry state remains unresolved in the literature. Before an avulsed tooth is replanted, the periodontal ligament (PDL) cells require an appropriate storage medium to preserve their viability and biological properties. Hank's balanced salt solution (HBSS) is the reference medium recommended for this purpose in the literature.

Objectives. The aim of the present study was to compare the effects of the reference solution – HBSS – and injectable platelet-rich fibrin (I-PRF) on the differentiation of PDL fibroblasts by examining the differentiation markers associated with osteoblastic, osteoclastic, fibroblastic, and cementoblastic lineages.

Material and methods. Twenty freshly extracted third molar teeth were used for cell isolation. After being left to dry for 30 min, the teeth were immersed for 30 min at room temperature in HBSS ($n = 7$) or I-PRF ($n = 7$). For the positive control group ($n = 3$), cells were isolated immediately after extraction, whereas for the negative control group ($n = 3$), cells were isolated after 30 min of dry time. Viable cells were counted, and immunofluorescence staining was performed to assess cell differentiation, using antibodies against Runt-related transcription factor 2 (RUNX2), receptor activator of nuclear factor kappa-B ligand (RANKL), collagen type I (COL1), and cementum attachment protein (CAP). Integrated density (IntDen) was measured using the ImageJ software.

Results. There were no statistically significant differences between the HBSS and I-PRF groups in the expression of RUNX2, RANKL or CAP. The I-PRF group showed significantly higher expression of COL1 ($p < 0.01$).

Conclusions. Injectable PRF may contribute to the regeneration of PDL cells when used as a rejuvenating medium before replantation following an extraoral dry period.

Keywords: replantation, cell differentiation, tooth avulsion, collagen type I, platelet-rich fibrin

Cite as

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Highlights

- Injectable platelet-rich fibrin (I-PRF) can be used as an autologous rejuvenating medium for avulsed teeth brought in a dry condition.
- Treatment with I-PRF significantly enhanced the expression of collagen type I (COL1) in periodontal ligament (PDL) cells as compared to controls.
- Periodontal ligament cells exposed to I-PRF maintained their typical spindle-shaped fibroblastic morphology without early signs of senescence.
- Injectable platelet-rich fibrin demonstrates promising potential to promote PDL regeneration and improve the prognosis of replanted teeth following extraoral dry storage.

Introduction

Replantation is often the primary treatment for avulsed teeth, although its success is significantly influenced by the timing of intervention and the immediate actions taken.^{1,2} A key factor for a favorable outcome is the viability and regenerative potential of the periodontal ligament (PDL), as the survival of a replanted tooth largely depends on the integrity of its PDL. However, the viability of PDL cannot be directly assessed by dental professionals through a clinical examination alone.³

The duration that a tooth remains outside the oral cavity, either in a dry state or in a storage medium, critically affects the viability of PDL cells. Studies have shown that PDL cells generally become non-viable after being maintained in a dry state for more than 30 min.⁴ However, evidence suggests that permanent teeth with closed apices may still be replanted when the extraoral dry time is less than 60 min, although they may eventually develop ankylosis or replacement resorption.⁵

The International Association for Dental Traumatology (IADT) guidelines classify PDL cells as “may be viable but compromised” in avulsed permanent teeth with closed apices that have been stored in media such as milk, Hank’s balanced salt solution (HBSS), saliva, or saline for less than 60 min of extraoral dry time.⁵ When an avulsed tooth is brought to the clinic by a patient, parent, guardian, or other caregiver in a dry condition, there is a concern that the heterogeneous PDL cell populations remaining on the root surface may differentiate into osteoblasts, osteoclasts or cementoblasts following replantation.⁶ Root resorption is closely related to the nature of these heterogeneous PDL cell populations.^{7,8}

Studies have shown that immersing an avulsed tooth in a nutrient-rich medium before replantation can rejuvenate the surviving cells, reduce the inflammatory response and decrease the risk of root resorption.⁹ Hank’s balanced salt solution has been commonly used by dental professionals because of its ability to support the recovery of compromised PDL cells after extended dry periods, unlike milk or saline.¹⁰

Blood derivatives, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), are rich sources of growth factors that play important roles in tissue repair, making them

valuable in dental treatment. Researchers have investigated the antimicrobial and anti-inflammatory effects of pure platelet-rich plasma (P-PRP) and leukocyte platelet-rich plasma (L-PRP) in periodontal treatment.¹¹ Leukocyte platelet-rich fibrin promotes angiogenesis, chemotaxis, mitosis, and cell proliferation.^{12,13} Titanium-prepared platelet-rich fibrin (T-PRF), which incorporates titanium particles, has also gained attention in regenerative medicine.^{14,15} These findings highlight the potential of platelet concentrates to both combat infection and promote tissue regeneration, suggesting their potential for more effective treatment.¹¹ Similarly, recent studies on tooth replantation have indicated that platelet concentrates may support tissue regeneration.^{3,16–18} An important advantage of these materials is that they can be obtained from the patients themselves. Recent modifications to the centrifugation protocols used for PRF preparation have led to the development of injectable platelet-rich fibrin (I-PRF). It is a flowable platelet concentrate that can release growth factors for up to 14 days and contains approx. 1.5 times more platelets than whole blood.¹⁹ These characteristics suggest that I-PRF may be a practical storage medium for avulsed teeth, as it can be obtained from the patient’s own blood and may serve as a rejuvenating medium to help maintain the viability of PDL cells.

There are many studies concerning the effects of storage solutions on the prognosis of replantation. However, no pretreatment of the root surface that is effective in restoring the depleted cellular components of PDL before replantation has yet been suggested.⁵ Although studies have reported on the growth factors released from I-PRF and their effects on fibroblast and osteoblast behavior,^{20,21} no study has examined the differentiation mechanisms of PDL cells from avulsed teeth in response to I-PRF.

The present study aimed to improve the prognosis of replanted avulsed teeth under the care of dental professionals. The avulsion treatment method proposed in this study seeks to provide an effective pretreatment approach for avulsed teeth that arrive at a dental clinic in a dry state. Therefore, we tested a novel procedure for treating avulsed teeth by comparing I-PRF and HBSS as pretreatment media. Since PDL cell differentiation can be assessed by the expression of specific markers associated with particular cell lineages, Runt-related transcription factor 2

(RUNX2), receptor activator of nuclear factor kappa-B ligand (RANKL), collagen type I (COL1), and cementum attachment protein (CAP) were used to assess differentiation toward osteoblastic, osteoclastic, fibroblastic, and cementoblastic lineages, respectively.

Material and methods

The study was conducted in accordance with the principles outlined in the 2013 revision of the Declaration of Helsinki. Participant recruitment and selection procedures were approved by the Ethics Committee for Clinical Research at the Faculty of Medicine, Kütahya Dumlupınar University, Turkey (approval date: August 16, 2017; approval No. 2017-10/3). All participants were informed about the study and voluntarily provided written informed consent before participation.

Sample preparation

Twenty third molars were prophylactically extracted from systemically healthy patients aged 18–35 years, and the freshly extracted teeth were immediately used for PDL fibroblast isolation. The teeth had no caries, periapical infection, restorations, periodontal disease, or hypoplasia. Patients were excluded from the study if they were taking anticoagulants, antiplatelet medications or antiresorptive drugs (such as bisphosphonates), had blood or bone metabolism disorders, or were pregnant or lactating.

Four 9-milliliter samples of peripheral venous blood were collected from each participant into non-coated tubes without anticoagulants (Vacusera; Disera, Izmir, Turkey) to obtain I-PRF. The white-capped polyethylene terephthalate tubes were centrifuged using a centrifuge device (PRF Duo Quattro; Process for PRF, Nice, France) at 1,300 rpm for 3 min, with modifications to previously described protocols.²² The rotor angulation of the device was 40°, with a relative centrifugal force (RCF) of 119 g at the minimum (RCF_{min}), 208 at the maximum (RCF_{max}), and 145 at the clot (RCF_{clot}). Injectable PRF was then collected from the tubes using a sterile syringe, taking care to avoid contamination with red blood cells (RBCs).

The extracted third molar teeth were cleaned with sterile saline, and all teeth except those in the positive control group were stored dry for 30 min at room temperature. For the negative control group, 3 teeth ($n = 3$) were used for PDL cell isolation after a 30-minute dry period. For the experimental groups ($n = 7$ teeth per group), the teeth were immersed in either HBSS (Capricorn Scientific, Ebsdorfergrund, Germany) or the I-PRF obtained from the blood of the respective patients for 30 min at room temperature following the 30-minute dry period. For the positive control group, 3 teeth ($n = 3$) were used immediately after extraction for PDL cell isolation, without a dry period or the immersion stage. Figure 1 outlines all stages of the study.

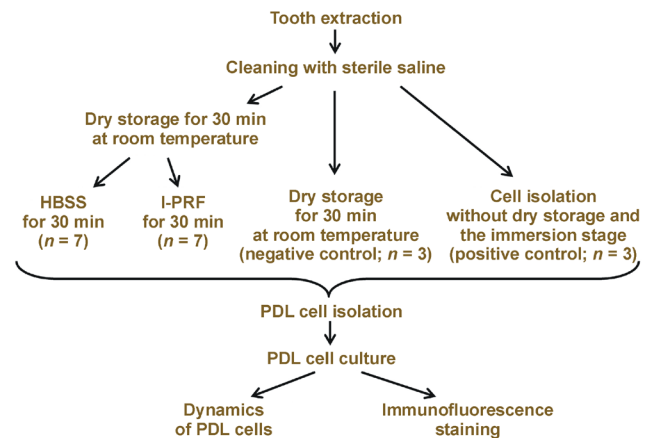


Fig. 1. Stages of the study

HBSS – Hank's balanced salt solution; I-PRF – injectable platelet-rich fibrin; PDL – periodontal ligament.

Cell isolation

Following the storage period, the teeth were thoroughly rinsed with phosphate-buffered saline (PBS) (Capricorn Scientific). The PDL tissue was carefully removed from the middle third of the root surface, using a scalpel blade. The collected tissue was placed in Eppendorf tubes containing 1 mL of Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F-12; Wisent Bioproducts, Saint-Jean-Baptiste, Canada), supplemented with 10% fetal bovine serum (FBS) (Capricorn Scientific) and 1% penicillin-streptomycin-amphotericin B solution (Capricorn Scientific). The tissue samples were then dissected into small fragments using sterile scissors and centrifuged at 500 rpm for 1 min. The resulting pellet was resuspended in 1 mL of a solution containing 2 mg/mL collagenase type I and 2 mg/mL dispase (Roche, Mannheim, Germany) and incubated in a 37°C water bath for 30–45 min. The released cells were collected by centrifugation at $111 \times g$ for 2 min and the resulting cell pellet was transferred to a T25 tissue culture flask. The culture flasks were maintained at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air. The culture medium was replaced every 3 days until the cells reached full confluence. The cells were detached from the flask surface using 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) in Dulbecco's phosphate-buffered saline (DPBS) (Capricorn Scientific). Following detachment, the cells were stained with trypan blue and counted using a hemocytometer under an inverted microscope. The cells were seeded at a density of 5×10^3 cells per T25 flask, and evaluated at the 3rd or 4th passage (P3 or P4).²³

A total of 5,000 cells were seeded onto coverslips placed in a six-well plate. Once the cells reached confluence, the culture medium was aspirated and the cells were washed 3 times with PBS. The cells were then fixed with 3.5% paraformaldehyde (Merck, Darmstadt, Germany) for 30–35 min at room temperature, followed by 3 additional washes with PBS.²⁴

Immunofluorescence staining

Three positive-control coverslips and 1 negative-control coverslip were selected for each tooth for each of the antibodies RUNX2, RANKL, COL1, and CAP. Following fixation, the coverslips were incubated with a blocking solution containing 0.1% Triton™ X-100 (BioShop Canada, Burlington, Canada) at 37°C for 30 min.

The primary antibodies against RUNX2 (polyclonal antibody; 5 µg/mL, ab114133; Abcam, Cambridge, UK), RANKL (polyclonal antibody; 1 µg/mL, ab9957; Abcam) and COL1 (polyclonal antibody; 1:500, ab34710; Abcam) were added to the respective groups and incubated overnight at 4°C. The CAP primary antibody (monoclonal antibody; 1:50, sc-53947; Santa Cruz Biotechnology Inc., Dallas, USA) was added and incubated at room temperature for 1 h. All samples were then rinsed 3 times with PBS. For the RUNX2, RANKL and COL1 treatment, a fluorescein isothiocyanate (FITC)-conjugated secondary antibody (1:50, SA00003-2; Proteintech Group Inc., Rosemont, USA) was applied at room temperature for 1 h. For the CAP treatment, an FITC-conjugated secondary antibody (1:50, SA00003-1; Proteintech Group Inc.) was applied at room temperature for 1 h. Following rinsing with PBS, the cell nuclei were stained by incubating the cells with 4',6-diamidino-2-phenylindole (DAPI) (FluorLast™ with DAPI; BioVision Inc., Milpitas, USA) at room temperature for 5 min. Control samples were processed without the addition of primary antibodies.

The stained samples were examined using a fluorescence microscope (Nikon ECLIPSE Ci-E, Nikon Instruments Inc., New York, USA). Images were acquired using a 5.0-megapixel charge-coupled device (CCD) camera (DS-Fi1c; Nikon Instruments Inc., Melville, USA). Image acquisition was performed using the NIS-Elements software, v. 4.10 (Nikon Instruments Inc., Melville, USA). The images were acquired at a resolution of 1,280 × 960 pixels with a bit depth of 24 bits. For fluorescence imaging, FITC (excitation filter: 480/30; barrier filter: 535/45) and DAPI (excitation filter: 375/28; barrier filter: 460/60) filter sets were used, with a filter diameter of 25 mm for both.

Image analysis

Image analysis was performed on the photographs acquired at a standard magnification of ×40. Fluorescence intensity was assessed using the ImageJ software, v. 1.51v (<https://imagej.net/ij>). Integrated density (IntDen) was used to quantify the total fluorescence intensity within the selected area, and was subsequently normalized to the cell count. For each experimental condition, a minimum of 3 slides were prepared, and fluorescence intensity was quantitatively assessed in the acquired microscopic images. The presented images are representative of at least 3 independent experiments.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 20.0 (IBM Corp., Armonk, USA). Descriptive statistics were calculated as minimum–maximum values (min–max), and the Kolmogorov–Smirnov test was used to assess the normality of data distribution. The one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was performed for comparisons among the groups. A *p*-value <0.05 was considered statistically significant.

Results

The cells derived from the digested tissue exhibited consistent adhesion to plastic surfaces across all experimental groups, and displayed similar growth patterns. No discernible differences were observed among the groups in terms of the ability of cells to adhere to plastic surfaces (Fig. 2). Consequently, the cells were successfully maintained through successive passages, with comparable mean cell viability observed across all groups (*p* > 0.05) (Fig. 3).

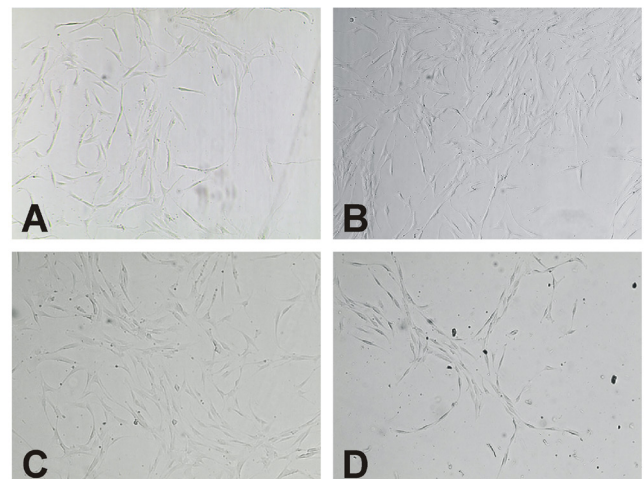


Fig. 2. Ability of cells to adhere to plastic (no significant difference between the groups was observed)

A – I-PRF; B – HBSS; C – positive control (PC); D – negative control (NC).

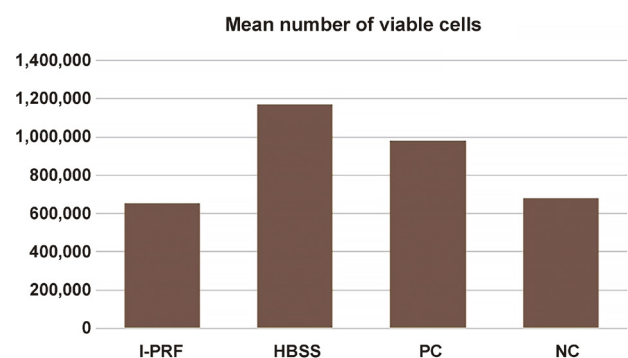


Fig. 3. Mean number of viable cells in various groups

The microscopic images of PDL fibroblasts were captured following exposure to different rejuvenating media, including HBSS and I-PRF. In all experimental groups, PDL fibroblasts demonstrated the ability to form cell colonies from a single cell, indicating their clonogenic potential. The morphological evaluation of the P3 cells revealed that the cells from the I-PRF-treated group retained their typical fibroblastic, spindle-shaped morphology. In contrast, the cells from the HBSS-treated group exhibited early signs of senescence, and the typical fibroblastic morphology was no longer evident (Fig. 4).

The RUNX2 antibody was used to assess PDL cell differentiation toward the osteoblastic lineage, RANKL to assess differentiation toward the osteoclastic lineage, COL1 to assess differentiation toward the fibroblastic lineage, and CAP to assess differentiation toward the cementoblastic lineage. All markers were evaluated in all experimental groups (Fig. 5).

RUNX2 expression was observed as bright spots within the nucleus. The IntDen values in the I-PRF, HBSS and positive control groups differed significantly from that of the negative control group. The negative control group exhibited a higher IntDen value than the other groups ($p < 0.01$) (Fig. 5).

RANKL expression was significantly higher in the I-PRF and HBSS groups than in both control groups ($p < 0.01$).

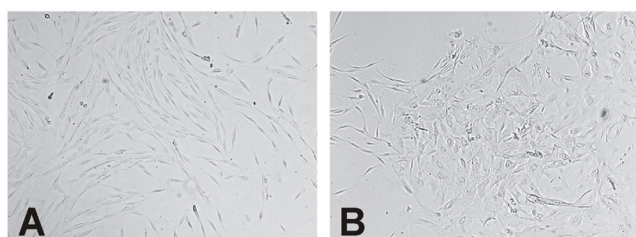


Fig. 4. Microscopic images – morphological evaluation

A – cells from the I-PRF group have typical fibroblastic, spindle-like cell morphology; B – cells from the HBSS group show early signs of senescence, and the typical fibroblastic morphology is no longer evident.

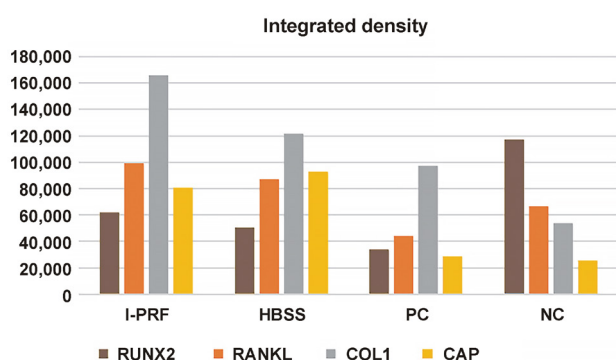


Fig. 5. Integrated density (IntDen) values for all groups

RUNX2 – Runt-related transcription factor 2; RANKL – receptor activator of nuclear factor kappa-B ligand; COL1 – collagen type I; CAP – cementum attachment protein.

Although RANKL expression was slightly lower in the I-PRF group than in the HBSS group, no statistically significant difference was observed between these 2 groups (Fig. 5).

COL1 expression was significantly higher in the I-PRF group than in both the positive and negative control groups ($p < 0.01$). The IntDen value for COL1 expression in the HBSS group was intermediate between those of the I-PRF and positive control groups (Fig. 5).

CAP expression was deficient in both control groups, whereas mild expression was observed in both the I-PRF and HBSS groups ($p < 0.01$) (Fig. 5).

Fluorescence microscope images for all groups with antibody expression are presented in Fig. 6.

Discussion

Since the awareness of the need to immediately replant avulsed teeth or store them in an appropriate liquid medium is still limited in the general population, avulsed teeth are frequently brought to the clinic wrapped in napkins. Although dry teeth can be replanted by the clinician, alternative treatment approaches should be investigated to improve the prognosis. Optimal recovery following dental trauma or regenerative periodontal treatment depends on

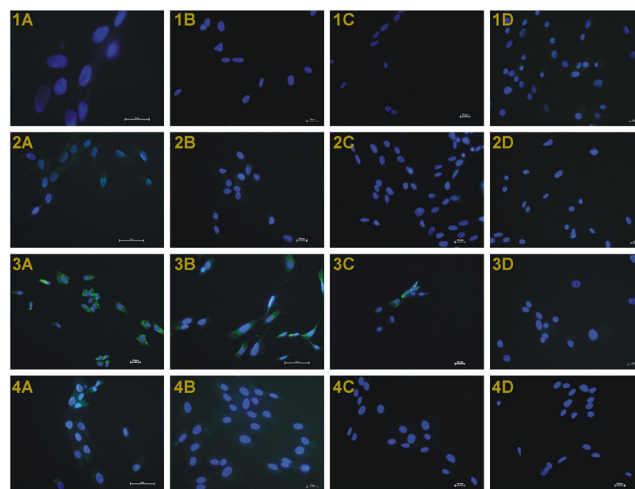


Fig. 6. Fluorescence microscope images for all groups with antibody expression (scale bar: 20 μ m)

I-PRF: 1A – RUNX2 expression was observed as bright green spots in cell nuclei, not brighter than in the NC group, 2A – RANKL expression was mild, 3A – COL1 expression was pronounced in the cytoplasm of PDL fibroblasts, 4A – CAP expression was mild and brighter than in the NC group; HBSS: 1B – RUNX2 expression was observed as bright green spots in cell nuclei, not brighter than in the NC group, 2B – RANKL expression was not clear, but it was brighter than in the control groups, 3B – COL1 expression was lower than in the I-PRF group, 4B – CAP expression was mild and brighter than in the NC group; PC: 1C – RUNX2 expression was observed as bright green spots in cell nuclei, not brighter than in the NC group, 2C – RANKL expression was lower than in the experimental groups, 3C – COL1 expression was pronounced in the cytoplasm of the cells, 4C – CAP expression was not clear; NC: 1D – RUNX2 expression was observed intensively in cell nuclei, 2D – RANKL expression was lower than in the experimental groups, 3D – COL1 expression was not clearly observed; 4D – CAP expression was not clear.

the complete regeneration of PDL and the establishment of a balanced connection between the cementum and the alveolar bone. In cellular regeneration following avulsion, it is essential to restore the depleted cellular metabolites and maintain cell viability.²⁵ Therefore, we investigated a novel approach for the rejuvenation of avulsed teeth by comparing I-PRF with HBSS. In the present study, the early-passage primary PDL cultures obtained from extracted human permanent teeth were used to mimic the cellular conditions of an avulsed tooth.

Although the highest cell number was observed in the HBSS group, no statistically significant difference was found among the groups. These findings demonstrate the successful cultivation of a substantial proportion of viable precursor cells derived from the PDL of representative avulsed teeth. Moreover, the data indicate that the PDL cells obtained from teeth that had not been stored in a rejuvenating medium and had an extraoral dry time of 30 min remained viable. As these cells were classified as “may be viable but compromised” with regard to their regenerative potential, the present study investigated whether I-PRF could provide a greater rejuvenating effect than HBSS. The literature suggested that I-PRF may be superior to HBSS as a rejuvenating medium.⁵

The periodontal tissue response to tooth avulsion involves mechanisms related to cell viability, regeneration and resorption, which are influenced by cellular identity and differentiation. The rationale for using HBSS as a rejuvenating agent in the present study was its proposed ability to maintain and replenish the depleted cellular components of PDL cells.¹⁰ Hank's balanced salt solution has been shown to maintain cell viability and reduce root resorption when used as a storage medium prior to replantation.³ In a previous study, HBSS enhanced osteogenic differentiation in PDL fibroblasts by upregulating RUNX2 expression, whereas milk preserved cell viability and cellular identity.²³ However, milk is not known to restore the depleted cellular metabolites.²⁶ Given the distinction between storage and rejuvenating media, milk was not used as a rejuvenating medium in the present study. Further research into its potential to restore the depleted cellular functions in avulsed teeth would be of interest.

Ideal healing would involve the regeneration of collagen fibers capable of attaching to the newly formed cementum and bone.²⁷ Collagen type I is the main component of PDL fibers, and its expression can be used as an indicator of PDL tissue regeneration. A reimplantation study demonstrated that PRF promoted the proliferation of human periodontal ligament stem cells (PDLSCs) and inhibited their osteoblastic differentiation by upregulating COL1 mRNA expression.¹⁶ Consistent with the effects of PRF, the present study demonstrated that I-PRF helped PDL cells maintain their fibroblastic identity, as evidenced by significantly higher COL1 expression compared with the positive control.

The present study suggests that I-PRF helps maintain the fibroblastic phenotype of PDL cells, as evidenced by the

strong expression of COL1. The concomitant high levels of COL1 and RANKL expression in the I-PRF group may indicate a balance between bone resorption and regeneration. However, the precise mechanisms underlying PDL cell-induced osteoclastogenesis remain unclear. The immersion of avulsed teeth in HBSS has been reported to induce osteoclastogenesis in PDL cells, resulting in the formation of osteoclast-like cells independently of RANKL.²⁸ Previous research also demonstrated stable RANKL expression in HBSS-treated teeth despite prolonged storage periods.²³ When PDL cells are co-cultured with CD14+ monocytes, osteoclast formation can occur, at least in part, through RANKL-mediated mechanisms.^{23,29} In the present study, RANKL expression was higher in the PDL cells treated with I-PRF than in the control groups. Given that osteoclast activity is associated with inflammatory root resorption, further investigation of osteoclastogenesis, inflammatory cytokines, and the effects of I-PRF on the RANKL/RANK (receptor activator of nuclear factor kappa-B)/OPG (osteoprotegerin) system may provide important insights into the mechanisms underlying periodontal healing following avulsion.

Injured periodontal tissues can regenerate through the action of proteins such as cementum-derived growth factor (CDGF), CAP and cementum protein-1 (CEMP1), which promote cell adhesion and differentiation, and may contribute to the formation of new cementum.³⁰ Platelet-rich fibrin has been shown to enhance human PDLSC proliferation while inhibiting osteoblastic differentiation by increasing the mRNA expression levels of COL1 and CEMP1.¹⁶ Furthermore, studies investigating PRP have demonstrated that regeneration is enhanced when stem cells, fibrin glue and PRP are used in combination.³¹ To our knowledge, the present study is the first to evaluate the effects of I-PRF on PDL cells in relation to cementogenesis. The significant difference in CAP expression between the experimental and control groups is consistent with previous findings, and suggests that healing following tooth avulsion may involve a regenerative response that helps minimize the resorption of the damaged root surface.^{32,33}

A key advantage of I-PRF is that it can be prepared at a low centrifugation speed (such as 700 rpm, 60 × g in original protocols or 1,300 rpm in modified protocols) within a short period (3 min). In contrast, the preparation of other platelet concentrates, such as PRF, requires more time, potentially increasing the extraoral dry time of avulsed teeth. The lower centrifugation speed also increases the proportion of platelets and leukocytes recovered, which may enhance tissue regeneration and promote the activity of PDL fibroblasts in the repair process.^{22,34}

Limitations

Although the small sample size is a major limitation of the present study, another limitation is that the effects of centrifugation force and duration, which may result in

different PRF-based matrices, were not evaluated in relation to osteoclastogenesis. Although numerous studies have investigated the use of I-PRF in cells of heterogeneous origin, the inability to assess individual cellular defense mechanisms, such as macrophage (M) differentiation, may also be considered a limitation of the present study. Studies evaluating macrophage polarization toward the M1 or M2 phenotypes are needed to comprehensively assess the effects of I-PRF on the RANKL pathway and osteoclastogenesis. Further in vitro and in vivo studies are warranted to elucidate the molecular mechanisms underlying the effects of I-PRF on osteoclastogenesis and to determine whether different PRF isolation protocols influence this process. In addition, before I-PRF can be used clinically as a rejuvenating medium, its platelet content should be characterized through biochemical and cellular analyses, and the platelet concentrates obtained from individual patients should be quantitatively evaluated to determine the biological activity and composition of I-PRF.

Conclusions

The present study investigated the use of I-PRF as a rejuvenating medium following tooth avulsion. One of the most common challenges encountered in avulsion cases is the lack of public awareness regarding the need to store avulsed tooth in an appropriate liquid medium; consequently, families frequently present to the clinic with the tooth in a dry condition. In conclusion, I-PRF may be considered as a regenerative medium for avulsed teeth presenting to the clinic after an extraoral dry period. Further studies involving different extraoral dry times and larger sample sizes are warranted to confirm these findings.

Ethics approval and consent to participate

The study was conducted in accordance with the principles outlined in the 2013 revision of the Declaration of Helsinki. Participant recruitment and selection procedures were approved by the Ethics Committee for Clinical Research at the Faculty of Medicine, Kütahya Dumlupınar University, Turkey (approval date: August 16, 2017; approval No. 2017-10/3). All participants were informed about the study and voluntarily provided written informed consent before participation.

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

Generative AI technology was used solely to assist in language editing and proofreading of the manuscript. ChatGPT 3.5 was used.

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References

- Andreasen JO, Andreasen FM, Tsilingaridis G. Avulsions. In: Andreasen JO, Andreasen FM, Andersson L, eds. *Textbook and Color Atlas of Traumatic Injuries to the Teeth*. 5th ed. Hoboken, NJ: Wiley-Blackwell; 2019:486–520.
- Glendor U, Halling A, Andersson L, Eilert-Petersson E. Incidence of traumatic tooth injuries in children and adolescents in the county of Västmanland, Sweden. *Swed Dent J*. 1996;20(1-2):15–28. PMID:8738905.
- Hiremath H, Kulkarni S, Sharma R, Hiremath V, Motiwala T. Use of platelet-rich fibrin as an autologous biologic rejuvenating media for avulsed teeth – an in vitro study. *Dent Traumatol*. 2014;30(6):442–446. doi:10.1111/edt.12119
- Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. *Int J Oral Surg*. 1981;10(1):43–53. doi:10.1016/s0300-9785(81)80007-5
- Fouad AF, Abbott PV, Tsilingaridis G, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Dent Traumatol*. 2020;36(4):331–342. doi:10.1111/edt.12573
- McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. *J Periodontol Res*. 1991;26(3 Pt 1):144–154. doi:10.1111/j.1600-0765.1991.tb01638.x
- Trope M. Root resorption of dental and traumatic origin: Classification based on etiology. *Pract Periodontics Aesthet Dent*. 1998;10(4):515–522. PMID:9655059.
- Goswami M, Chaitra T, Chaudhary S, Manuja N, Sinha A. Strategies for periodontal ligament cell viability: An overview. *J Conserv Dent*. 2011;14(3):215–220. doi:10.4103/0972-0707.85789
- Pettiette M, Hupp J, Mesaros S, Trope M. Periodontal healing of extracted dogs' teeth air-dried for extended periods and soaked in various media. *Endod Dent Traumatol*. 1997;13(3):113–118. doi:10.1111/j.1600-9657.1997.tb00023.x
- Qian H, Ding Y, Wu Y, Li S. The effects of three modified Hank's balanced salt solutions on root resorption of late replanted teeth: A pilot study. *J Craniomaxillofac Surg*. 2018;46(5):808–814. doi:10.1016/j.jcms.2018.02.009
- Pietruszka P, Chruścicka I, Duś-Ilnicka I, Paradowska-Stolarz A. PRP and PRF – subgroups and divisions when used in dentistry. *J Pers Med*. 2021;11(10):944. doi:10.3390/jpm11100944
- Miron RJ, Zucchelli G, Pikos OA, et al. Use of platelet-rich fibrin in regenerative dentistry: A systematic review. *Clin Oral Investig*. 2017;21(6):1913–1927. doi:10.1007/s00784-017-2133-z
- Crisci A, Manfredi S, Crisci M. Fibrin rich in leukocyte-platelets (L-PRF) and injectable fibrin rich platelets (i-PRF), two opportunity in regenerative surgery: Review of the sciences and literature. *J Dent Med Sci*. 2019;18(4):66–79. doi:10.9790/0853-1804106679
- Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E. In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): A new platelet concentrate. *Br J Oral Maxillofac Surg*. 2013;51(5):438–443. doi:10.1016/j.bjoms.2012.08.003
- Bhattacharya HS, Gummaluri SS, Rani A, Verma S, Bhattacharya P, Rayashettygura 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

16. Zhao YH, Zhang M, Liu NX, et al. The combined use of cell sheet fragments of periodontal ligament stem cells and platelet-rich fibrin granules for avulsed tooth reimplantation. *Biomaterials*. 2013;34(22):5506–5520. doi:10.1016/j.biomaterials.2013.03.079
17. Kobayashi E, Fujioka-Kobayashi M, Sculean A, et al. Effects of platelet rich plasma (PRP) on human gingival fibroblast, osteoblast and periodontal ligament cell behaviour. *BMC Oral Health*. 2017;17(1):91. doi:10.1186/s12903-017-0381-6
18. Verboket RD, Anbar B, Söhling N, et al. Changes in platelet-rich fibrin composition after trauma and surgical intervention. *Platelets*. 2020;31(8):1069–1079. doi:10.1080/09537104.2020.1714575
19. Serafini G, Lopreiato M, Lollobrigida M, et al. Platelet rich fibrin (PRF) and its related products: Biomolecular characterization of the liquid fibrinogen. *J Clin Med*. 2020;9(4):1099. doi:10.3390/jcm9041099
20. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Behavior of gingival fibroblasts on titanium implant surfaces in combination with either injectable-PRF or PRP. *Int J Mol Sci*. 2017;18(2):331. doi:10.3390/ijms18020331
21. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma. *Platelets*. 2018;29(1):48–55. doi:10.1080/09537104.2017.1293807
22. Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable platelet rich fibrin (i-PRF): Opportunities in regenerative dentistry? *Clin Oral Investig*. 2017;21(8):2619–2627. doi:10.1007/s00784-017-2063-9
23. Bağ İ, Yıldırım S. Effect of avulsion storage media on periodontal ligament fibroblast differentiation. *Dent Traumatol*. 2017;33(6):458–464. doi:10.1111/edt.12356
24. Oktar PA, Yildirim S, Balci D, Can A. Continual expression throughout the cell cycle and downregulation upon adipogenic differentiation makes nucleostemin a vital human MSC proliferation marker. *Stem Cell Rev Rep*. 2011;7(2):413–424. doi:10.1007/s12015-010-9201-y
25. Thomas T, Gopikrishna V, Kandaswamy D. Comparative evaluation of maintenance of cell viability of an experimental transport media “coconut water” with Hank’s balanced salt solution and milk, for transportation of an avulsed tooth: An in vitro cell culture study. *J Conserv Dent*. 2008;11(1):22–29. doi:10.4103/0972-0707.43414
26. Gamson EK, Dumsha TC, Sydskis R. The effect of drying time of periodontal ligament cell viability. *J Endod*. 1992;18(4):186–189. doi:10.1016/S0099-2399(06)81441-X
27. Liu J, Ruan J, Weir MD, et al. Periodontal bone–ligament–cementum regeneration via scaffolds and stem cells. *Cells*. 2019;8(6):537. doi:10.3390/cells8060537
28. Zhan X, Zhang C, Dissanayaka WL, et al. Storage media enhance osteoclastogenic potential of human periodontal ligament cells via RANKL-independent signaling. *Dent Traumatol*. 2013;29(1):59–65. doi:10.1111/j.1600-9657.2012.01138.x
29. Uchiyama M, Nakamichi Y, Nakamura M, et al. Dental pulp and periodontal ligament cells support osteoclastic differentiation. *J Dent Res*. 2009;88(7):609–614. doi:10.1177/0022034509340008
30. Arzate H, Zeichner-David M, Mercado-Celis G. Cementum proteins: Role in cementogenesis, biomineralization, periodontium formation and regeneration. *Periodontol 2000*. 2015;67(1):211–233. doi:10.1111/prd.12062
31. Rezaei M, Jamshidi S, Saffarpour A, et al. Transplantation of bone marrow-derived mesenchymal stem cells, platelet-rich plasma, and fibrin glue for periodontal regeneration. *Int J Periodontics Restorative Dent*. 2019;39(1):e32–e45. doi:10.11607/prd.3691
32. Andreasen J. O. A time-related study of periodontal healing and root resorption activity after replantation of mature permanent incisors in monkeys. *Swed Dent J*. 1980;4(3):101–110. PMID:6933704.
33. Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. *Int J Oral Surg*. 1981;10(1):43–53. doi:10.1016/S0300-9785(81)80007-5
34. Miron RJ, Xu H, Chai J, et al. Comparison of platelet-rich fibrin (PRF) produced using 3 commercially available centrifuges at both high (~700 g) and low (~200 g) relative centrifugation forces. *Clin Oral Investig*. 2020;24(3):1171–1182. doi:10.1007/s00784-019-02981-2

Effectiveness of a combination of calcium hydroxide and the anchovy nano-extract on the odontoblast-like cell count and alkaline phosphatase expression in rats' dental pulp

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Abstract

Background. There is growing interest in the use and processing of natural products for potential applications in dentistry, including endodontic treatment. Anchovy (*Stolephorus insularis*) is one such natural product.

Objectives. The aim of the present study was to evaluate odontoblast-like cells (OLCs) and alkaline phosphatase (ALP) expression in the pulp of Wistar rats after the administration of a combination of calcium hydroxide (CH) and the anchovy nano-extract as a potential material for pulp regeneration.

Material and methods. Fifty-four Wistar rats were divided into 3 groups: the control group (group 1) received CH and distilled water; the 1st treatment group (group 2) received a 1:1 ratio combination of CH and the anchovy nano-extract; and the 2nd treatment group (group 3) received a 1:2 ratio combination of CH and the anchovy nano-extract. Each group was further divided into 3 subgroups according to the observation periods of 7, 14 and 21 days. Pulp perforation was performed on the rats' maxillary right first molars, followed by the placement of the tested materials into the perforated pulp. The rats were sacrificed according to the respective observation periods. The specimens were processed, and evaluated histopathologically (staining with hematoxylin and eosin (H&E)) and through immunohistochemistry (IHC). The data were analyzed using the one-way ANOVA followed by the Tukey HSD post hoc test. Statistical significance was set at $p < 0.05$.

Results. There were significant increases in OLCs and ALP expression following the application of the anchovy nano-extract at both 1:1 and 1:2 ratios, with significant differences as compared to the control group ($p < 0.05$). The highest OLC count and ALP expression were observed on day 21. The higher 1:2 ratio resulted in faster OLC formation, which occurred on day 14; in the case of the 1:1 ratio, increased OLC formation occurred on day 21. Both ratios increased ALP expression on day 21.

Conclusions. The application of a combination of CH and the anchovy nano-extract to the perforated pulp increased the number of OLCs and ALP expression.

Keywords: regeneration, medicine, alkaline phosphatase, calcium hydroxide, odontoblast

Highlights

- The application of a combination of calcium hydroxide (CH) and the anchovy nano-extract to the perforated pulp increased the number of OLCs and alkaline phosphatase (ALP) expression.
- Both the 1:1 and 1:2 ratio combinations of CH and the anchovy nano-extract increased the OLC count and ALP expression.
- The highest OLC count and ALP expression were observed on day 21 after application.

Introduction

Oral health has a significant impact on a person's quality of life (QoL), affecting their ability to speak, eat, and maintain overall health. Therefore, comprehensive treatment is necessary to address oral health holistically.¹ Vital pulp exposure can occur due to trauma, caries removal, or during mechanical cavity preparation.² Consequently, prompt pulp capping is imperative when pulp exposure occurs.³ Preserving pulp vitality is crucial in all of these scenarios to ensure that the tooth remains vital, receives adequate nutrients, maintains innervation, and has effective immunological defense. Pulp capping treatment aims to promote the regeneration of the damaged pulp by employing bioactive substances.²

The regeneration process in the pulp, involving stem cells, odontoblasts, inflammatory cells, and fibroblasts, takes place following pulp capping treatment. An increase in stem cell activity occurs during the pulp healing phase, characterized by the proliferation and differentiation of these cells into odontoblast-like cells (OLCs). To initiate mineralization, alkaline phosphatase (ALP) is synthesized by these newly formed OLCs.³ Alkaline phosphatase plays an important role in the tissue response to injury.^{4,5} It is a marker of OLC differentiation, and its presence indicates that the cells are undergoing differentiation.⁶

Bioactive substances have been reported to support tissue regeneration.⁷ These substances may be in the form of stem cells or bioceramic materials, such as mineral trioxide aggregate (MTA) and Biodentine™, which are currently considered among the most reliable materials for pulp capping. They offer several advantages over calcium hydroxide (CH), but are relatively expensive.⁸ A recent study reported that both bioceramic materials and CH are effective for the direct pulp capping of permanent teeth.⁹ Calcium hydroxide has been widely used for direct pulp capping.^{10,11} One of the advantageous characteristics of CH is its high pH, which contributes to stem cell activation.^{12,13} It increases the pH of an acidic environment, inhibits the growth of microorganisms, and supports the healing process and defense mechanisms of the pulp tissue.¹⁴ However, several studies have reported various drawbacks of CH, including its high alkalinity, which may result in pulp necrosis and inflammation, as well as its high solubility, inadequate adhesion and poor sealing ability.^{2,3}

In recent decades, there has been growing interest in the use of pharmaceuticals and natural products in dentistry.^{15–17} Several natural products have been explored for use in endodontic treatment.^{18–21} Anchovy (*Stolephorus insularis*) is one such natural material that has been investigated as a potential alternative treatment option.^{22,23} The mineral composition of 100 g of anchovy includes 500 mg of calcium, 500 mg of phosphorus and 1 mg of iron.²⁴ The anchovy extract is currently being investigated for its potential to stimulate bone and tooth formation. The bioactivity of the anchovy extract is attributed to its mineral composition, particularly its calcium and phosphorus content.²² Previous studies investigating the anchovy extract against *Streptococcus mutans* have demonstrated its antibacterial efficacy.^{24,25}

The present study aimed to analyze the effect of 1:1 and 1:2 combinations of CH and the anchovy nano-extract on the OLC count and ALP expression following application to the perforated vital pulp in rats on days 7, 14 and 21. The null hypothesis was that the combination of CH and the anchovy nano-extract would have no effect on the number of OLCs or ALP expression following application to the perforated vital pulp in rats.

Material and methods

Animals

This post-test-only control group study followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) 2.0 guidelines. Ethical approval for the study was obtained from the institutional ethics committee at the Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia (approval No. 835/HRECC.FODM/XI/2022). This in vivo experimental study used 54 healthy adult male rats (*Rattus norvegicus*) aged 12–16 weeks, with body weight ranging from 250 to 300 g. The sample size was calculated using the Lemeshow formula based on the estimated proportion from a previous study.²⁶ The animals were randomly assigned using a lottery system to 9 groups, with 6 rats in each group. The animals were divided into 3 main groups: group 1 as the control group; group 2 receiving a 1:1 ratio combination; and group 3 receiving a 1:2 ratio combination of CH and the anchovy

nano-extract. Each main group was further divided into 3 subgroups according to the observation periods of 7, 14 and 21 days. The rats had free access to food and water, and were housed under a 12-hour light/dark cycle at a constant temperature of 25°C and relative humidity of 45–55%.²⁶ The rats were continuously cared for and monitored by 3 laboratory veterinarians.

Preparation of the anchovy nano-extract

The anchovy nano-extract was prepared using 1 kg of anchovy, which was boiled for 1 h and then oven-dried for 24 h. A total of 100 g of dried anchovy was macerated in 1 M hydrochloric acid for 2 h, followed by neutralization through washing according to the standard maceration procedure.²⁷ The anchovy was then desiccated in an oven at 105°C for 2 h. Subsequently, the dried anchovy was pulverized using a mortar, passed through a 60-mesh sieve, and further processed using high-efficiency milling for 1 h at 3,000 rpm with a milling ball diameter of 0.5 mm. The milling process was continued until nanosized particles were achieved, with periodic particle-size assessments performed using dynamic light scattering.²⁸

In this study, the anchovy nano-extract was used in 2 ratios – a 1:1 ratio and a 1:2 ratio of CH to the anchovy nano-extract, both dissolved in distilled water. The control group (group 1) received CH dissolved in distilled water. Each rat received 0.1 mg of the respective mixture.

Pulp capping

The rats were anesthetized with ketamine 40 mg/kg (Ketalar; Pfizer Healthcare, Dublin, Ireland) and xylazine 5 mg/kg (Xyla; Interchemie, Venray, The Netherlands) by intraperitoneal injection. Povidone–iodine 1% (Betadine; Watsons Indonesia, South Jakarta, Indonesia) was applied to the gingiva and oral mucosa using cotton pellets. The involved maxillary right first molar was then swabbed with Consepsis™ (Ultradent Products Inc., South Jordan, USA) for aseptic preparation. The tooth was prepared using a dental handpiece (NSK, Kanuma, Japan) with a No. ¼ round diamond bur (Edenta, Au, Switzerland) to perforate the pulp.²⁹ The perforated pulp was identified by the appearance of a pink spot. The respective material was then placed directly over the perforated area. Group 1 (control) received CH only, group 2 received a 1:1 mixture of CH and the anchovy nano-extract, and group 3 received a 1:2 mixture of CH and the anchovy nano-extract. The material was placed over the perforated pulp, and the cavity was restored with glass ionomer cement (GIC) (GC, Tokyo, Japan) to cover the capping material and prevent contamination. Following the pulp capping procedure, the rats were euthanized on days 7, 14 and 21 for the evaluation of OLCs and ALP expression. Euthanasia was performed by an overdose of intraperitoneal anesthetic injection

consisting of ketamine 450 mg/kg (Ketalar) and xylazine 45 mg/kg (Xyla), in accordance with the American Veterinary Medical Association (AVMA) guidelines, followed by a standard decalcification process as described in a previous study.²⁶

Histopathological and immunohistochemical analysis

Histological sections were prepared at an approximate thickness of 4 µm and oriented parallel to the long axis of the tooth. Odontoblast-like cells were evaluated histopathologically, while ALP expression was evaluated using immunohistochemistry (IHC). Primary monoclonal antibodies (Santa Cruz Biotechnology, Dallas, USA) were used to specifically detect the target protein through antigen–antibody binding. The enzyme was then reacted with a chromogenic substrate, and the resulting staining was observed using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) at ×1,000 magnification.

Statistical analysis

The data obtained in this study were analyzed using SPSS Statistics for Windows, v. 24 (IBM Corp., Armonk, USA), and presented as mean ± standard deviation ($M \pm SD$). The Shapiro–Wilk test was used to assess data normality, followed by Levene's test to assess the homogeneity of variance. The one-way analysis of variance (ANOVA) was performed to determine significant differences among the groups. Subsequently, the Tukey honestly significant difference (HSD) post hoc test was conducted to identify specific differences between the groups. Statistical significance was set at $p < 0.05$.

Results

The OLC and ALP data were normally distributed and homogeneous. The one-way ANOVA was performed to assess the effects of the treatment on OLCs and ALP expression. The results showed a significant difference among the groups ($p < 0.05$). Therefore, the null hypothesis was rejected. The mean values for the OLC count and ALP expression are presented in Table 1, while the comparisons of OLCs and ALP expression among the different groups and observation periods are presented in Table 2 and Table 3, respectively.

Significant differences in OLCs and ALP expression were observed between both groups receiving CH with the added anchovy nano-extract at 1:1 and 1:2 ratios and the CH-only group ($p < 0.05$). No significant difference was observed within the CH-only group with regard to different observation periods ($p > 0.05$). Significant increases in the OLC count and ALP expression were observed on days 14 and 21 as compared to day 7 ($p < 0.05$).

Table 1. Odontoblast-like cell (OLC) count and alkaline phosphatase (ALP) expression in the study groups

Groups and time points	<i>n</i>	OLC count	ALP expression
Group 1, day 7	6	2.33 ±1.106	4.50 ±2.217
Group 1, day 14	6	3.00 ±0.816	4.16 ±1.067
Group 1, day 21	6	3.83 ±1.344	2.83 ±1.067
Group 2, day 7	6	7.00 ±1.414	8.66 ±1.972
Group 2, day 14	6	7.00 ±1.291	8.50 ±1.708
Group 2, day 21	6	9.00 ±1.915	11.83 ±1.572
Group 3, day 7	6	6.33 ±1.491	9.66 ±1.247
Group 3, day 14	6	9.33 ±1.700	10.16 ±1.067
Group 3, day 21	6	9.66 ±1.247	13.50 ±2.062

Groups: group 1 (control) – calcium hydroxide (CH) only; group 2 – CH and the anchovy nano-extract in a 1:1 ratio; group 3 – CH and the anchovy nano-extract in a 1:2 ratio.

Odontoblast-like cells

The histopathological identification of OLCs on days 7, 14 and 21 is shown in Fig. 1. The arrows indicate the OLCs observed in each group.

Significant differences in the OLC count were observed between both groups receiving CH with the added anchovy nano-extract at 1:1 and 1:2 ratios and the CH-only group ($p < 0.05$). The addition of the anchovy nano-extract to CH at a 1:1 ratio significantly increased the OLC count as compared to the CH-only group on days 7, 14 and 21 ($p = 0.000$ for all). Similarly, the 1:2 ratio significantly increased the OLC count as compared to the CH-only group on day 7 ($p = 0.001$), day 14 ($p = 0.000$) and day 21 ($p = 0.000$). A significant difference in the OLC count between the 1:1 and 1:2 ratios was observed on day 14 ($p = 0.034$) (Table 2).

Table 2. Significance of differences in the odontoblast-like cell (OLC) count and alkaline phosphatase (ALP) expression between the study groups with regard to the same observation day

	Comparisons	OLC count (<i>p</i> -value)	ALP expression (<i>p</i> -value)
Day 7	group 1 to group 2	0.000*	0.008*
	group 1 to group 3	0.001*	0.001*
	group 2 to group 3	0.719	0.678
Day 14	group 1 to group 2	0.000*	0.000*
	group 1 to group 3	0.000*	0.001*
	group 2 to group 3	0.034*	0.146
Day 21	group 1 to group 2	0.000*	0.000*
	group 1 to group 3	0.000*	0.000*
	group 2 to group 3	0.801	0.265

Groups: group 1 (control) – calcium hydroxide (CH) only; group 2 – CH and the anchovy nano-extract in a 1:1 ratio; group 3 – CH and the anchovy nano-extract in a 1:2 ratio.

* statistically significant.

Table 3. Significance of differences in the odontoblast-like cell (OLC) count and alkaline phosphatase (ALP) expression within the study groups with regard to different observation days

	Comparisons	OLC count (<i>p</i> -value)	ALP expression (<i>p</i> -value)
Group 1	day 7 to day 14	0.689	0.938
	day 7 to day 21	0.141	0.237
	day 14 to day 21	0.180	0.345
Group 2	day 7 to day 14	1.000	0.988
	day 7 to day 21	0.014*	0.031*
	day 14 to day 21	0.023*	0.023*
Group 3	day 7 to day 14	0.016*	0.863
	day 7 to day 21	0.008*	0.008*
	day 14 to day 21	0.934	0.009*

Groups: group 1 (control) – calcium hydroxide (CH) only; group 2 – CH and the anchovy nano-extract in a 1:1 ratio; group 3 – CH and the anchovy nano-extract in a 1:2 ratio.

* statistically significant.

No significant change in the OLC count throughout the whole observation period was observed within the control group, which received CH only ($p > 0.05$). In group 2, a significant increase in the OLC count was observed on day 21 as compared to day 7 ($p = 0.014$) and day 14 ($p = 0.023$). In group 3, a significant increase in the OLC count was observed on days 14 ($p = 0.016$) and 21 ($p = 0.008$) as compared to day 7, while no significant difference was observed between days 14 and 21 ($p = 0.934$) (Table 3).

Alkaline phosphatase expression

The immunohistochemical detection of ALP on days 7, 14 and 21 is shown in Fig. 2. The arrows indicate ALP expression in macrophage cells in each group.

Significant differences in ALP expression were observed between both groups receiving CH with the added anchovy nano-extract at 1:1 and 1:2 ratios and the CH-only group ($p < 0.05$). The addition of the anchovy nano-extract to CH at a 1:1 ratio significantly increased ALP expression as compared to the CH-only group on day 7 ($p = 0.008$), day 14 ($p = 0.000$) and day 21 ($p = 0.000$). Similarly, the 1:2 ratio significantly increased ALP expression as compared to the CH-only group on day 7 ($p = 0.001$), day 14 ($p = 0.001$) and day 21 ($p = 0.000$). No significant difference in ALP expression was observed between the 1:1 and 1:2 ratios on any of the observation days (Table 2).

No significant difference in ALP expression throughout the whole observation period was observed within the control group, which received CH only ($p > 0.05$). In group 2, a significant increase in ALP expression was observed on day 21 as compared to day 7 ($p = 0.031$) and day 14 ($p = 0.023$). Also in group 3, a significant increase in ALP expression was observed on day 21 as compared to day 7 ($p = 0.008$) and day 14 ($p = 0.009$) (Table 3).

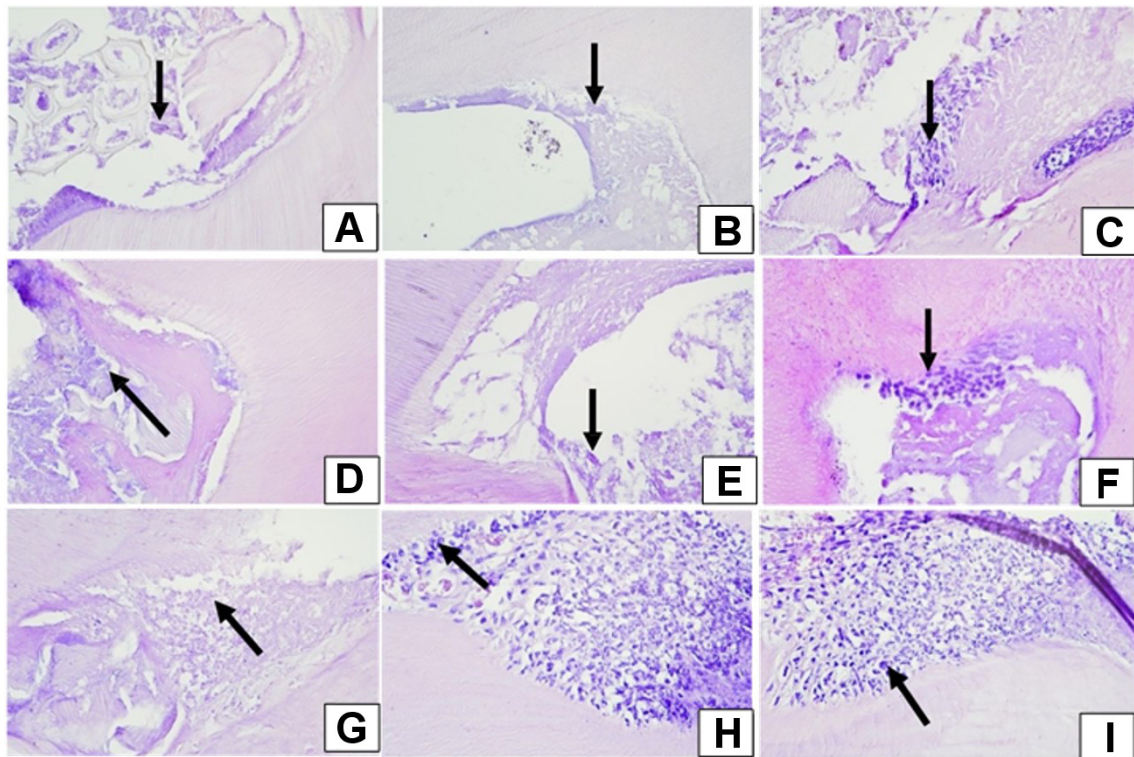


Fig. 1. Detection of odontoblast-like cells (OLCs) (indicated by arrows) in the study groups at different time points (histopathological examination)

Groups: group 1 (control) – calcium hydroxide (CH) only; group 2 – CH and the anchovy nano-extract in a 1:1 ratio; group 3 – CH and the anchovy nano-extract in a 1:2 ratio.

A, B, C – groups 1, 2 and 3, respectively, on day 7; D, E, F – groups 1, 2 and 3, respectively, on day 14; G, H, I – groups 1, 2 and 3, respectively, on day 21.

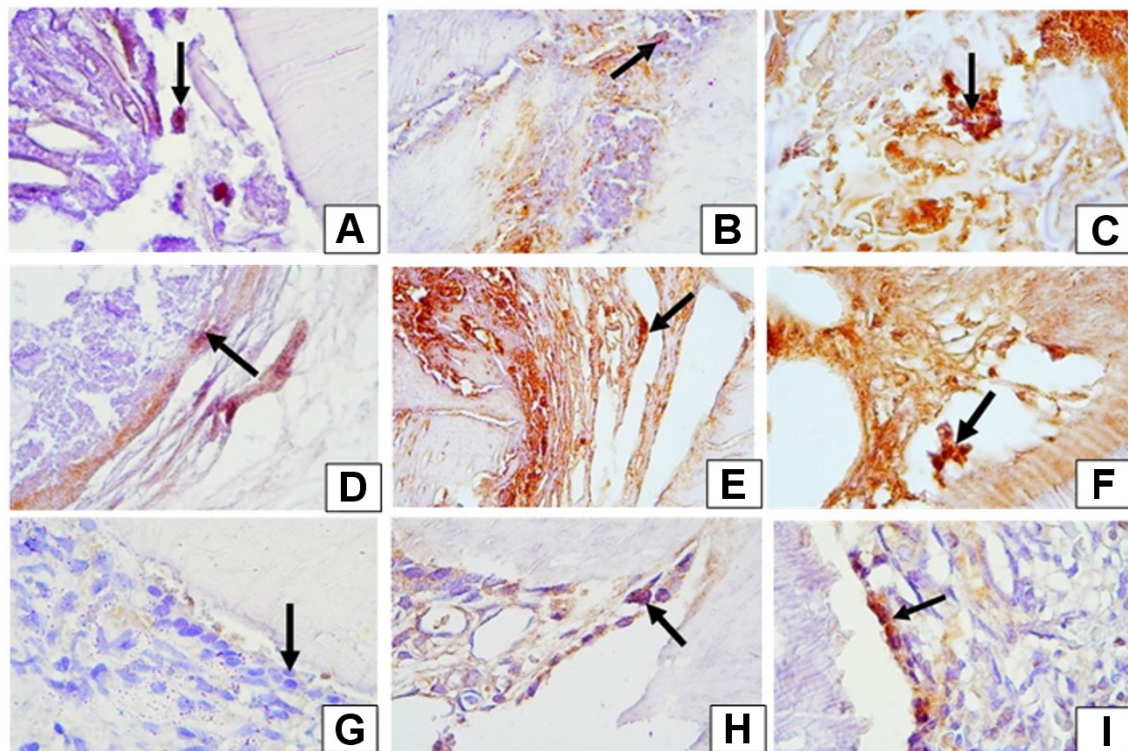


Fig. 2. Immunohistochemistry (IHC) examination of alkaline phosphatase (ALP) expression in macrophages (indicated by arrows) in the study groups at different time points

Groups: group 1 (control) – calcium hydroxide (CH) only; group 2 – CH and the anchovy nano-extract in a 1:1 ratio; group 3 – CH and the anchovy nano-extract in a 1:2 ratio.

A, B, C – groups 1, 2 and 3, respectively, on day 7; D, E, F – groups 1, 2 and 3, respectively, on day 14; G, H, I – groups 1, 2 and 3, respectively, on day 21.

Discussion

In this study, the rats were subjected to mechanical pulp injury in the form of perforation to stimulate the formation of reparative dentin.²⁹ Rats were selected, as the structure of their teeth resembles that of human teeth. Several previous studies have used rat maxillary and mandibular molars as experimental models.^{26,29} The formation of reparative dentin is the primary goal of vital pulp treatment following injury.³⁰ Stem cells migrate from richly vascularized tissues to the injured area to facilitate tissue repair. In general, the repair process following pulp capping involves moderate inflammation, the recruitment and migration of stem or progenitor cells, cell proliferation, and subsequent differentiation. Stem cells play a crucial role in tissue regeneration.³¹ Various materials and methods have been investigated to optimize the proliferation and differentiation of mesenchymal stem cells (MSCs).^{32–35} Exploring new materials and approaches to enhance the proliferation and differentiation of MSCs, including dental pulp stem cells, is important for supporting pulp regeneration.^{36–39} Previous studies have demonstrated the positive effects of MSCs on tissue regeneration, including in medically compromised subjects.^{40–44} Odontoblast-like cells originate from dental pulp stem cells, which are activated by the cytokines released during the inflammatory process.³⁰ However, excessive or prolonged inflammation, particularly in the presence of bacteria and their by-products such as lipopolysaccharides, may interfere with the proliferation and differentiation of these cells and consequently impair pulp regeneration.^{45,46} Therefore, pulp capping materials should not only protect the injured pulp from bacterial contamination, but also support the cellular processes involved in pulp repair and regeneration.

This study evaluated ALP expression and the number of OLCs as markers involved in the formation of reparative dentin. The ability of the anchovy nano-extract to stimulate reparative dentin formation in the perforated pulp of Wistar rat molars may be attributed to its calcium and phosphorus content, which can contribute to the formation of calcium phosphate and hydroxyapatite. Hydroxyapatite plays an important role in supporting reparative dentin formation and maintaining the vitality of the injured pulp. Calcium hydroxide mixed with distilled water showed lower OLC count and ALP expression than the anchovy nano-extract treatment groups. Calcium hydroxide has a strongly alkaline pH.⁴⁷ Its high alkalinity causes the formation of a necrotic region, known as the cauterization zone, shortly after contact with the pulp tissue, subsequently leading to inflammation in the adjacent tissue.⁴⁸ Other limitations of CH as a pulp capping material include its high solubility and inadequate sealing ability, which may compromise the long-term integrity of reparative dentin.⁴⁹

In this study, significant differences in OLC formation were observed between the CH–anchovy nano-extract 1:1 combination group and the CH–anchovy nano-extract 1:2

combination group between days 14 and 21. This finding may be attributed to the phosphorus content of anchovies, which may contribute to odontoblast cell proliferation. Anchovies contain phosphorus, which, when mixed with water, can form phosphate. Phosphate can react with calcium to form calcium phosphate compounds, which play an important role in the differentiation of stem cells into odontoblasts. The anchovy extract contains 76% calcium phosphate compounds in the form of hydroxyapatite.²⁵ Calcium and phosphate ions are essential for the mineralization of the reparative dentin matrix. The results of a previous study indicated that the number of odontoblast cells increased with increasing concentrations of the anchovy extract.²⁴ Calcium and phosphate support mineralization by promoting protein adsorption.⁵⁰ This process is beneficial for dentinogenesis and provides a three-dimensional (3D) framework for pre-odontoblasts and odontoblasts, thereby supporting new dentin formation. During the early stage of MSC differentiation, Runt-related transcription factor 2 (Runx2) and bone morphogenetic protein-2 (BMP-2) expression is significantly increased, followed by the expression of ALP, osteopontin (OPN) and osteocalcin (OC) as later markers of mineralization.⁵⁰

In this study, ALP expression increased among the treatment groups. Alkaline phosphatase is an enzyme that promotes mineralization, reported to be expressed after 14 and 21 days, with greater expression observed on day 21.⁵¹ These findings are consistent with our study. The anchovy nano-extract contains calcium and phosphate as bioactive components. Calcium and phosphate may influence the Wnt/ β -catenin signaling pathway or activate the p38/MAPK signaling pathway, thereby promoting the differentiation of MSCs in the pulp and potentially increasing the expression of mineralization-related genes.⁵² The expression of ALP by pre-odontoblasts and odontoblasts indicates that these cells are undergoing the mineralization phase.

The nanoparticle size of the anchovy provides finely dispersed calcium and phosphate bioactive particles, which may result in a larger surface area and greater interaction with the pulp, and may also contribute to a dose-dependent effect. Microenvironmental factors, such as the degradation or dissolution of calcium and phosphate from the anchovy nano-extract, may also influence the results. In the *in vivo* environment, a series of biological events, including immune regulation and immune responses, may affect the observed outcomes. Further studies are needed to evaluate the interaction between the anchovy nano-extract and the inflammatory responses of the pulp.

Conclusions

Our study demonstrated that the application of CH and the anchovy nano-extract at both 1:1 and 1:2 ratios increased the number of OLCs and ALP expression in the dental pulp of rats. The higher 1:2 ratio resulted in faster

OLC formation as compared to the 1:1 ratio. Both ratios increased ALP expression on day 21.

Ethics approval and consent to participate

Ethical approval for the study was obtained from the institutional ethics committee at the Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia (approval No. 835/HRECC.FODM/XI/2022).

Data availability

The datasets supporting the findings of the current study are all contained within the article.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

- Mills A, Berlin-Broner Y, Levin L. Improving patient well-being as a broader perspective in dentistry. *Int Dent J.* 2023;73(6):785–792. doi:10.1016/j.identj.2023.05.005
- Nie E, Yu J, Jiang R, et al. Effectiveness of direct pulp capping bioactive materials in dentin regeneration: A systematic review. *Materials (Basel).* 2021;14(22):6811. doi:10.3390/ma14226811
- Islam R, Readul Islam MR, Tanaka T, Alam MK, Aly Ahmed HM, Sano H. Direct pulp capping procedures – evidence and practice. *Jpn Dent Sci Rev.* 2023;59:48–61. doi:10.1016/j.jdsr.2023.02.002
- Nugraha AP, Narmada IB, Ernawati DS, et al. Bone alkaline phosphatase and osteocalcin expression of rat's gingival mesenchymal stem cells cultured in platelet-rich fibrin for bone remodeling (in vitro study). *Eur J Dent.* 2018;12(04):566–573. doi:10.4103/ejd.ejd_261_18
- Nugraha AP, Narmada IB, Winoto ER, et al. Gingiva mesenchymal stem cells normoxic or hypoxic preconditioned application under orthodontic mechanical force on osterix, osteopontin, and ALP expression. *Eur J Dent.* 2024;18(2):501–509. doi:10.1055/s-0043-1772699
- Nugroho JJ. Increase in ALP levels at odontoblast cell line after haruan (*Channa striata*) extract administration as remineralization material. *J Dentomaxillofac Sci.* 2022;7(1):54–56. doi:10.15562/jdmfs.v7i1.1289
- Palczewska-Komsa M, Puszkars A, Dalewski B, et al. Analysis of selected parameters of reparative dentin after direct pulp capping with MTA repair HP in human teeth, using CBCT and micro-CT. *Dent Med Probl.* 2025;62(6):1237–1243. doi:10.17219/dmp/189747
- Wannakajeepiboon M, Sathorn C, Kornuthisopon C, Santiwong B, Wasanapiarnpong T, Linsuwanont P. Evaluation of the chemical, physical, and biological properties of a newly developed bioceramic cement derived from cockle shells: An in vitro study. *BMC Oral Health.* 2023;23(1):354. doi:10.1186/s12903-023-03073-0
- Salem SA, Abbas AN, Aboelsoud MG, et al. Bioceramic putty versus calcium hydroxide for direct pulp capping in immature permanent molars: A 12-month randomized controlled trial. *J Stomatol.* 2025;78(2):100–107. doi:10.5114/jos.2025.151570
- Darmadi EY, Soesilo D. Antimicrobial activity of calcium hydroxide, calcium oxide, and mineral trioxide aggregate paste against α -hemolytic *Streptococcus*. *Conserv Dent J.* 2024;14(1):24–28. doi:10.20473/cdj.v14i1.2024.24-28
- Saraswati W, Juniarti DE, Lestari V, et al. Differences of compressive strength between calcium carbonate from blood clam shells and calcium hydroxide as a candidate for pulp capping material. *Conserv Dent J.* 2024;14(1):11–14. doi:10.20473/cdj.v14i1.2024.11-14
- Prasetyo EP, Juniarti DE, Sampoerno G, et al. The antibacterial efficacy of calcium hydroxide–iodophors and calcium hydroxide–barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis* in vitro. *Dent J.* 2022;55(2):62–66. doi:10.20473/j.djmk.v55.i2.p62-66
- Prasetyo EP, Widjiastuti I, Cahyani F, et al. Cytotoxicity of calcium hydroxide on human umbilical cord mesenchymal stem cells. *Pesqui Bras Odontopediatria Clin Integr.* 2020;20:e0044. doi:10.1590/pboci.2020.141
- Prasetyo EP, Kuntjoro M, Goenhartho S, et al. Calcium hydroxide increases human umbilical cord mesenchymal stem cells expressions of apoptotic protease-activating factor-1, caspase-3 and caspase-9. *Clin Cosmet Investig Dent.* 2021;13:59–65. doi:10.2147/CCIDE.S284240
- Mohamed HSE, Hegazy RH, Bashir MH, et al. Potential protective role of parsley on induced tongue carcinogenesis in albino rats. *Dent Med Probl.* 2025;62(1):79–87. doi:10.17219/dmp/161507
- Rady D, Abbass MM, Hakam H, Rady R, Aboushady I. Correlation between the expression of the *iNOS*, *caspase-3* and *α -SMA* genes in the parotid glands of albino rats following the administration of two antihistamines from two different generations. *Dent Med Probl.* 2024;61(3):335–343. doi:10.17219/dmp/157346
- Radwan IA, Rady D, Ramadan M, El Moshly 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
- Adi Santoso CM, Samadi K, Prasetyo EP, Wahjuningrum DA. Root canal cleanliness between mangosteen peel extract irrigant and NaOCl 2.5%. *Conserv Dent J.* 2020;10(1):40–43. doi:10.20473/cdj.v10i1.2020.40-43
- Ismiyatin K, Yuanita T, Saraswati W, et al. Mixing ratio of nano hydroxyapatite and epigallocatechin-3-gallate (EGCG) towards viscosity and antibacterial effect as a potential pulp capping material: An experimental study. *Saudi Dent J.* 2024;36(7):1006–1009. doi:10.1016/j.sdentj.2024.04.009
- Juniarti DE, Kusumaningsih T, Soetojo A, Prasetyo EP, Sunur YK. Antibacterial activity and phytochemical analysis of ethanolic purple leaf extract (*Graptophyllum pictum* (L.) Griff) on *Lactobacillus acidophilus*. *Malays J Med Health Sci.* 2021;17:71–73.
- Cahyani F, Saraswati W, Prasetyo EP, et al. The effect of α -mangostin on TLR-4 and PGE2 in the dentin–pulp complex exposed to HEMA. *J Int Dent Med Res.* 2022;15(1):21–26.
- Yuanita T, Juniarti DE, Marbun T. Inhibitory effect of nano *Stolephorus insularis* and calcium hydroxide on glucosyltransferase (GTF) activity of *Lactobacillus aciophilus*. *Conserv Dent J.* 2023;13(1):29–32. doi:10.20473/cdj.v13i1.2023.29-32
- Yuanita T, Sukaton, Moza Paramitha SM, Putri Ulfadi BT, Salma AF, Setyabudi. Inhibition of *Streptococcus mutans* biofilm formation using combination of nano brown anchovy (*Stolephorus insularis*) and calcium hydroxide. *Conserv Dent J.* 2023;13(2):69–73. doi:10.20473/cdj.v13i2.2023.69-73
- Prahasti AE, Yuanita T. Utilization of anchovy in dentistry. *Malays J Med Health Sci.* 2021;17(Suppl 6):87–90. https://medic.upm.edu.my/upload/dokumen/2021092200470214_2021_0339.pdf. Accessed March 24, 2025.
- Achmad H, Utamy TD, Inayah Y. The effectiveness of anchovy as antibacterial agent for children oral health. *J Dent Oral Sci.* 2022;4(2):1–11. doi:10.37191/Mapsci-2582-3736-4(2)-124
- Prasetyo EP, Sampoerno G, Juniarti DE, et al. Effect of lipopolysaccharide-induced apical periodontitis in diabetes mellitus rats on periapical inflammation. *Eur J Dent.* 2023;17(4):1146–1152. doi:10.1055/s-0042-1758790

27. Prahasati AE, Yuanita T, Rahayu RP. Nanoscale *Stolephorus* sp. powder fabrication using high-energy milling for bioactive materials in dentistry. *Dent Med Probl.* 2024;61(4):585–592. doi:10.17219/dmp/163634
28. Hariani PL, Muryati M, Said M, Salni S. Synthesis of nano-hydroxyapatite from snakehead (*Channa striata*) fish bone and its antibacterial properties. *Key Eng Mater.* 2020;840:293–299. doi:10.4028/www.scientific.net/KEM.840.293
29. Pribadi N, Budiarti D, Kurniawan HJ, Widjiastuti I. The NF- κ B and collagen type 1 expression in dental pulp after treated calcium hydroxide combined with propolis. *Eur J Dent.* 2021;15(1):122–126. doi:10.1055/s-0040-1716319
30. Ali H, Shahriar M, Gaffer MA, et al. Induction of reparative dentin following pulpotomy with Biodentine™ at four week follow up visit: A case report. *Int J Adv Multi Res Studies.* 2024;4(1):973–975. doi:10.62225/2583049X.2024.4.1.2321
31. Hendrijantini N, Kusumaningsih T, Rostiny R, Mulawardhana P, Danudiningrat CP, Rantam FA. A potential therapy of human umbilical cord mesenchymal stem cells for bone regeneration on osteoporotic mandibular bone. *Eur J Dent.* 2018;12(3):358–362. doi:10.4103/ej. ejd_342_17
32. Nugraha AP, Ihsan IS, Dinariyanti A, et al. Cobalt (II) chloride in enhancing hypoxia inducible factor-1 α expression of gingival derived mesenchymal stem cells in vitro. *Res J Pharm Tech.* 2021;14(5):2639–2642. doi:10.52711/0974-360X.2021.00465
33. Kuntjoro M, Hendrijantini N, Prasetyo EP, et al. Cobalt chloride as a hypoxia mimicking agent induced HIF-1 α and mTOR expressions of human umbilical cord mesenchymal stem cells. *Pesqui Bras Odontopediatria Clin Integr.* 2024;24:e220128. doi:10.1590/pboci.2024.020
34. Nugraha AP, Prasetyo EP, Kuntjoro M, et al. The effect of cobalt (II) chloride in the viability percentage and the induced hypoxia inducible factor-1 α of human adipose mesenchymal stem cells (HAMSCs): An in vitro study. *Sys Rev Pharm.* 2020;11(6):308–314. doi:10.31838/srp.2020.6.49
35. Ariestania V, Hendrijantini N, Prahasanti C, et al. Cytotoxicity of HA-TCP scaffold on human umbilical cord mesenchymal stem cells using MTT assay. *Int J Integ Eng.* 2022;14(2):80–85. doi:10.30880/ijie.2022.14.012
36. Akgun OM, Tekinay AB, Tansik G, Yildirim C, Polat GG. Pulp regeneration using a peptide nanofiber artificial scaffold on animal models: A preliminary study. *Adv Clin Exp Med.* 2025;34(3):407–419. doi:10.17219/acem/189639
37. Amriel MF, Prasetyo EP, Fepiosandi RA, et al. Human dental pulp mesenchymal stem cells regenerative potential. *Conserv Dent J.* 2025;15(1):27–32. doi:10.20473/cdj.v15i1.2025.27-32
38. Darmadi EY, Fauziah YA. The synergistic role of the immune system and stem cells in dental tissue regeneration. *Conserv Dent J.* 2025;15(1):18–22. doi:10.20473/cdj.v15i1.2025.18-22
39. Prasetyo EP, Wahjuningrum DA, Samporno G, et al. A review of tissue engineering in regenerative endodontic treatment. *Conserv Dent J.* 2024;14(1):1–5. doi:10.20473/cdj.v14i1.2024.1-5
40. Kuntjoro M, Agustono B, Prasetyo EP, Salim S, Rantam FA, Hendrijantini N. The effect of advanced glycation end products (AGEs) on human umbilical cord mesenchymal stem cells (hUCMSCs) with regard to osteogenesis and calcification. *Res J Pharm Tech.* 2021;14(8):4019–4024. doi:10.52711/0974-360X.2021.00696
41. Prasetyo EP, Juniarti DE, Kuntjoro M, et al. Mesenchymal stem cells from human umbilical cord decrease inflammation and increase vascularization of induced apical periodontitis model in diabetes mellitus rats. *J Appl Oral Sci.* 2024;32:e20240225. doi:10.1590/1678-7757-2024-0225
42. Kuntjoro M, Hendrijantini N, Prasetyo EP, et al. Human umbilical cord mesenchymal stem cells accelerate and increase implant osseointegration in diabetic rats. *J Appl Oral Sci.* 2023;31:e20220375. doi:10.1590/1678-7757-2022-0375
43. Hendrijantini N, Kuntjoro M, Agustono B, et al. Bone formation and mineralization around the implant in osteoporotic animal models enhanced by mesenchymal stem cells. *Dent J.* 2024;57(2):91–96. doi:10.20473/j.djmk.v57.i2.p91-96
44. Prasetyo EP, Saravanan P, Kharisna D, et al. Umbilical cord mesenchymal stem cells improve bone regeneration in diabetes mellitus animal model with apical periodontitis. *J Oral Biol Craniofac Res.* 2025;15(1):16–21. doi:10.1016/j.jobcr.2024.11.009
45. Kuntjoro M, Prasetyo EP, Cahyani F, et al. Lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells. *Pesqui Bras Odontopediatria Clin Integr.* 2020;20:e0048. doi:10.1590/pboci.2020.153
46. Kuntjoro M, Hendrijantini N, Prasetyo EP, Agustono B, Hong G. Hypoxia-preconditioned human umbilical cord mesenchymal stem cells transplantation ameliorates inflammation and bone regeneration in peri-implantitis rat model. *Eur J Dent.* 2025;19(2):420–427. doi:10.1055/s-0044-1791530
47. Kahler B, Taha NA, Lu J, Saoud TM. Vital pulp therapy for permanent teeth with diagnosis of irreversible pulpitis: Biological basis and outcome. *Aust Dent J.* 2023;68(Suppl 1):S110–S122. doi:10.1111/adj.12997
48. Chandra R, Tripathi S, Jain J, Mehrotra A, De R, Dixit M. Vital pulp therapy. *J Adv Med Dent Sci Res.* 2023;11(4):62–81.
49. Abdelaz P, ElZoghbi A, Shokry M, Ahmed AZ, Rasha H. Reparative dentin formation using stem cell therapy versus calcium hydroxide in direct pulp capping: An animal study. *Braz Dent J.* 2019;30(6):542–549. doi:10.1590/0103-6440201902711
50. Jin P, Liu L, Cheng L, Chen X, Xi S, Jiang T. Calcium-to-phosphorus releasing ratio affects osteoinductivity and osteoconductivity of calcium phosphate bioceramics in bone tissue engineering. *Biomed Eng Online.* 2023;22(1):12. doi:10.1186/s12938-023-01067-1
51. Sutthavas P, Habibovic P, Van Rijt SH. The shape-effect of calcium phosphate nanoparticle based films on their osteogenic properties. *Biomater Sci.* 2021;9(5):1754–1766. doi:10.1039/d0bm01494j
52. Christie B, Musri N, Djustiana N, et al. Advances and challenges in regenerative dentistry: A systematic review of calcium phosphate and silicate-based materials on human dental pulp stem cells. *Mater Today Bio.* 2023;23:100815. doi:10.1016/j.mtbio.2023.100815

Dose-dependent effects of gamma-ray irradiation on SLA-treated titanium Grade 4: An in vitro evaluation of its physical, chemical and surface properties

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Conflict of interest

None declared

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Abstract

Background. Gamma-ray sterilization is commonly used for dental implants, but may alter their physical, chemical and surface properties.

Objectives. The present study compared gamma-ray irradiation doses of 15 kGy and 25 kGy in terms of their effects on the physical (microhardness), chemical (titanium (Ti) ion release) and surface (morphology and hydrophilicity) properties of sand-blasted, large-grit, acid-etched (SLA) Ti Grade 4 (G4) implants.

Material and methods. A total of 48 cylindrical Ti G4 samples (4 mm in diameter, 8 mm in thickness) were irradiated using cobalt-60 (Co-60) gamma radiation at 0 kGy (non-irradiated), 15 kGy or 25 kGy doses. Post-irradiation analyses included testing Vickers hardness (HV), Ti ion release in simulated body fluid (SBF) after 2 weeks, the water contact angle (θ), and scanning electron microscopy (SEM) for morphology assessment. Statistical significance was set at $\alpha = 0.05$.

Results. Gamma-ray irradiation significantly impacted all measured properties. The mean hardness decreased from 536.5 HV (non-irradiated) to 251.3 HV (15 kGy) and 203.8 HV (25 kGy) ($p < 0.001$); no significant difference was observed between 15 kGy and 25 kGy. Titanium ion release increased with a radiation dose: 44.68 $\mu\text{g/L}$ (non-irradiated); 93.75 $\mu\text{g/L}$ (15 kGy; $p = 0.0292$ vs. control); and 218.98 $\mu\text{g/L}$ (25 kGy; $p < 0.001$ vs. control and 15 kGy). The water contact angles approached 0° post-irradiation, indicating a shift to superhydrophilicity, significantly different from the moderately hydrophilic control ($p = 0.0085$), with no difference between the radiation doses ($p = 0.1266$). The SEM analysis revealed more pronounced micro-damage and roughness at 25 kGy.

Conclusions. Both 15 kGy and 25 kGy significantly altered surface properties, but 25 kGy induced greater Ti ion release and micro-damage. Within the study limitations, 15 kGy is recommended as the preferred sterilization dose, as it maintains sterility while minimizing mechanical degradation and excessive Ti ion release as compared to 25 kGy.

Keywords: gamma radiation, sterilization, titanium implant

Highlights

- This study demonstrates that gamma-ray irradiation alters the morphology and hydrophilicity, as well as physical (microhardness) and chemical (titanium (Ti) ion release) properties of SLA (sand-blasted, large-grit, acid-etched)-treated Ti Grade 4 (G4) implants.
- A 15 kGy dose optimally sterilizes Ti while maintaining mechanical integrity and minimizing Ti ion release.
- A 25 kGy dose weakens Ti hardness and increases Ti ion release beyond safe limits, affecting long-term implant stability.
- Gamma-ray irradiation enhances surface hydrophilicity, which may improve osseointegration and bone healing in dental implant applications.
- The findings support the safe and effective use of gamma-ray sterilization for Ti implants in clinical and industrial settings.

Introduction

Dental implant fixtures must be completely sterile before clinical use to prevent peri-implant infections that jeopardize osseointegration. Biomaterial guidelines emphasize that sterilization methods should eliminate pathogens without degrading the properties of the implant. Various sterilization techniques include steam autoclaving, chemical disinfectants and radiation (gamma or electron-beam).¹ Among these, gamma-ray irradiation is widely used due to its deep penetration, decisive microbial lethality, and ability to sterilize without thermal damage or chemical residues.^{2–4} Using high-energy photons (usually from a cobalt-60 (Co-60) source), gamma-ray irradiation effectively inactivates bacteria, viruses and spores by inducing irreparable DNA damage, ensuring sterility without leaving toxic byproducts.^{1,2} Gamma rays can sterilize devices in their final packaging at ambient temperature, making them suitable for mass production.⁵ These advantages have led to gamma-ray sterilization becoming an FDA (Food and Drug Administration)- and EMA (European Medicines Agency)-approved standard method in the dental implant industry.⁶

Standard gamma sterilization protocols often employ doses around 25 kGy, as it meets the requirements regarding the sterility assurance level for medical devices.⁷ However, ensuring that such radiation does not compromise the physical and chemical integrity of titanium (Ti) implants during the terminal sterilization process is critical.⁷ Studies have shown that exposure to gamma-ray irradiation alters the physical (microhardness), chemical (Ti ion release) and surface (morphology and hydrophilicity) properties of Ti implants.² Surface roughness and hydrophilicity play a crucial role in osseointegration.^{8,9} Implants with moderate roughness (*Sa* 1–2 μm) and high hydrophilicity exhibit enhanced osseointegration.¹ Ueno et al. demonstrated that gamma-ray irradiation enhanced the hydrophilicity of Ti surfaces, reducing the water contact angle (θ) to approx. 10°, which might improve osseointegration.²

Similarly, El-Bediwi et al. reported that gamma-ray exposure increased surface roughness and microstructural changes in Ti, affecting its mechanical integrity.¹⁰ Research has shown that gamma-ray irradiation can disrupt the titanium oxide (TiO_2) layer, potentially increasing Ti ion release and raising concerns about cytotoxic effects.^{1,11} Zhou et al. further report that mechanical wear during implantation and prolonged chemical corrosion may also compromise this oxide layer.¹¹ Despite these findings, the issue of Ti ion release and its clinical implications remain relatively underexplored, particularly in relation to peri-implant conditions and long-term biocompatibility.^{12,13}

Despite the widespread use of 25 kGy in sterilization protocols, emerging evidence suggests that lower doses, such as 15 kGy, might be sufficient to achieve sterility while minimizing adverse effects on Ti implants.^{11,14–16} Preliminary bioburden testing, which quantifies microbial contamination before sterilization, has shown that a dose of 15 kGy can be substantiated under the International Organization for Standardization (ISO) 11137-2 V_{Dmax} 15 protocols as sufficient to achieve microbial reduction to acceptable levels, provided the natural bioburden is very low.¹⁷ Moreover, the ISO standards for medical device sterilization recommend dose adjustments based on bioburden assessments, rather than a fixed-dose approach.² Preliminary research on the prototype Ti implant sterilization using gamma rays identified a bioburden threshold at a 15 kGy dose.⁶ However, studies that have directly compared the effects of 15 kGy vs. 25 kGy on Ti implant properties are limited.

Therefore, this study aims to analyze the effects of gamma-ray irradiation at 15 kGy and 25 kGy on the mechanical properties (microhardness), surface characteristics (morphology and hydrophilicity) and Ti ion release of SLA (sand-blasted, large-grit, acid-etched)-treated Ti implants. Understanding the impact of different doses on implant integrity is crucial for optimizing sterilization protocols while preserving implant performance.

Material and methods

This quasi-experimental study, conducted from April to July 2023, aimed to investigate the effects of gamma-ray irradiation on cylindrical SLA-treated Ti surfaces. The research sample consisted of 48 cylindrical, non-threaded Ti implants (4 mm in diameter, 8 mm in thickness) made from pure Grade 4 (G4) Ti, manufactured by Pudak Scientific (Bandung, Indonesia). Titanium samples were prepared using a dual microtopography surface treatment called sand-blasted, large-grit, acid-etched (SLA). First, the samples were sand-blasted with alumina particles (200–300 μm). Next, they were immersed in 48% hydrofluoric acid at room temperature for 30–60 s. Subsequently, the samples were immersed in a mixture of 37% hydrochloric acid and 97% sulfuric acid in a 2:1 ratio at temperatures ranging from 80°C to 100°C for 5–6 min, which created a secondary micro-scale topography. Finally, the samples were rinsed thoroughly with distilled water, ultrasonically cleaned, and air-dried before gamma-ray irradiation.

A total of 48 cylindrical SLA-treated Ti samples were used in this study. The samples were divided into 3 irradiation groups depending on the dose used (0 kGy, 15 kGy and 25 kGy), with 16 samples per group. Within each group, the samples were allocated to 4 assessment categories: physical properties (Vickers hardness); surface morphology (scanning electron microscopy (SEM)); hydrophilicity (the contact angle); and Ti ion release (graphite furnace atomic absorption spectrometry (GFAAS)). Each assessment was performed on the samples from all 3 irradiation groups to enable consistent comparisons across doses. The Ti surface was the independent variable, while the dependent variables included physical characteristics, ion release, morphology, and hydrophilicity. Gamma-ray dose levels (0 kGy, 15 kGy and 25 kGy) were controlled to maintain uniformity.

Gamma-ray irradiation was performed using a Co-60 radioisotope device (Gammacell 220; Nordion Inc., Ottawa, Canada), which emits electromagnetic radiation onto Ti samples packed in polyethylene (PE) plastic. The samples were exposed to irradiation doses of 15 kGy and 25 kGy. The physical analysis was conducted at the Bandung Institute of Technology (ITB), the morphological and ion release analyses at the Indonesian Institute of Sciences (LIPI), and hydrophilicity testing at the Faculty of Dentistry, Universitas Padjadjaran, Indonesia.

Assessment methods

Physical properties

Hardness was measured with a Vickers hardness tester (HV-10; Mitutoyo, Kawasaki, Japan), which uses a diamond pyramid-shaped indenter (136°) that is pressed into the surface of the test material (Ti) under a controlled load. The test was conducted on the irradiated (15 kGy

and 25 kGy) and non-irradiated samples. Hardness was expressed in Vickers hardness units (HV).

Morphology

Surface morphology was observed using a scanning electron microscope (SEM) (JSM-IT300; JEOL USA Inc., Peabody, USA), with a resolution of 0.1–0.2 nm. Observations were conducted at an acceleration voltage of 10 kV with a working distance of 10 mm, using the secondary electron imaging (SEI) mode. The samples were analyzed at magnifications of $\times 150$, $\times 500$, $\times 1,000$, $\times 2,500$, $\times 5,000$, and $\times 10,000$.

Hydrophilicity

The contact angle (θ) of a 5-microliter water droplet on the Ti surface (non-irradiated, and irradiated at 15 kGy and 25 kGy) was measured using the ImageJ contact angle measurement device (<https://imagej.net/ij>). The contact angle was calculated using the convexity formula $\theta = 2\alpha$. The water contact angle classification was as follows: superhydrophilic ($\theta \approx 0^\circ$); hydrophilic ($0^\circ < \theta < 90^\circ$); hydrophobic ($90^\circ < \theta < 120^\circ$); ultrahydrophobic ($120^\circ < \theta < 150^\circ$); and superhydrophobic ($\theta > 150^\circ$).

Chemical properties

Titanium ion release was measured after 2 weeks of immersion in simulated body fluid (SBF), using GFAAS (AAAnalyst 800; PerkinElmer, Waltham, USA), with results expressed in $\mu\text{g/L}$.

Materials and equipment

The materials used in this study include cylindrical Ti G4 samples and the SBF solution. The equipment comprised six 250-milliliter glass jars, 3 dropper pipettes, tweezers, label paper, gloves, masks, a Vickers hardness tester, a SEM, a contact angle measurement system (Image J), a GFAAS device, and an incubator.

Procedure and data collection

Titanium sample grouping

The Ti samples were divided into 3 groups: group 1 served as the control group (non-irradiated samples); group 2 included samples irradiated at a 15 kGy gamma-ray dose; and group 3 consisted of samples exposed to a 25 kGy gamma-ray dose.

Gamma-ray irradiation

The Ti samples underwent gamma-ray irradiation at doses of 15 kGy and 25 kGy, while the control group

remained non-irradiated at 0 kGy, which ensured a comparative analysis of the effects of gamma-ray exposure on the Ti surface.

Vickers hardness tests

The gamma-irradiated (15 kGy, 25 kGy) and non-irradiated samples were mounted onto specimen holders. A Vickers hardness tester was then used – a 50-gram load was applied to the central top surface of the Ti cylinders for 10 s to measure their hardness.

Morphology analysis using SEM

The Ti samples from all groups were affixed to specimen holders for the surface morphology analysis. The surface structure was examined using a SEM at $\times 150$, $\times 500$, $\times 1,000$, $\times 2,500$, $\times 5,000$, and $\times 10,000$ magnifications, enabling a detailed observation of morphological changes.

Hydrophilicity testing

The water contact angle measurements for the 0 kGy, 15 kGy and 25 kGy samples were categorized as superhydrophilic at 0° , hydrophilic for angles between 0° and 90° , hydrophobic within the 90 – 120° range, ultra-hydrophobic between 120° and 150° , and superhydrophobic when exceeding 150° . A scoring system was used to quantify these properties, with a score of 1 assigned for superhydrophilic (0°), 2 for hydrophilic ($<90^\circ$), 3 for hydrophobic (90 – 120°), 4 for ultrahydrophobic (120 – 150°), and 5 for superhydrophobic ($>150^\circ$). A 5-microliter water droplet was applied to the surface of each Ti sample using a micropipette. The contact angle between the droplet and the Ti surface was measured using a contact angle measurement device to determine the hydrophilicity properties of each sample.

Ion release testing

The Ti samples from all groups were immersed in SBF inside small plastic containers. The containers were incubated at 37°C for 2 weeks, with the SBF solution replaced every 2 days to simulate in vivo conditions. After 2 weeks of immersion, the collected SBF solution was analyzed for Ti ion release, using GFAAS.

Data analysis design

Data analysis involved a mixed-method approach, integrating quantitative and qualitative methods. Quantitative data was analyzed using the analysis of variance (ANOVA) and post-hoc pairwise tests for hardness and ion release, while the Kruskal–Wallis and Wilcoxon–Mann–Whitney tests were applied for hydrophilicity (Table 1). Statistical test selection was based explicitly on data distribution and variance characteristics, assessed via the Shapiro–Wilk test for normality and Levene's test for the homogeneity of variance. The significance level was set at $p < 0.05$. Effect sizes (e.g., partial eta-squared (η_p^2) for ANOVA, Cohen's d) were also calculated to interpret the magnitude of differences between the groups. Qualitative analysis involved the descriptive evaluation of the morphological characteristics observed through SEM imaging, where surface features such as striations, micro-cracks and roughness variations were compared across the experimental groups.

The sample size was determined using Federer's formula, chosen for its practical suitability in exploratory laboratory conditions with limited resources and unknown prior variance estimates. Multiple identical samples per group provided internal replication, ensuring robust statistical comparisons. To minimize confounding variables, environmental conditions, sample purity and preparation consistency were strictly controlled, including uniform temperature and humidity monitoring, consistent batch sourcing of Ti G4, and rigorous standardization of SLA treatment.

Results

Vickers hardness tests

The mean Vickers hardness values decreased substantially with gamma-ray irradiation. The non-irradiated control group showed the highest hardness (536.5 ± 5.3 HV), whereas the values for the 15 kGy and 25 kGy groups were markedly lower at 251.3 ± 8.1 HV and 203.8 ± 63.2 HV, respectively. The one-way ANOVA confirmed a highly significant overall difference among the 3 groups ($p < 0.0001$; $\eta_p^2 \approx 0.95$). Post-hoc comparisons indicated that both 15 kGy and 25 kGy irradiation led to significantly reduced hardness as compared to the control ($p < 0.001$ for each dose). For example, the drop in hardness at 25 kGy

Table 1. Quantitative data statistical analysis

Variable tested	Normality test	Variance homogeneity	Difference test	Post-hoc test
Vickers hardness	Shapiro–Wilk test	Levene's test	one-way ANOVA	Tukey's HSD test
Ion release	Shapiro–Wilk test	Levene's test	one-way ANOVA	Tukey's HSD test
Hydrophilicity	Shapiro–Wilk test	–	Kruskal–Wallis test	Wilcoxon–Mann–Whitney test

HSD – honest significant difference.

corresponded to a very large effect size (Cohen's $d \approx 7$). There was no statistically significant difference between the 15 kGy and 25 kGy groups ($p > 0.05$), suggesting that most mechanical degradation occurs by 15 kGy (Table 2).

Morphological analysis using SEM

Scanning electron microscopy revealed dose-dependent surface damage on the SLA-treated Ti (Fig. 1–3).

Table 2. Vickers hardness values for the study groups and post-hoc test results

Group	Vickers hardness [HV]	<i>p</i> -value (post-hoc test)		
		0 kGy	15 kGy	25 kGy
0 kGy	536.5 ± 5.3	–	0.0000*	0.0000*
15 kGy	251.3 ± 8.1	0.0000*	–	0.1023
25 kGy	203.8 ± 63.2	0.0000*	0.1023	–

Data presented as mean ± standard deviation ($M \pm SD$).

* statistically significant.

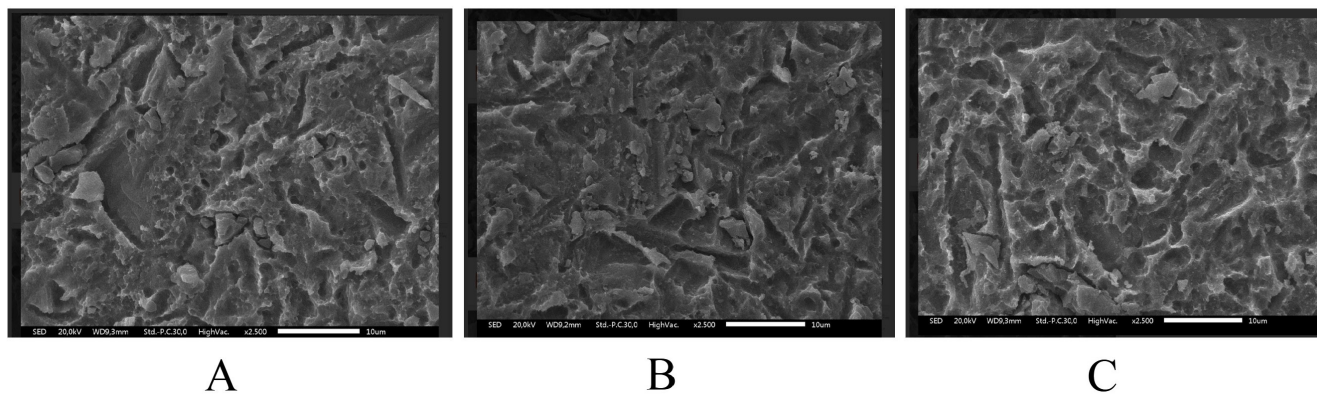


Fig. 1. Scanning electron microscopy (SEM) images at x2,500 magnification
A – 0 kGy; B – 15 kGy gamma radiation; C – 25 kGy gamma radiation.

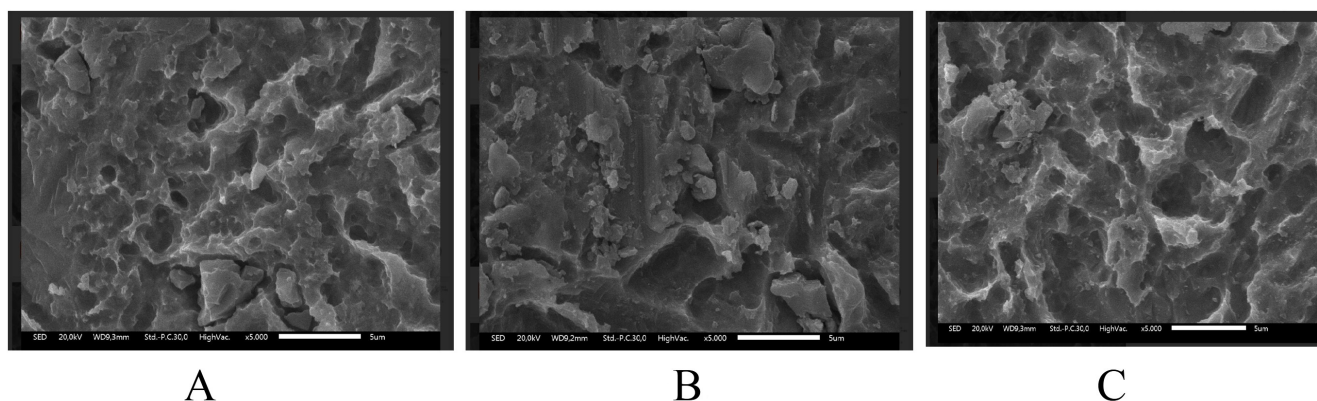


Fig. 2. Scanning electron microscopy (SEM) images at x5,000 magnification
A – 0 kGy; B – 15 kGy gamma radiation; C – 25 kGy gamma radiation.

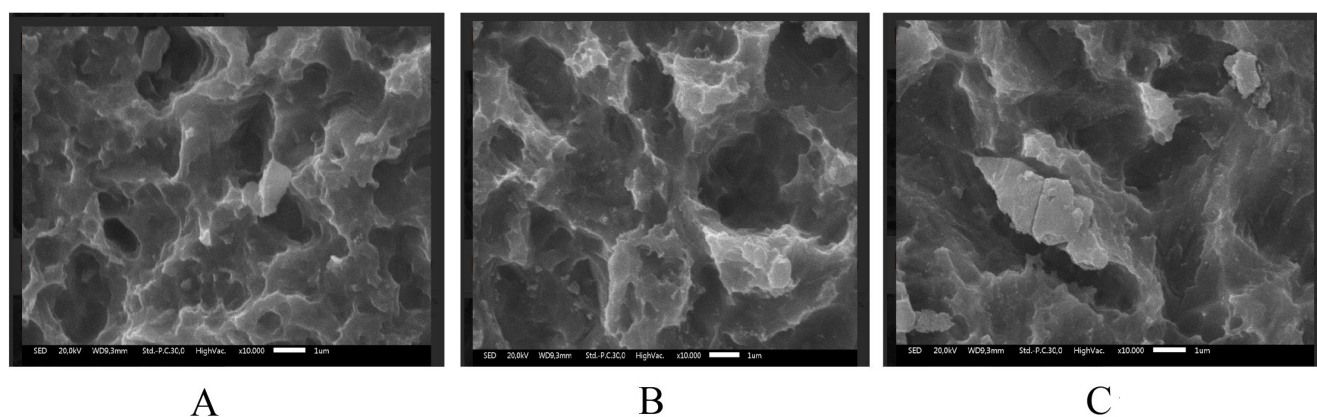


Fig. 3. Scanning electron microscopy (SEM) images at x10,000 magnification
A – 0 kGy; B – 15 kGy gamma radiation; C – 25 kGy gamma radiation.

The non-irradiated surfaces showed rough micro-texture, characteristic of SLA treatment, while higher radiation doses produced more pronounced microstructural alterations. The 25 kGy samples exhibited deeper striations, pits and micro-cracks than the 15 kGy and control samples. Although quantitative roughness measurements were not performed, SEM imaging demonstrated increased micro-damage and structural irregularities correlating with a radiation dose.

Hydrophilicity testing

Both irradiated groups showed a shift toward superhydrophilicity, with near-zero water contact angles, whereas the control surface remained moderately hydrophilic. The overall Kruskal–Wallis test result shown in Table 3 indicates a statistically significant difference in hydrophilicity among the 3 groups ($p = 0.0085$). Pairwise comparisons revealed that even the lower dose of 15 kGy significantly improved surface wettability as compared to the control group ($p = 0.0082$), supporting the meaningful effect of gamma-ray irradiation at this dose. However, no statistically significant difference was observed between the 15 kGy and 25 kGy groups ($p = 0.1266$), suggesting a saturation effect where hydrophilicity reaches its maximum potential beyond 15 kGy.

Table 3. Hydrophilicity values for the study groups and Kruskal–Wallis test results

Group	Hydrophilicity (values acc. to the scoring system applied)	<i>p</i> -value (Kruskal–Wallis test)
0 kGy	3.00 ± 10.50	0.0085*
15 kGy	2.00 ± 5.50	–
25 kGy	1.50 ± 3.50	–

Data presented as median ± average rank (*Me* ± *Avg. Rank*).

* statistically significant.

Ion release testing

The ion release analysis demonstrated a clear dose-dependent response. The one-way ANOVA revealed a highly significant overall difference in Ti ion release among the 3 groups ($p < 0.0001$) (Table 4). Post-hoc pairwise comparisons showed significantly higher Ti ion release in the 15 kGy group ($93.75 \pm 16.98 \mu\text{g/L}$) than in the

Table 4. Ion release values for the study groups and one-way ANOVA results

Group	Ti ion release [$\mu\text{g/L}$]	<i>p</i> -value (one-way ANOVA)
0 kGy	44.68 ± 2.48	<0.0001*
15 kGy	93.75 ± 16.98	–
25 kGy	218.98 ± 43.11	–

Data presented as $M \pm SD$.

Ti – titanium; * statistically significant.

control group ($44.68 \pm 2.48 \mu\text{g/L}$) ($p = 0.0292$). Furthermore, the 25 kGy group exhibited significantly higher Ti ion release ($218.98 \pm 43.11 \mu\text{g/L}$) than both the control and 15 kGy groups ($p < 0.001$ for both comparisons), indicating a dose-dependent increase in ion release.

Discussion

Gamma-ray exposure significantly reduced implant hardness, with the highest hardness observed in the non-irradiated control group ($536.5 \pm 5.3 \text{ HV}$), and progressively lower hardness values observed at doses of 15 kGy ($251.3 \pm 8.1 \text{ HV}$) and 25 kGy ($203.8 \pm 63.2 \text{ HV}$). Titanium G4 dental implants typically require a surface hardness of at least 280 HV to withstand masticatory loads. Although gamma-ray irradiation reduced the measured surface hardness to $251.3 \pm 8.1 \text{ HV}$ at 15 kGy, it is important to note that the depth of hardness deterioration was not assessed in this study. Therefore, while surface weakening is evident, it remains unclear whether the bulk mechanical integrity necessary for masticatory loading is significantly compromised. Further depth-resolved hardness analyses are warranted to confirm the clinical implications. Excessive occlusal stress beyond the tolerance of the bone and the implant can cause micro-fractures, reduce bone density at the implant neck and lead to crater-like defects. Studies show that irradiation at 10 kGy and 20 kGy alters Ti microstructure, affecting the crystal size and atomic bonds. High-energy gamma rays induce surface defects, atomic loss and movement, ultimately leading to Ti weakening.^{2,18} Excessive gamma-ray irradiation at 25 kGy caused significant surface destruction, as shown in the SEM analysis (Fig. 2). The irradiated samples displayed irregular, rough surfaces, with the most severe damage in the 25 kGy group. Similar studies have shown that higher irradiation doses, such as 30 kGy, create deeper grooves and increase roughness. Optimal implant surface roughness (1–100 μm) enhances cellular activity, osseointegration and bone apposition by facilitating fibrin protein adhesion and osteogenic cell attachment, and improving implant stability within the host bone.^{19,20}

In this research, all samples were surface-treated using the SLA technique, which made the sample's surface topography rough or irregular. Gamma-ray irradiation further altered surface characteristics by inducing disruptions in atomic bonding within the TiO_2 layer, thereby increasing surface roughness while simultaneously inducing Ti weakness. The SEM morphology results correlate with the physical properties of gamma-irradiated Ti. However, this study did not include quantitative surface roughness measurements; therefore, exact roughness values could not be determined.^{21–23}

Gamma rays, in addition to their excellent sterilization effects, can also enhance the osseointegration capability of implants. The hydrophilicity of the Ti surface can

impact various biological aspects, including facilitating the adhesion of macromolecular proteins to the Ti surface, influencing cell interactions in both hard and soft tissues, and affecting bacterial adhesion and biofilm formation.^{24,25} This pronounced shift toward complete wetting reflects radiation-induced alterations in the surface chemistry, such as removing hydrophobic contaminants and creating hydroxyl groups on the oxide layer. These changes enhance the ability of the surface to attract and bind water molecules, contributing to improved protein adsorption and subsequent cell attachment, which are crucial for successful osseointegration.

After the deposition of blood proteins on a hydrophilic implant surface, successful integration between the implant and the bone requires precursor osteoblast cells, followed by differentiation, extracellular matrix synthesis and soft tissue growth. A hydrophilic surface facilitates the invasion of mesenchymal stem cells (MSCs), promoting osteoblast maturation at the implantation site. Alongside hard tissue formation, the growth of soft tissue, such as the connective tissue layer beneath stratified squamous epithelial cells, is essential for covering the implantation site, and protecting against infection and peri-implantitis.^{26,27} Gittens et al. reported that keratinocyte proliferation on superhydrophilic surfaces improved epithelial closure.²⁸

Another biological effect of surface hydrophilicity is its influence on bacterial colonization. Hydrophilic bacterial strains tend to adhere more easily to other hydrophilic surfaces; the same applies to hydrophobic bacteria. Bacteria on hydrophilic surfaces can contribute to plaque formation, a key factor in peri-implantitis. Therefore, surface treatment that inhibits biofilm maturation, such as polyethylene coating, may be beneficial.^{28,29}

The findings of this study demonstrate that increased gamma-ray exposure resulted in a surface property shift from hydrophobic to hydrophilic, particularly in the 15 kGy and 25 kGy groups. When metals like Ti are irradiated with gamma rays, their surface properties change to hydrophilic due to a phenomenon known as radiation-induced surface activation (RISA).^{30,31} The working principle of RISA involves excitation and altered spacing between oxide particles, leading to atomic separation within the Ti-O-Ti bonds. Consequently, oxygen atoms (O₂) attract water molecules from the air, further enhancing surface hydrophilicity.

Ueno et al. described another mechanism contributing to hydrophilicity enhancement – organic molecules on the Ti implant surface being effectively decomposed by gamma-ray irradiation.² This process results in a clean Ti surface, facilitating the binding of oxygen atoms from the surrounding air, further promoting the hydrophilic transformation of the implant surface.^{2,32,33} Gamma rays alter the hydrophilicity of the implant surface and disrupt the TiO₂ bonds, leading to ion release.³⁴ In the present study, ion release increased significantly with higher

gamma-ray doses, with the highest ion concentration observed in the 25 kGy group (218.98 ± 43.11 µg/L), followed by the 15 kGy group (93.75 ± 16.98 µg/L), and the lowest in the non-irradiated group (44.68 ± 2.48 µg/L). Notably, ion release in the 25 kGy group slightly exceeded the safe threshold for human exposure (214.28 µg/L). Excessive Ti ion release can provoke systemic responses, including inflammatory reactions mediated by interleukin (IL)-1β activation and macrophage recruitment, potentially leading to peri-implant bone resorption (peri-implantitis).

Elevated ionic dissolution might influence peri-implant tissues or raise biocompatibility concerns over time, although the absolute levels observed here are still relatively low. Notably, a 15 kGy dose approx. doubled ion release relative to the non-irradiated control. In contrast, a 25 kGy dose quintupled it, highlighting the disproportionate impact of higher gamma radiation doses on Ti dissolution. This emphasizes the importance of carefully selecting sterilization parameters to ensure long-term implant stability.^{35–37} Clinically, minimizing ion release is critical to preventing potential inflammatory or cytotoxic effects. Therefore, a gamma-ray sterilization dose of 15 kGy is recommended for Ti implants, as it effectively sterilizes while maintaining Ti ion release within safe human exposure limits.^{38,39} According to the ISO 11137 standard, the determination of gamma-ray sterilization doses (15 kGy or 25 kGy) should be based on bioburden assessment. This study found that 15 kGy and 25 kGy doses showed no significant differences, except in ion release properties. A 15 kGy dose was identified as optimal, effectively sterilizing the implant material without significantly compromising its hardness, morphology or hydrophilicity. However, this study was limited by the absence of quantitative surface roughness measurements, pointing to the need for further in vitro research on Ti bioactivity and osseointegration after gamma-ray irradiation.^{40,41}

Several limitations of this in vitro evaluation should be acknowledged. Aside from the lack of quantitative roughness data mentioned above, the study was conducted on smooth cylindrical specimens in a controlled laboratory setting, which might not capture all aspects of clinical implant performance. In vivo conditions (such as bone remodeling dynamics and long-term body fluid exposure) could modulate the observed effects. Additionally, our focus was on short-term outcomes (immediate post-irradiation properties and ion release at 2 weeks), so the long-term effects of gamma-ray irradiation on implant surfaces remain uncertain. Despite these limitations, the consistently large effect sizes and clear trends observed here strengthen confidence in our findings. The observed gamma-induced changes in hardness, hydrophilicity and ion release are not only statistically significant, but also of substantial magnitude, underlining their potential clinical relevance.

Within the limitations of this study, a 15 kGy gamma dose emerges as a promising sterilization level that

achieves sterility while limiting adverse alterations to implant properties. In comparison, although effective for sterilization, the standard 25 kGy dose significantly compromises mechanical hardness and dramatically increases ion release, which could negatively affect implant longevity and biocompatibility. These findings highlight the importance of optimizing gamma-ray sterilization parameters for dental implants. While achieving sterility remains paramount, preserving Ti mechanical strength, surface morphology and biocompatibility is essential for ensuring long-term clinical success. Adopting a 15 kGy dose offers a promising balance between microbial safety and material preservation, potentially improving implant longevity and patient outcomes.

Conclusions

This study shows that gamma-ray irradiation at 15 kGy and 25 kGy significantly influences SLA-treated Ti implants. The results indicate a reduction in surface hardness, alterations in surface morphology, the enhancement of hydrophilicity, and an increase in Ti ion release. Notably, a 25 kGy dose induces considerable surface micro-damage, leads to excessive ion release, exceeding the established safe limits, and results in substantial mechanical degradation, which may jeopardize long-term implant stability and biocompatibility. In contrast, a 15 kGy dose effectively achieves sterilization while mitigating adverse effects, maintaining surface hardness closer to clinically acceptable thresholds, and ensuring that ion release remains within safe parameters.

While this research is constrained by the absence of quantitative surface roughness measurements and focuses on short-term in vitro evaluations, the findings suggest that a sterilization dose of 15 kGy strikes an advantageous balance between achieving sterility and preserving critical implant surface and mechanical properties. Further studies incorporating quantitative roughness profiling, in vivo evaluations and long-term assessment are essential to validate these conclusions and inform clinical practice.

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

Language editing was assisted by ChatGPT (OpenAI). All content accuracy and scientific integrity were independently verified by the authors.

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References

1. Park JH, Olivares-Nevarrete R, Baier RE, et al. Effect of cleaning and sterilization on titanium implant surface properties and cellular response. *Acta Biomater.* 2012;8(5):1966–1975. doi:10.1016/j.actbio.2011.11.026
2. Ueno T, Takeuchi M, Hori N, et al. Gamma ray treatment enhances bioactivity and osseointegration capability of titanium. *J Biomed Mater Res B Appl Biomater.* 2012;100(8):2279–2287. doi:10.1002/jbm.b.32799
3. International Atomic Energy Agency (IAEA). *Trends in Radiation Sterilization of Health Care Products*. 2008. <https://www.iaea.org/publications/7691/trends-in-radiation-sterilization-of-health-care-products>. Accessed February 4, 2025.
4. Selcan Türker N, Yekta Özer A, Kutlu B. et al. The effect of gamma radiation sterilization on dental biomaterials. *Tissue Eng Regen Med.* 2014;11(5):341–349. doi:10.1007/s13770-014-0016-9
5. Govindaraj S, Muthuraman MS. Systematic review on sterilization methods of implants and medical devices. *Int J ChemTech Res.* 2015;8(2):897–911.
6. El Bediwi A, Kashita E, Salman SM. Effect of gamma radiation on creep behavior, hardness and microstructure of titanium dental material. *J Adv Phys.* 2017;13(8):5078–5084. doi:10.24297/jap.v13i8.6319
7. Guo T, Kocak Oztug NA, Han P, Ivanovski S, Gulati K. Influence of sterilization on the performance of anodized nanoporous titanium implants. *Mater Sci Eng C Mater Biol Appl.* 2021;130:112429. doi:10.1016/j.msec.2021.112429
8. Da Silva Remísio MJ, Borges T, Castro F, Gehrke SA, Fernandes JCH, Fernandes GVdO. Histologic osseointegration level comparing titanium and zirconia dental implants: Meta-analysis of preclinical studies. *Int J Oral Maxillofac Implants.* 2023;38(4):667–680. doi:10.11607/jomi.10142
9. Łukaszewska-Kuska M, Krawczyk P, Buchwald T, et al. Electrodeposited hydroxyapatite coating on titanium after ultrashort-pulsed laser processing for a novel surface of endosseous implants. *Dent Med Probl.* 2024;61(6):909–918. doi:10.17219/dmp/175612
10. El-Bediwi AB, Saad M, El-Fallal A. Modification of titanium surface by magnetic resonance signals and gamma radiation. *Mater Sci Indian J.* 2013;9(10):401–410. <https://www.tsijournals.com/articles/modification-of-titanium-surface-by-magnetic-resonance-signals-and-gamma-radiation.pdf>. Accessed February 4, 2025.
11. Zhou Z, Shi Q, Wang J, et al. The unfavorable role of titanium particles released from dental implants. *Nanotheranostics.* 2021;5(3):321–332. doi:10.7150/ntno.56401
12. 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 Probl.* 2024;61(5):637–640. doi:10.17219/dmp/187215
13. Fernandes GVdO, Fernandes JCH, Castilho RM. Epigenetic modifications as a biomarker for periodontitis and peri-implantitis: A review. *Oral Implantol.* 2025;17(1):5–8. doi:10.11138/oi.v17i1.111
14. Honjo Y, Furuya M, Takamasa T, Okamoto K. Mechanism of hydrophilicity by radiation-induced surface activation. *J Power Energy Syst.* 2009;3(1):216–227. doi:10.1299/jpes.3.216
15. Dohshi S, Anpo M, Okuda S, Kojima T. Effect of γ -ray irradiation on the wettability of TiO₂ single crystals. *Top Catal.* 2005;35:327–330. doi:10.1007/s11244-005-3841-1

16. Vernon JJ, Raif EM, Aw J, Attenborough E, Jha A, Do T. Dental implant surfaces and their interaction with the oral microbiome. *Dent Rev.* 2022;2(4):100060. doi:10.1016/j.dentre.2022.100060
17. Nguyen H, Morgan DA, Sly LI, Benkovich M, Cull S, Forwood MR. Validation of 15 kGy as a radiation sterilisation dose for bone allografts manufactured at the Queensland Bone Bank: Application of the VDmax 15 method. *Cell Tissue Bank.* 2008;9(2):139–147. doi:10.1007/s10561-008-9064-5
18. Printzell L, Reseland JE, Jeppesen Edin NF, Tiainen H, Ellingsen JE. The dose-dependent impact of γ -radiation reinforced with backscatter from titanium on primary human osteoblasts. *Biomater Investig Dent.* 2023;10(1):2209116. doi:10.1080/26415275.2023.2209116
19. Mohamed NA, Kiong TS, Ismail AF, Mat Teridi MA. Novel gamma-ray enhanced TiO₂ nanoparticles photoanode for efficient photoelectrochemical (PEC) water splitting. *Appl Surf Sci.* 2024;642(1):158602. doi:10.1016/j.apsusc.2023.158602
20. Bae EB, Yoo JH, Jeong SI, et al. Effect of titanium implants coated with radiation-crosslinked collagen on stability and osseointegration in rat tibia. *Materials (Basel).* 2018;11(12):2520. doi:10.3390/ma11122520
21. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: A systematic review. *Clin Oral Implants Res.* 2009;20(Suppl 4):172–184. doi:10.1111/j.1600-0501.2009.01775.x
22. Jemat A, Ghazali MJ, Razali M, Otsuka Y. Surface modifications and their effects on titanium dental implants. *Biomed Res Int.* 2015;2015:791725. doi:10.1155/2015/791725
23. Pabst A, Asran A, Lüers S, et al. Osseointegration of a new, ultrahydrophilic and nanostructured dental implant surface: A comparative in vivo study. *Biomedicines.* 2022;10(5):943. doi:10.3390/biomedicines10050943
24. Zhao G, Schwartz Z, Wieland M, et al. High surface energy enhances cell response to titanium substrate microstructure. *J Biomed Mater Res A.* 2005;74(1):49–58. doi:10.1002/jbm.a.30320
25. Lang NP, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res.* 2011;22(4):349–356. doi:10.1111/j.1600-0501.2011.02172.x
26. Choe H, Tatro JM, Hausman BS, et al. *Staphylococcus aureus* and *Acinetobacter baumannii* inhibit osseointegration of orthopedic implants. *Infect Immun.* 2022;90(3):e0066921. doi:10.1128/iai.00669-21
27. Da Silva IR, Da Silva Barreto AT, Seixas RS, et al. Novel strategy for surface modification of titanium implants towards the improvement of osseointegration property and antibiotic local delivery. *Materials (Basel).* 2023;16(7):2755. doi:10.3390/ma16072755
28. Gittens RA, Scheideler L, Rupp F, et al. A review on the wettability of dental implant surfaces II: Biological and clinical aspects. *Acta Biomater.* 2014;10(7):2907–2918. doi:10.1016/j.actbio.2014.03.032
29. Greenstein G, Cavallaro J. Failed dental implants: Diagnosis, removal and survival of reimplantations. *J Am Dent Assoc.* 2014;145(8):835–842. doi:10.14219/jada.2014.28
30. Sirin M. The effect of titanium (Ti) additive on radiation shielding efficiency of Al₂₅Zn alloy. *Prog Nucl Energy.* 2020;128:103470. doi:10.1016/j.pnucene.2020.103470
31. Oza U, Parikh H, Duseja S, Agrawal C. Dental implant biomaterials: A comprehensive review. *Int J Dent Res.* 2020;5(2):87–92. doi:10.31254/dentistry.2020.5212
32. Department of Agriculture, Australia. *Gamma irradiation as a treatment to address pathogens of animal biosecurity concern. Final policy review.* Canberra, Australia: Department of Agriculture; 2014. <https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/ba/memos/2014/gamma-irradiation-review.pdf>. Accessed January 28, 2025.
33. Chebwogen M, Nalyanya KA, Amollo TA, Githaiga BM. Effect of gamma radiation on microbial and bacterial load of hide at preservation stage. *Int J Phys.* 2023;11(6):291–293. doi:10.12691/ijp-11-6-3
34. Harrell CR, Djonov V, Fellabaum C, Volarevic V. Risks of using sterilization by gamma radiation: The other side of the coin. *Int J Med Sci.* 2018;15(3):274–279. doi:10.7150/ijms.22644
35. Cho WT, Kim SY, Jung SI, et al. Effects of gamma radiation-induced crosslinking of collagen type I coated dental titanium implants on osseointegration and bone regeneration. *Materials (Basel).* 2021;14(12):3268. doi:10.3390/ma14123268
36. Morena D, Leitão-Almeida B, Pereira M, et al. Comparative clinical behavior of zirconia versus titanium dental implants: A systematic review and meta-analysis of randomized controlled trials. *J Clin Med.* 2024;13(15):4488. doi:10.3390/jcm13154488
37. Biju D, Arumugam P, Kannan S, et al. Development, characterization, and biocompatibility and corrosion analyses of a silver-decorated graphene oxide and chitosan surface coating for titanium dental implants: A preliminary report. *Dent Med Probl.* 2024;61(4):627–632. doi:10.17219/dmp/187107
38. Yasunaga M, Kobayashi F, Hara Y, Yamazaki M, Ohno T, Ito A. Biological activity of terminally gamma-ray-sterilized titanium and hydroxyapatite coated with a growth factor–apatite composite layer. *Mater Today Commun.* 2020;24:101098. doi:10.1016/j.mtcomm.2020.101098
39. Korsch M, Marten SM, Stoll D, Prechtel C, Dötsch A. Microbiological findings in early and late implant loss: An observational clinical case-controlled study. *BMC Oral Health.* 2021;21(1):112. doi:10.1186/s12903-021-01439-w
40. IAEA code of practice for the radiation sterilisation of tissue allografts: Requirements for validation and routine control. In: Phillips GO, ed. *Allografts in Bone Healing: Biology and Clinical Applications: Volume 3. Clinical Applications of Bone Allografts and Substitutes: Biology and Clinical Applications.* International Atomic Energy Agency (IAEA): Vienna, Austria: 2005:1–55. doi:10.1142/9789812701114_0001
41. Bonsignore LA, Colbrunn RW, Tatro JM, et al. Surface contaminants inhibit osseointegration in a novel murine model. *Bone.* 2011;49(5):923–930. doi:10.1016/j.bone.2011.07.013

Influence of surface treatment and simulated aging on the shear bond strength of different luting agents to zirconia

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Abstract

Background. Zirconia, or stabilized zirconium oxide (ZrO₂), is a widely used dental ceramic for full-coverage crowns owing to its favorable biological and esthetic properties. Upon cooling after high-temperature sintering, zirconia undergoes a tetragonal-to-monoclinic phase transformation, which causes volume expansion and brittleness. Adhesive cementation (AC) is crucial for the clinical performance of zirconia restorations, enhancing their retention, marginal adaptation and fracture resistance, thereby contributing to their suitability for dental restorations.

Objectives. The aim of the present study was to evaluate how different kinds of surface treatment affect the shear bond strength (SBS) of zirconia, to analyze the influence of various luting agents on the SBS of zirconia, and to investigate the effect of simulated aging through water storage on the SBS of zirconia.

Material and methods. An experimental study design was used to assess the impact of 2 luting agents (RelyX™ Unicem and Panavia™ F 2.0), different surface treatment methods (STMs) and variable water-storage durations (1day (1D), 1 month (1M) and 6 months (6M)) on the SBS of zirconia.

Results. The study revealed that RelyX consistently showed lower mean SBS values than Panavia across the different water-storage durations. For instance, in group 1 at 1D, the mean SBS for RelyX was 17.12 ± 0.71 MPa as compared to 21.76 ± 2.81 MPa for Panavia. ANOVA indicated significant differences in SBS between RelyX and Panavia in each group (group 1: $F = 13.892$; $p < 0.000$). Post-hoc tests confirmed significant differences in the mean SBS across the different aging durations. Overall, the findings provide comprehensive statistical evidence regarding the performance of the 2 dental luting agents under different surface treatment and aging conditions.

Conclusions. RelyX consistently demonstrated lower mean SBS values than Panavia across the different water-storage durations, indicating differences in their bonding performance. Statistical analyses confirmed significant differences in SBS between the 2 luting agents.

Keywords: dental materials, zirconia, surface treatment, shear bond strength, luting agents

Highlights

- Surface treatment significantly affected shear bond strength (SBS), as acid etching, particularly with silane, generally produced stronger and more durable zirconia–resin bonds than air abrasion alone.
- Cement selection influenced bonding performance, with Panavia™ F 2.0 generally showing higher initial SBS than RelyX™ Unicem, although performance varied with surface treatment and aging.
- Aging significantly reduced SBS across most surface treatment groups and both cements, indicating decreased bond durability over time.
- Group 6 produced the most stable bonding, as the combination of silane, air abrasion and HF treatment resulted in the highest and most stable SBS throughout storage.
- Combined surface treatment modalities improved bond durability, suggesting that integrating chemical and micromechanical treatment is more effective than using either approach alone, although further clinical validation beyond this in vitro model is needed.

Introduction

Zirconia, or stabilized zirconium oxide (ZrO_2), is a high-strength dental ceramic widely recognized for its excellent fracture toughness and biocompatibility. Its tetragonal phase, stabilized by a small addition of yttria, enhances its mechanical properties and dimensional stability, making it an ideal material for dental prosthetics.^{1–3}

Although zirconia inherently possesses high fracture toughness, the manner in which the tooth is prepared remains crucial in influencing stress distribution within the crown–tooth complex. A well-performed tooth preparation reduces the risk of fracture and enhances the mechanical retention of the zirconia crown, particularly in posterior regions, where greater occlusal forces are exerted.⁴ Until recently, zirconia ceramic crowns were fabricated similarly to metal-ceramic crowns, with an external layer of sintered porcelain and an internal zirconia core. This combination was intended to provide the crown with improved esthetic appearance as well as enhanced mechanical strength.^{5–7}

Fractures of veneering porcelain pose significant challenges in fixed dental prostheses, primarily due to compatibility issues between the zirconia framework and the veneering material. In recent years, monolithic zirconia has emerged as an alternative to veneered zirconia restorations. This innovative approach involves the fabrication of full-contour zirconia restorations using a dental computer-aided design/computer-aided manufacturing (CAD/CAM) system, thereby eliminating the complications associated with the bonding of zirconia and veneering porcelain.

Despite this progress, it is important to recognize that monolithic zirconia may not provide the same esthetic outcomes as zirconia cores veneered with porcelain. This difference may be attributed to the relatively uniform color and limited optical properties of monolithic zirconia crowns, which can result in a less natural and esthetically pleasing appearance.⁸

The prosthetic fabrication of zirconia has evolved from traditional layering techniques to advanced CAD/CAM processes. These methods not only ensure precise

anatomical conformity and improved esthetic outcomes, but also significantly reduce the potential for manual errors.⁹ The present study specifically focuses on CAD/CAM-fabricated restorations, given their increasing prevalence in clinical applications and their relevance to the evaluation of surface treatment and bonding efficiency.

Cementation is crucial to the clinical effectiveness of ceramic restorations. Zirconia ceramics can benefit from adhesive cementation (AC), which may improve retention, marginal adaptation and fracture resistance. The use of zirconia and resin cements may also permit more conservative tooth preparation while reducing the risk of restoration failure. Various surface treatment and bonding strategies have been proposed to achieve a strong and durable bond between zirconia and resin cements. These include incorporating phosphate-modified monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (MDP), into resin cement; air abrasion, performed either in the laboratory or chairside, using 30- or 110-micrometer silica-coated aluminum oxide particles; the use of zirconate-coupling primers, tetraethoxysilane flame-treatment devices, organofunctional silanes, laser irradiation, silicon vapor-phase deposition, and selective infiltration etching.^{7,10,11}

Despite the availability of various surface treatment methods (STMs) aimed at enhancing the strength of bond to ZrO_2 , conflicting results persist, primarily due to variations in bond-strength testing methodologies.

Due to its chemical inertness and lack of a silica-rich glassy phase, establishing an effective bond between zirconia and resin cements remains challenging, rendering conventional acid-etching methods less effective. Achieving a strong and durable resin–zirconia bond generally requires a comprehensive approach combining chemical and micromechanical surface pretreatment.¹² Acid etching can increase the wettability and surface area of silica-containing ceramics by altering their surface energy and bonding potential. However, unlike silica-based ceramics, zirconia has a highly crystalline, silica-free microstructure that is resistant to conventional acid etching. Consequently, acid etching produces limited topographical changes on the

zirconia surface, and is generally insufficient to achieve a strong and durable bond with a resin cement.¹³

Alternatively, acid-resistant ceramics can be air-abraded with aluminum oxide particles to produce surface irregularities and enhance micromechanical retention. However, research suggests that although air-abraded zirconia ceramics may initially improve bonding with certain luting agents, this bond may not remain stable during prolonged storage in artificial saliva or thermocycling in water. This instability may be attributed to limited chemical interaction between the treated zirconia surface and certain luting agents, as well as to the degradation of the resin–zirconia interface under aging conditions. In summary, acid etching may not produce substantial topographical alterations in highly crystalline ceramics, while air abrasion can initially enhance bonding through micromechanical retention, but may not provide sufficient long-term bond durability when used alone.¹⁴

To improve resin bonding to metal and highly crystalline ceramics, various silica-coating systems were initially introduced as experimental surface treatment techniques. Notable systems, such as Rocatec, CoJet and Silicoater MD, have demonstrated adequate bond strength in various studies. Monolithic zirconia crowns can benefit from resin-based cements, particularly when enhanced retention and bonding are required; however, achieving a durable bond remains challenging because of the inert, silica-free nature of zirconia. Air abrasion is commonly recommended to enhance micromechanical retention, while resin-based cements can provide additional chemical interaction when functional monomers are incorporated. Despite the high strength and fracture toughness of zirconia, the properties and durability of the resin cement are also important for maintaining adequate support and long-term stability of the ceramic restoration.¹⁵

The study specifically employed RelyX™ Unicem and Panavia™ F 2.0, selected for their distinct adhesive formulations and widespread clinical use. RelyX Unicem, a self-adhesive resin cement, is characterized by the ease of use and favorable initial bond strength, making it suitable for a broad range of clinical indications. Panavia F 2.0, a resin cement used in conjunction with an adhesive primer, was selected for its high bond durability and effectiveness under moist conditions. These properties make both cements suitable for evaluating the effects of different STMs and aging conditions, providing insights into their performance under varied clinical scenarios.¹⁶

The present study aimed to assess the effects of 2 luting agents, different STMs and variable water-storage durations on the shear bond strength (SBS) of zirconia.

Surface preparation techniques, such as air abrasion, selective etching, and the application of functional primers, can play an important role in enhancing the bond strength between zirconia and resin cements. These treatment modalities can improve micromechanical interlocking and promote chemical interaction at the zirconia–resin cement interface, thereby contributing to bond durability. The present study explores different surface treatment

protocols to determine their effects on the SBS of various luting agents to zirconia, an aspect that is critical to the long-term clinical success of ceramic restorations.

Hypotheses

The following hypotheses were tested:

- null hypothesis 1: Different STMs do not significantly affect the SBS of zirconia;
- null hypothesis 2: Different types of resin cement do not significantly affect the SBS of zirconia;
- null hypothesis 3: Artificial aging through water storage does not significantly affect the SBS of zirconia.

Material and methods

Methodology

Each zirconia block was sectioned into specimens measuring 10 mm × 4 mm × 2 mm, using an EM-DC2 die-cutting machine (Aixin Medical Equipment, Tianjin, China). The specimens were then sintered and polished. This uniform rectangular shape was selected to standardize the testing area and ensure the consistent application of the surface treatment and bonding procedures across all specimens. The specimens were randomly divided into 6 groups, each of which received specific surface treatment before bonding with the luting cement (Fig. 1).

Surface treatment groups

The zirconia specimens were divided into 6 distinct groups, each subjected to a different surface treatment protocol to evaluate its effect on bond strength:

- group 1: Air abrasion using 50-micrometer alumina (Al_2O_3) particles at a pressure of 3.5 bar, a working distance of 10 mm, and a blasting time of 15 s;
- group 2: Air abrasion using 100-micrometer Al_2O_3 particles under the same conditions as group 1;
- group 3: Immersion in 10% hydrofluoric acid (HF) at 60°C for 60 min, followed by rinsing with deionized water and air-drying;
- group 4: Immersion in 20% HF under the same conditions as group 3;
- group 5: Immersion in 30% HF under the same conditions as groups 3 and 4;
- group 6: Combination treatment consisting of air abrasion with 110-micrometer Al_2O_3 particles at 3.5 bar for 10 s, followed by immersion in 30% HF at 60°C for 60 min and subsequent silanization.

A total of 360 specimens were prepared and equally distributed among the 6 surface treatment groups. Each group comprised 30 specimens for RelyX Unicem and 30 specimens for Panavia F 2.0, resulting in 180 specimens for each type of resin cement.

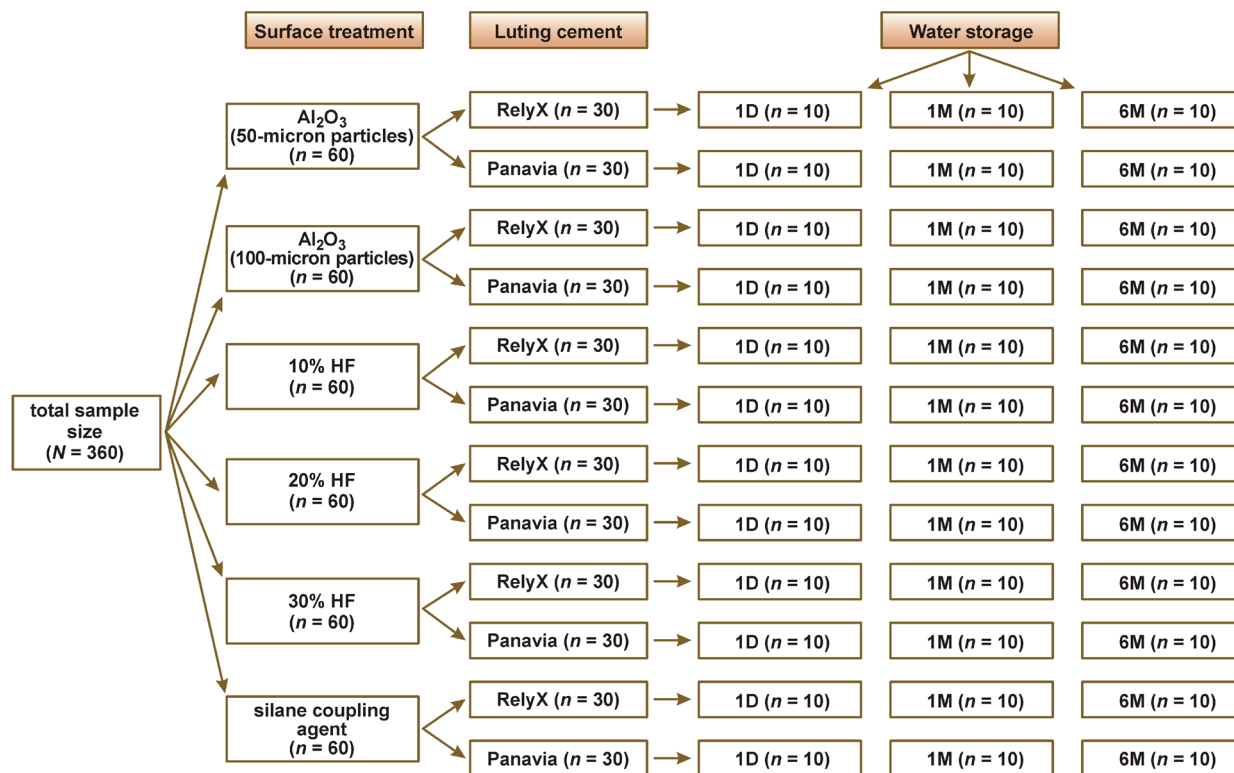


Fig. 1. Study design

Water-storage durations: 1D – 1 day (24 h); 1M – 1 month; 6M – 6 months.
Al₂O₃ – aluminum oxide (alumina); HF – hydrofluoric acid.

Bonding of zirconia specimens with resin cement blocks

Of the 360 specimens prepared, 120 specimens – 60 bonded with RelyX Unicem and 60 with Panavia F 2.0 – underwent immediate testing to assess the initial bond strength. The remaining 240 specimens were allocated for extended testing following different water-storage durations to evaluate the effects of aging on bond strength. Prior to bonding, the specimens were cleaned and primed according to the respective surface treatment protocols. Bonding was performed at room temperature under a constant load of 75 N.

Water storage

Following the bonding procedure, the specimens were immersed in distilled water according to the following storage periods:

- category 1: Specimens were immersed in distilled water for 24 h (1 day –1D) to assess the initial bond strength following water exposure;
- category 2: Specimens were stored in distilled water for 1 month (1M), with weekly water changes, to assess the intermediate-term stability of the bond;
- category 3: Specimens were stored in distilled water for 6 months (6M), with weekly water changes, to assess the long-term durability and performance of the bonded interface.

Shear bond strength testing

Shear bond strength was evaluated using a universal testing machine (UTM) at a crosshead speed of 1 mm/min. The maximum load recorded at the time of debonding was measured in kilograms (kg), converted to newtons (N), and then divided by the bonding area to calculate SBS, expressed in megapascals (MPa).

Results

In a comparative analysis of the RelyX and Panavia dental cements, the mean SBS values and standard deviations ($M \pm SD$) were assessed across 6 groups at 3 water-storage durations – 1D, 1M and 6M. Overall, the results indicated a tendency toward decreased SBS with prolonged water storage, although the pattern varied among the groups and luting agents (Table 1).

Specimens in category 1 generally showed the highest SBS values, whereas those in categories 2 and 3 exhibited greater reductions following prolonged water storage. In group 1, the mean SBS for RelyX decreased from 17.12 ± 0.71 MPa at 1D to 8.54 ± 6.42 MPa at 6M, while for Panavia, the values decreased from 21.76 ± 2.81 MPa to 7.10 ± 2.80 MPa. Group 2 showed a different pattern, with RelyX reaching its highest mean value at 1M (24.47 ± 2.95 MPa), whereas Panavia exhibited its highest mean value at 1D

Table 1. Shear bond strength (SBS) values [MPa] for the RelyX and Panavia cements according to different surface treatment modalities (groups) under different water-storage (aging) conditions

Group	Water-storage duration	RelyX		Panavia	
		<i>n</i>	SBS [MPa]	<i>n</i>	SBS [MPa]
1	1D	10	17.12 ±0.71	10	21.76 ±2.81
	1M	10	10.76 ±1.06	10	17.61 ±3.48
	6M	10	8.54 ±6.42	10	7.10 ±2.80
	total	30	12.14 ±5.20	30	15.49 ±6.93
2	1D	10	23.89 ±10.04	10	32.41 ±10.04
	1M	10	24.47 ±2.95	10	22.96 ±15.86
	6M	10	16.84 ±10.35	10	17.38 ±6.48
	total	30	21.73 ±8.93	30	24.25 ±12.74
3	1D	10	20.45 ±3.37	10	19.01 ±4.93
	1M	10	16.91 ±1.34	10	13.03 ±3.54
	6M	10	10.81 ±1.95	10	9.62 ±2.90
	total	30	16.06 ±4.66	30	13.89 ±5.44
4	1D	10	18.58 ±3.49	10	20.52 ±2.36
	1M	10	16.05 ±2.11	10	10.31 ±2.85
	6M	10	9.92 ±5.18	10	8.06 ±4.73
	total	30	14.85 ±5.21	30	12.96 ±6.45
5	1D	10	18.31 ±2.89	10	21.37 ±0.95
	1M	10	14.53 ±4.39	10	8.65 ±3.99
	6M	10	9.73 ±4.36	10	5.44 ±2.17
	total	30	14.19 ±5.22	30	11.82 ±7.46
6	1D	10	31.07 ±3.28	10	31.94 ±3.59
	1M	10	26.47 ±2.86	10	23.97 ±3.27
	6M	10	19.98 ±3.56	10	17.94 ±3.68
	total	30	25.84 ±5.59	30	24.62 ±6.75

Data presented as mean ± standard deviation (*M* ± *SD*).

Groups: 1 – air abrasion (50-micron Al₂O₃ particles); 2 – air abrasion (100-micron Al₂O₃ particles); 3 – acid etching with 10% HF; 4 – acid etching with 20% HF; 5 – acid etching with 30% HF; 6 – combination treatment with a silane coupling agent.

Table 2. ANOVA results for the RelyX and Panavia cements under different water-storage conditions for each group

Group	Cement	Comparison between the groups (<i>F</i> ; <i>p</i> -value)	Comparison within the groups (mean square)	Total (sum of squares)
1	RelyX	13.892; <i>p</i> < 0.000*	14.293	783.1
	Panavia	61.500; <i>p</i> < 0.000*	9.300	1,392.4
2	RelyX	2.499; <i>p</i> = 0.101*	72.227	2,311.1
	Panavia	4.400; <i>p</i> = 0.022*	131.500	4,706.1
3	RelyX	42.046; <i>p</i> < 0.000*	5.660	628.8
	Panavia	15.000; <i>p</i> < 0.000*	15.100	859.1
4	RelyX	13.702; <i>p</i> < 0.000*	14.485	788.0
	Panavia	36.600; <i>p</i> < 0.000*	12.000	1,206.1
5	RelyX	11.898; <i>p</i> < 0.000*	15.563	790.5
	Panavia	98.900; <i>p</i> < 0.000*	7.200	1,613.3
6	RelyX	29.449; <i>p</i> < 0.000*	10.542	905.5
	Panavia	39.800; <i>p</i> < 0.000*	12.400	1,320.6

Groups: 1 – air abrasion (50-micron Al₂O₃ particles); 2 – air abrasion (100-micron Al₂O₃ particles); 3 – acid etching with 10% HF; 4 – acid etching with 20% HF; 5 – acid etching with 30% HF; 6 – combination treatment with a silane coupling agent.

* statistically significant.

(32.41 ±10.04 MPa). Groups 3, 4, 5, and 6 generally demonstrated decreasing SBS values with prolonged water storage. For example, in Group 4, the mean SBS for RelyX decreased from 18.58 ±3.49 MPa at 1D to 9.92 ±5.18 MPa at 6M, while Panavia decreased from 20.52 ±2.36 MPa to 8.06 ±4.73 MPa). Overall, these findings suggest differences in the stability and bonding performance of the 2 luting agents under different water-storage conditions.

Table 2 summarizes differences in SBS among the 2 luting agents, STMs and aging conditions. It provides a comparative assessment of the performance of RelyX and Panavia under the various experimental conditions and illustrates the effect of prolonged water storage on bond strength.

Table 3 presents the results of the post hoc tests evaluating differences in SBS between zirconia specimens bonded

Table 3. Post hoc test results for the RelyX and Panavia cements

Group	Comparison	Cement	<i>MD</i>	<i>SE</i>	<i>p</i> -value
1	1D vs. 1M	RelyX	6.36	1.691	0.001*
		Panavia	4.15	1.360	0.005*
	1D vs. 6M	RelyX	8.58	1.691	0.000*
		Panavia	14.66	1.360	0.000*
	1M vs. 6M	RelyX	2.22	1.691	0.200
		Panavia	10.51	1.360	0.000*
2	1D vs. 1M	RelyX	−0.58	3.800	0.880
		Panavia	9.45	5.128	0.076
	1D vs. 6M	RelyX	7.05	3.800	0.074
		Panavia	15.03	5.128	0.007*
	1M vs. 6M	RelyX	7.63	3.800	0.055
		Panavia	5.58	5.128	0.286
3	1D vs. 1M	RelyX	3.54	1.064	0.003*
		Panavia	5.98	1.735	0.002*
	1D vs. 6M	RelyX	9.64	1.064	0.000*
		Panavia	9.39	1.735	0.000*
	1M vs. 6M	RelyX	6.10	1.064	0.000*
		Panavia	3.41	1.735	0.060
4	1D vs. 1M	RelyX	2.53	1.702	0.041*
		Panavia	10.21	1.551	0.000*
	1D vs. 6M	RelyX	8.66	1.702	0.000*
		Panavia	12.46	1.551	0.000*
	1M vs. 6M	RelyX	6.13	1.702	0.001*
		Panavia	2.25	1.551	0.159
5	1D vs. 1M	RelyX	3.78	1.764	0.004*
		Panavia	12.72	1.198	0.000*
	1D vs. 6M	RelyX	8.58	1.764	0.000*
		Panavia	15.93	1.198	0.000*
	1M vs. 6M	RelyX	4.80	1.764	0.011*
		Panavia	3.21	1.198	0.012*
6	1D vs. 1M	RelyX	4.60	1.452	0.004*
		Panavia	7.97	1.573	0.000*
	1D vs. 6M	RelyX	11.09	1.452	0.000*
		Panavia	14.00	1.573	0.000*
	1M vs. 6M	RelyX	6.49	1.452	0.000*
		Panavia	6.03	1.573	0.001*

Groups: 1 – air abrasion (50-micron Al₂O₃ particles); 2 – air abrasion (100-micron Al₂O₃ particles); 3 – acid etching with 10% HF; 4 – acid etching with 20% HF; 5 – acid etching with 30% HF; 6 – combination treatment with a silane coupling agent.

MD – mean difference; *SE* – standard error; * statistically significant.

with the 2 luting agents, RelyX and Panavia, across the different aging durations. In group 1, significant mean differences in SBS were observed with regard to 1D, 1M and 6M, indicating significant differences for the 2 luting agents at nearly all tested durations (except 1M vs. 6M for RelyX). In group 2, a significant difference emerged at 6M vs. 1D for Panavia, suggesting differences in bond performance following prolonged aging. In group 3, significant differences in SBS were observed with regard to all 3 aging durations (1D, 1M and 6M). Similarly, Groups 4 and 6 demonstrated significant mean differences across all tested durations. Group 5 also showed significant differences at

1D vs. 1M and 6M. Overall, these findings underscore the significant impact of simulated aging on the performance of Relyx and Panavia when applied to zirconia, providing valuable insights for their clinical application.

Table 4 presents a statistical comparison of groups 1–6 under different water-storage conditions. In group 1, a significant difference was observed at 1D ($p < 0.001$; $t = -5.063$), with a mean difference of -4.64 and a standard error (SE) of 0.92 , a trend consistent at a duration of 1M, but not sustained at 6M ($p = 0.465$), where the direction reversed. Group 2 showed a highly significant difference at 1M ($p < 0.001$; $t = 0.295$), with a mean difference of 1.51 and

Table 4. Comparisons between the 2 cements in different groups and under different water-storage conditions

Comparison	<i>F</i>	<i>p</i> -value	<i>t</i>	df	<i>p</i> -value (2-tailed)	<i>MD</i>	<i>SE</i>
1-1D	8.099	0.011*	−5.063	18	0.000*	−4.64	0.92
			−5.063	10.141	0.000*	−4.64	0.92
1-1M	3.299	0.086	−5.955	18	0.000*	−6.85	1.15
			−5.955	10.653	0.000*	−6.85	1.15
1-6M	2.121	0.162	0.746	18	0.465	1.60	2.14
			0.746	12.560	0.469	1.60	2.14
2-1D	0.187	0.671	−1.897	18	0.074	−8.52	4.49
			−1.897	18.000	0.074	−8.52	4.49
2-1M	29.251	0.000*	0.295	18	0.771	1.51	5.10
			0.295	9.622	0.774	1.51	5.10
2-6M	3.406	0.081	−0.140	18	0.890	−0.54	3.86
			−0.140	15.123	0.891	−0.54	3.86
3-1D	1.847	0.191	0.761	18	0.457	1.44	1.89
			0.761	15.923	0.458	1.44	1.89
3-1M	10.063	0.005*	3.240	18	0.005*	3.88	1.20
			3.240	11.522	0.007*	3.88	1.20
3-6M	0.770	0.392	1.076	18	0.296	1.19	1.10
			1.076	15.763	0.298	1.19	1.10
4-1D	0.530	0.476	−1.452	18	0.164	−1.93	1.33
			−1.452	15.821	0.166	−1.93	1.33
4-1M	0.486	0.494	5.114	18	0.000*	5.74	1.12
			5.114	16.562	0.000*	5.74	1.12
4-6M	0.251	0.622	0.837	18	0.414	1.86	2.22
			0.837	17.852	0.414	1.86	2.22
5-1D	8.223	0.010*	−3.177	18	0.005*	−3.06	0.96
			−3.177	10.927	0.009*	−3.06	0.96
5-1M	0.134	0.718	3.135	18	0.006*	5.88	1.88
			3.135	17.837	0.006*	5.88	1.88
5-6M	5.171	0.035*	2.781	18	0.012*	4.28	1.54
			2.781	13.193	0.015*	4.28	1.54
6-1D	0.036	0.851	−0.568	18	0.577	−0.87	1.54
			−0.568	17.853	0.577	−0.87	1.54
6-1M	0.019	0.892	1.813	18	0.086	2.49	1.37
			1.813	17.681	0.087	2.49	1.37
6-6M	0.232	0.636	1.258	18	0.225	2.04	1.62
			1.258	17.982	0.225	2.04	1.62

Groups: 1 – air abrasion (50-micron Al_2O_3 particles); 2 – air abrasion (100-micron Al_2O_3 particles); 3 – acid etching with 10% HF; 4 – acid etching with 20% HF; 5 – acid etching with 30% HF; 6 – combination treatment with a silane coupling agent. Water storage durations: 1D – 1 day (24 h); 1M – 1 month; 6M – 6 months. df – degrees of freedom; * statistically significant.

$SE = 5.10$, whereas differences at 1D and 6M were not statistically significant. Group 3 demonstrated a significant difference at 1M ($p = 0.005$; $t = 3.240$), with a mean difference of 3.88 and $SE = 1.20$, while no significant differences were observed at 1D or 6M. In Group 4, a highly significant difference was observed at 1M ($p < 0.001$; $t = 5.114$), with a mean difference of 5.74 and $SE = 1.12$, whereas differences at 1D and 6M were not significant. Group 5 showed a significant difference at 1D ($p = 0.010$; $t = -3.177$), with a mean difference of -3.06 and $SE = 0.96$, with similar significant differences observed at 1M and 6M. Finally, Group 6 showed no statistically significant difference at 1D, 1M or 6M. Overall, these findings provide insights into the comparative performance of these dental cements across different conditions.

Discussion

The outcomes of this study provide important insights into the application of zirconia in dental prosthetics, highlighting the crucial role of surface treatment in the longevity and performance of zirconia-based restorations. Acid-etched surfaces demonstrated higher bond strength under the experimental conditions, suggesting the potential for more effective and durable adhesive techniques in clinical practice. These findings underscore the importance of appropriate surface preparation in optimizing the mechanical properties of zirconia restorations, which are often subjected to substantial mechanical stress and wear over time.

The superior bond strength observed with the acid-etching treatment compared with air abrasion in the present study is consistent with findings reported in other studies.^{14,17–19} Collectively, these findings suggest that acid treatment may enhance the surface characteristics of zirconia and promote improved interaction with resin cements under certain experimental conditions. This may contribute to greater bond durability and potentially reduce the risk of restoration failure. Further research is warranted to establish standardized and clinically validated surface treatment protocols to incorporate acid etching more broadly, thus enhancing the overall success rates of zirconia restorations.

Moreover, the observed decrease in bond strength over time, particularly following prolonged water storage, highlights an important area for improvement in dental cement formulations. Both RelyX and Panavia demonstrated reductions in bond strength under the aging conditions evaluated, which may have implications for the long-term performance of zirconia restorations. This degradation presents an important challenge in adhesive dentistry and underscores the need for further research into more durable bonding systems capable of maintaining stable adhesion under prolonged exposure to the challenging conditions encountered in the oral environment.^{19–24}

Furthermore, the findings regarding the differential performance of the various luting agents and surface treatment modalities under simulated aging conditions provide valuable insights into the potential long-term performance of zirconia restorations. Dental practitioners and materials scientists should consider these variables when selecting and developing restorative materials, respectively. Such considerations are important for advancing patient care through the use of materials and bonding protocols that provide greater resistance to degradation over time.^{18,20,21,24,25}

Additionally, the differences observed among the tested groups warrant further investigation into how specific surface modifications influence micromechanical retention and chemical interaction at the zirconia–resin cement interface. A better understanding of these mechanisms may help optimize surface treatment protocols and improve the bonding performance and long-term stability of zirconia-based restorations.^{14,15,26–28}

Our findings support further consideration of surface treatment and cementation protocols in the clinical management of zirconia restorations. By providing empirical evidence on the behavior of different luting agents and surface treatment modalities under the tested aging conditions, this study contributes to a better understanding of zirconia–resin cement interaction and may help guide the refinement of bonding protocols. Further clinical research is warranted to determine whether these findings translate into improved long-term durability and reliability of zirconia restorations.

Limitations

While this study provides valuable insights into the bond strength of zirconia following specific surface treatment and cementation protocols, its laboratory setting limits the direct extrapolation of the findings to clinical scenarios. The use of water storage to simulate aging, although useful for preliminary assessment, may not fully reproduce the complex biological, chemical, thermal, and mechanical conditions present in the oral environment or capture long-term clinical outcomes. Additionally, mechanical testing was performed under controlled uniaxial loading, which does not fully represent the complex and multidirectional stresses experienced by dental restorations in vivo. Further research incorporating longer-term clinical follow-up and more clinically representative, multidirectional loading conditions is recommended to validate and extend these findings.

Conclusions

In conclusion, the study rejects the 3 null hypotheses, demonstrating that surface treatment protocols significantly affect the SBS of zirconia, that different luting agents, particularly RelyX and Panavia, exhibit different

bonding performance, and that artificial aging through water storage significantly reduces bond strength. These findings highlight the importance of both surface treatment and aging conditions in determining the performance of luting agents used with zirconia restorations. Additionally, the study found that the application of a silane coupling agent enhanced the bond strength of RelyX Unicem to yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) before aging, whereas HF treatment reduced the surface roughness of Y-TZP. Overall, water storage generally resulted in decreased bond strength for both luting agents, emphasizing the importance of long-term bond durability in zirconia-based restorations.

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

During the preparation of this manuscript, the authors used DeepL AI platform for language checking and grammar correction.

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References

- Nistor L, Grădinaru M, Rică R, et al. Zirconia use in dentistry – manufacturing and properties. *Curr Health Sci J*. 2019;45(1):28–35. doi:10.12865/CHSJ.45.01.03
- Elissalde C, Chung UC, Josse M, et al. Single-step sintering of zirconia ceramics using hydroxide precursors and spark plasma sintering below 400°C. *Scr Mater*. 2019;168:134–138. doi:10.1016/j.scripta-mat.2019.04.037
- Deng B, Luo J, Harris JT, Smith CM. Critical stress map for ZrO₂ tetragonal to monoclinic phase transformation in ZrO₂-toughened glass-ceramics. *Materialia*. 2020;9:100548. doi:10.1016/j.mtla.2019.100548
- Soleimani F, Jalali H, Mostafavi AS, Zeighami S, Memarian M. Retention and clinical performance of zirconia crowns: A comprehensive review. *Int J Dent*. 2020;2020:8846534. doi:10.1155/2020/8846534
- Raigrodski AJ. Contemporary materials and technologies for all-ceramic fixed partial dentures: A review of the literature. *J Prosthet Dent*. 2004;92(6):557–562. doi:10.1016/j.prosdent.2004.09.015
- Abdulazeez MI, Majeed MA. Fracture strength of monolithic zirconia crowns with modified vertical preparation: A comparative in vitro study. *Eur J Dent*. 2022;16(1):209–214. doi:10.1055/s-0041-1735427
- Miyazaki T, Nakamura T, Matsumura H, Ban S, Kobayashi T. Current status of zirconia restoration. *J Prosthodont Res*. 2013;57(4):236–261. doi:10.1016/j.jpor.2013.09.001
- Ezzat Y, Sharka R, Rayyan M, Al-Rafee M. Fracture resistance of monolithic high-translucency crowns versus porcelain-veneered zirconia crowns after artificial aging: An in vitro study. *Cureus*. 2021;13(12):e20640. doi:10.7759/cureus.20640
- Vaid NR, Adel SM. Contemporary orthodontic workflows: A panacea for efficiency? *Semin Orthod*. 2023;29(1):1–3. doi:10.1053/j.sodo.2023.02.002
- Russo DS, Cinelli F, Sarti C, Giachetti L. Adhesion to zirconia: A systematic review of current conditioning methods and bonding materials. *Dent J (Basel)*. 2019;7(3):74. doi:10.3390/dj7030074
- Özcan M, Bernasconi M. Adhesion to zirconia used for dental restorations: A systematic review and meta-analysis. *J Adhes Dent*. 2015;17(1):7–26. doi:10.3290/j.jad.a33525
- Snyder T. Keys to bonding esthetic, durable zirconia restorations: Two case reports. *Compend Contin Educ Dent*. 2023;44(8):e1–e4. PMID:37708046.
- Rapani A, Berton F, Tramontin A, et al. Surface roughness of enamel and dentin after preparation finishing with rotary burs or piezoelectric instruments. *Prosthesis*. 2023;5(3):711–720. doi:10.3390/prosthesis5030050
- Kim SH, Cho SC, Lee MH, Kim HJ, Oh NS. Effect of 9% hydrofluoric acid gel hot-etching surface treatment on shear bond strength of resin cements to zirconia ceramics. *Medicina (Kaunas)*. 2022;58(10):1469. doi:10.3390/medicina58101469
- Su Z, Li M, Zhang L, et al. A novel porous silica-zirconia coating for improving bond performance of dental zirconia. *J Zhejiang Univ Sci B*. 2021;22(3):214–222. doi:10.1631/jzus.B2000448
- Fidalgo-Pereira R, Torres O, Carvalho Ó, et al. A scoping review on the polymerization of resin-matrix cements used in restorative dentistry. *Materials (Basel)*. 2023;16(4):1560. doi:10.3390/ma16041560
- Roy AK, Prasad GN, Bhagat TV, et al. Analysis of surface conditioning methods on core-veneer bond strength of CAD/CAM zirconia restorations. *Technol Health Care*. 2021;29(3):467–477. doi:10.3233/THC-202539
- Muhammed HA, Mahmoud EM, Fahmy AE, Nasr DM. The effect of sandblasting versus acid etching on the surface roughness and biaxial flexural strength of CAD/CAM resin-matrix ceramics (in vitro study). *BMC Oral Health*. 2023;23(1):169. doi:10.1186/s12903-023-02883-6
- Amaral R, Özcan M, Bottino MA, Valandro LF. Microtensile bond strength of a resin cement to glass infiltrated zirconia-reinforced ceramic: The effect of surface conditioning. *Dent Mater*. 2006;22(3):283–290. doi:10.1016/j.dental.2005.04.021
- Rigos AE, Sarafidou K, Kontonasaki E. Zirconia bond strength durability following artificial aging: A systematic review and meta-analysis of in vitro studies. *Jpn Dent Sci Rev*. 2023;59:138–159. doi:10.1016/j.jdsr.2023.04.002
- Badarneh A, Choi JJE, Lyons K, Porter G, Waddell N, Li KC. The effect of aging on the wear performance of monolithic zirconia. *Dent Mater*. 2022;38(5):e136–e146. doi:10.1016/j.dental.2022.04.018
- Levartovsky S, Cartier L, Brand M, Blasbalg JJ, Pilo R. The retentive strength of zirconium oxide crowns cemented by self-adhesive resin cements before and after 6 months of aging. *Materials (Basel)*. 2020;13(18):3998. doi:10.3390/ma13183998
- Vaddamanu SK, Vyas R, Bavabeedu SS, Arora S, Badiyani BK, Kumar A. In vitro results of scanning technique on assessing cement thickness and interfacial nanoleakage of luted CAD/CAM-fabricated fiber posts. *J Pharm Bioallied Sci*. 2021;13(Suppl 1):S676–S678. doi:10.4103/jpbs.JPBS_761_20

24. Alrabeah G, Alomar S, Almutairi A, Alali H, ArRejaie A. Analysis of the effect of thermocycling on bonding cements to zirconia. *Saudi Dent J.* 2023;35(6):734–740. doi:10.1016/j.sdentj.2023.07.009
25. Sandes Farias DC, Gonçalves LM, Walter R, Chung Y, Blatz MB. Bond strengths of various resin cements to different ceramics. *Braz Oral Res.* 2019;33:e095. doi:10.1590/1807-3107bor-2019.vol33.0095
26. Heboyen A, Vardanyan A, Karobari MI, et al. Dental luting cements: An updated comprehensive review. *Molecules.* 2023;28(4):1619. doi:10.3390/molecules28041619
27. Leung GKH, Wong AWY, Chu CH, Yu OY. Update on dental luting materials. *Dent J (Basel).* 2022;10(11):208. doi:10.3390/dj10110208
28. Giti R, Zarkari R. The effect of a zirconia primer on the shear bond strength of Y-TZP ceramic to three different core materials by using a self-adhesive resin cement. *J Indian Prosthodont Soc.* 2019;19(2):134–140. doi:10.4103/jips.jips_348_18

Remineralization potential after a single application of silver diamine fluoride and casein phosphopeptide–amorphous calcium phosphate fluoride on demineralized dentin

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Abstract

Background. Non-invasive treatment involves the use of various remineralizing and cariostatic agents, including sodium fluoride, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), casein phosphopeptide–amorphous calcium phosphate fluoride (CPP-ACPF), silver nitrate, and silver diamine fluoride (SDF).

Objectives. The purpose of the present study was to evaluate the surface morphology and mineral uptake of demineralized dentin following a single application of SDF and CPP-ACPF.

Material and methods. Twenty-four caries-free premolar sections with marked areas of specific dimensions on the dentin surface were exposed to a demineralizing solution for 96 h and randomly allocated to 2 groups: group 1 (SDF); and group 2 (CPP-ACPF). Surface morphology was analyzed using scanning electron microscopy (SEM), while mineral uptake was assessed using energy-dispersive X-ray spectroscopy (EDX) following demineralization and the application of the test agents. Paired and unpaired *t* tests were used to analyze the continuous data.

Results. The EDX revealed a difference in the mean increase in the calcium(Ca)/phosphorus (P) ratio between the SDF group (0.34 ± 0.92) and the CPP-ACPF group (0.01 ± 0.22); however, this difference was not statistically significant ($p = 0.400$). In contrast, the mean increase in fluoride uptake was significantly higher in the SDF group (2.07 ± 0.66 at%) than in the CPP-ACPF group (0.37 ± 0.38 at%) ($p = 0.001$). The SEM images showed dense, granular structures in the intertubular areas, with partial occlusion of the dentinal tubules in the SDF group, whereas the CPP-ACPF group exhibited mineral deposits partially occluding the dentinal tubules, with non-homogeneous sediment formation.

Conclusions. A single application of both SDF and CPP-ACPF demonstrated potential to promote mineral uptake in demineralized dentin, with comparable effects on calcium and phosphate incorporation. However, SDF showed a greater capacity for fluoride uptake and produced a denser, more compact surface morphology, whereas CPP-ACPF resulted in a more porous mineral deposition pattern. Overall, both agents demonstrated remineralization potential, although their effects on the mineral composition and surface characteristics of demineralized dentin differed.

Keywords: CPP-ACP, scanning electron microscopy, silver diamine fluoride, energy-dispersive X-ray spectroscopy

Highlights

- The samples treated with silver diamine fluoride (SDF) and casein phosphopeptide–amorphous calcium phosphate fluoride (CPP-ACPF) showed higher Ca/P ratio and fluoride uptake in comparison with demineralized dentin.
- The SDF samples showed higher Ca/P ratio and fluoride uptake than the CPP-ACPF samples, indicating higher remineralization potential of SDF.
- The SEM evaluation exhibited dense, granular structures in the SDF group and porous, non-homogenous deposits in the CPP-ACPF group.

Introduction

Dental caries results from an imbalance between the physiological processes of demineralization and remineralization of dental hard tissues. The current approach to caries management emphasizes the early detection and targeted, non-invasive management of reversible lesions.¹ The materials or agents used for this purpose act mainly through 2 mechanisms: preventing further demineralization; or restoring the lost minerals and thereby promoting remineralization.² In this regard, various remineralizing and cariostatic agents have been introduced, including sodium fluoride, tricalcium phosphate, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), casein phosphopeptide–amorphous calcium phosphate fluoride (CPP-ACPF), silver nitrate, and silver diamine fluoride (SDF), which have demonstrated varying degrees of effectiveness.

Silver diamine fluoride is a safe and effective caries-arresting agent that was first explored as a treatment option for managing carious lesions in Japan in 1969.³ It contains silver and fluoride ions in an ammoniacal solution, with silver ions stabilized through complexation with ammonia.⁴ Silver ions exert a strong antimicrobial effect and can react with phosphate and chloride ions to form relatively insoluble silver compounds, contributing to the hardening of soft carious lesions.⁵ The high fluoride concentration in SDF promotes the remineralization of demineralized dentin, and can contribute to the formation of fluoridated apatite and calcium fluoride. Furthermore, the alkaline nature of the ammoniacal solution may contribute to an acid-buffering effect and promote mineral precipitation.⁶

Another commonly used non-fluoridated remineralizing agent, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), is a naturally derived agent obtained from the casein protein of milk.⁷ CPP-ACP stabilizes and localizes calcium and phosphate ions, maintaining a state of supersaturation with respect to hydroxyapatite, thereby reducing demineralization and promoting remineralization.⁸ CPP-ACPF is a nanocomplex formed by incorporating fluoride (900 ppm) into CPP-ACP. CPP-ACPF enhances fluoride incorporation into dental biofilm and subsurface enamel, thereby promoting enamel remineralization.⁹

Kumar and Kumari stated that the silver component of SDF exerts an oligodynamic effect within the dentinal tubules by inhibiting bacterial growth and reducing dentin demineralization.¹⁰ According to Cabalén et al., SDF can arrest active dentinal lesions by inhibiting demineralization and promoting remineralization.¹¹

To date, limited evidence is available regarding the effects of a single application of SDF and CPP-ACPF on mineral uptake in demineralized dentin. Therefore, an in vitro study was conducted to evaluate the surface morphology and mineral uptake of demineralized dentin following a single application of SDF and CPP-ACPF, using scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDX).

Material and methods

The present in vitro study was conducted in affiliation with the Department of Pedodontics and Preventive Dentistry, Vishnu Dental College, Bhimavaram, India. Surface morphology and mineral content were evaluated using SEM and EDX, respectively, at Osmania University, Hyderabad, India. The study protocol was approved by the institutional ethics committee at Vishnu Dental College (IECVDC/2021/PG01/PPD/IVT/30).

Sample size

The sample size was calculated based on the results of a pilot study using the G*Power 3.1 software (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>), with the level of significance set at 5% and the statistical power at 80%. A total of 24 teeth were included in the study, with 12 teeth allocated to each group.

Methodology

Extracted non-carious premolars were disinfected in 10% formalin for 1 week¹² and cleaned of debris, using an ultrasonic scaler (DTE D3; Guilin Woodpecker Medical Instruments, Guilin, China). The teeth were stored in double-deionized distilled water in a glass beaker at room temperature until the experimental procedure was initiated.

The teeth were decoronated at the cemento-enamel junction (CEJ) and the roots were discarded. Each coronal portion was sectioned mesiodistally into buccal and lingual halves, using a double-faced diamond disc (Diatech USA, Mount Pleasant, USA) under continuous water cooling. The enamel was removed using a water-cooled diamond disc to expose the underlying dentin, and the specimens were mounted in self-cure acrylic resin blocks (DPI RR Cold Cure; DPI, Mumbai, India). The specimens were ultrasonicated in deionized water for 5 min, using an ultrasonic cleaner (PHUC-50; Phoenix, USA). A 3 mm × 3 mm window was marked on the dentin surface of each specimen, and the remaining tooth surface was coated with acid-resistant nail varnish (Clarins, Paris, France).

The specimens were randomly allocated into 2 groups: group 1 – SDF (38%, e-SDF; Kids-e-Dental, Mumbai, India); and group 2 – CPP-ACPF (10% w/v, GC Tooth Mousse Plus, GC, Tokyo, Japan). Each specimen was individually immersed in 15 mL of a demineralizing solution containing 2.2 mM calcium chloride, 2.2 mM monosodium dihydrogen phosphate, and 0.05 M acetic acid. The pH was adjusted to 4.5, using 50% sodium hydroxide. The specimens were maintained in the demineralizing solution for 96 h at 37°C.

Following demineralization, the specimens were subjected to SEM with EDX (Hitachi S-3700N; Hitachi Ltd., Tokyo, Japan) after dehydration, gold sputtering and conductive adhesive fixation using a sputter coater (Hitachi E-1010 Ion Sputter, Hitachi Ltd.). The SEM was used for the qualitative evaluation of surface morphology, whereas EDX was used for the quantitative assessment of mineral content. The specimens were evaluated before and after the application of the respective test agents for 3 min at a beam voltage of 10 kV and a magnification of ×3,000 (Fig. 1).

Statistical analysis

Statistical analysis was performed after assessing the normality of data distribution. The data were tabulated and analyzed using parametric tests. An independent-samples *t* test was used to compare the 2 groups, while a paired-samples *t* test was used to compare the measurements obtained after demineralization with those obtained following the application of the respective test agents. A *p*-value ≤0.05 was considered statistically significant.

Results

Comparison of the mean Ca/P ratio

On intragroup comparison, group 1 showed a higher mean calcium (Ca)/phosphorus (P) ratio after the application of SDF as compared to the baseline value; however, the difference was not statistically significant (*p* = 0.400). Similarly, group 2 showed a higher mean Ca/P ratio after

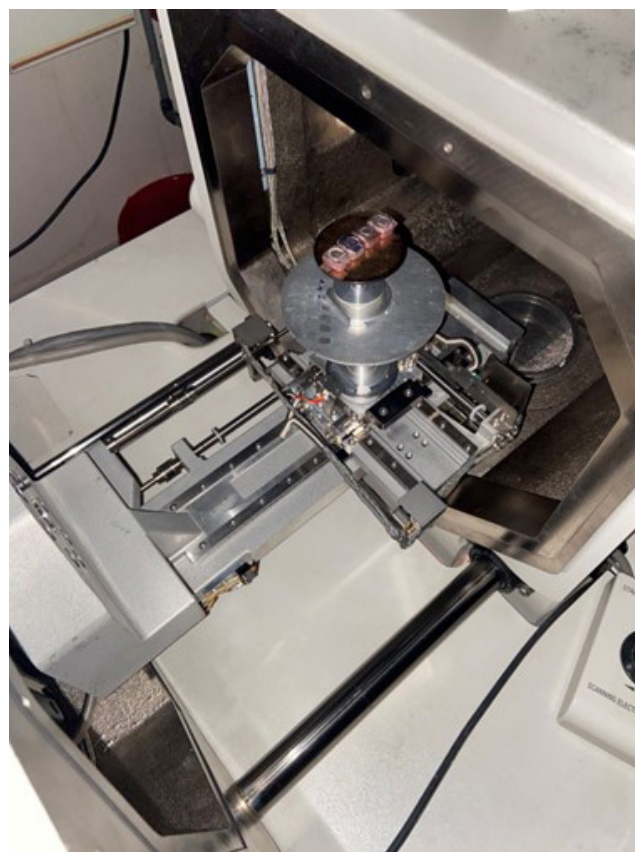


Fig. 1. Specimens for the scanning electron microscopy (SEM) evaluation

the application of CPP-ACPF as compared to the baseline value, although the difference was not statistically significant (*p* = 0.920) (Table 1).

Table 1. Intragroup comparison of the mean Ca/P ratio values (paired-samples *t* test)

Groups	<i>n</i>	Time point	<i>M</i> ± <i>SD</i>	<i>MD</i>	<i>p</i> -value
Group 1 (SDF)	12	baseline	2.16 ± 0.45	0.34	0.400
		after application	2.50 ± 0.56		
Group 2 (CPP-ACPF)	12	baseline	2.60 ± 0.36	0.01	0.920
		after application	2.61 ± 0.55		

SDF – silver diamine fluoride; CPP-ACPF – casein phosphopeptide–amorphous calcium phosphate; *M* – mean; *SD* – standard deviation; *MD* – mean difference.

On intergroup comparison, the SDF group demonstrated a greater increase in the mean Ca/P ratio than the CPP-ACPF group; however, the difference between the groups was not statistically significant (*p* = 0.400) (Table 2).

Table 2. Intergroup comparison of the mean Ca/P ratio values (independent-samples *t* test)

Groups	<i>n</i>	<i>MD</i> ± <i>SD</i>	<i>p</i> -value
Group 1 (SDF)	12	0.34 ± 0.92	0.400
Group 2 (CPP-ACPF)	12	0.01 ± 0.22	

Comparison of the mean fluoride uptake

On intragroup comparison, the SDF group showed a higher mean fluoride content value than at the baseline, and the difference was statistically significant ($p = 0.001$). The CPP-ACPF group also showed a higher mean fluoride content value than at the baseline; however, the difference was not statistically significant ($p = 0.064$) (Table 3).

Table 3. Intragroup comparison of the mean fluoride content values [at%] (paired-samples t test)

Groups	n	Time point	$M \pm SD$	MD	p -value
Group 1 (SDF)	12	baseline	4.74 ± 0.93	2.07	0.001*
		after application	6.81 ± 0.96		
Group 2 (CPP-ACPF)	12	baseline	2.01 ± 1.17	0.37	0.064
		after application	2.38 ± 1.51		

* statistically significant.

On intergroup comparison, the SDF group demonstrated a greater increase in the mean fluoride content value (2.07 ± 0.66 at%) than the CPP-ACPF group (0.37 ± 0.38 at%), and the difference between the groups was statistically significant ($p = 0.001$) (Table 4).

Table 4. Intergroup comparison of the mean fluoride content values [at%] (independent-samples t test)

Groups	n	$MD \pm SD$	p -value
Group 1 (SDF)	12	2.07 ± 0.66	0.001*
Group 2 (CPP-ACPF)	12	0.37 ± 0.38	

* statistically significant.

Scanning electron microscopy evaluation

On evaluation at $\times 3,000$ magnification, all demineralized dentin specimens showed clearly exposed dentinal tubules (Fig. 2). The SDF-treated specimens exhibited dense, granular structures in the intertubular areas, with partial

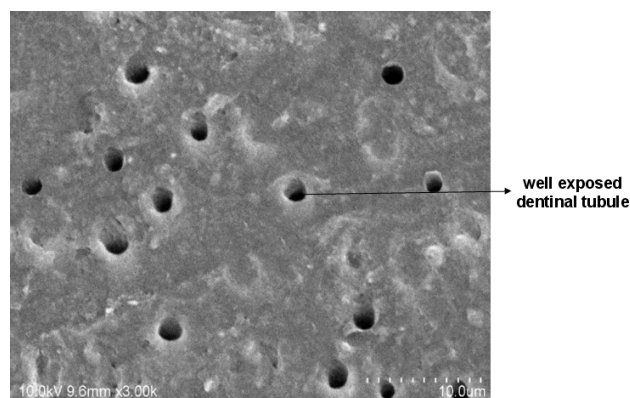


Fig. 2. SEM micrograph of demineralized dentin at $\times 3,000$ magnification, showing well exposed dentinal tubules

occlusion of the dentinal tubules (Fig. 3). The CPP-ACPF-treated specimens showed mineral deposits partially occluding the dentinal tubules, with non-homogeneous sediment deposits observed on the dentinal surface (Fig. 4).

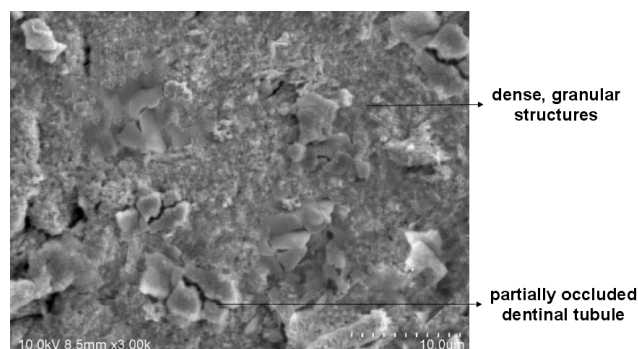


Fig. 3. SEM micrograph after the application of silver diamine fluoride (SDF) at $\times 3,000$ magnification, showing dense, granular structures in the intertubular areas, with partially occluded dentinal tubules

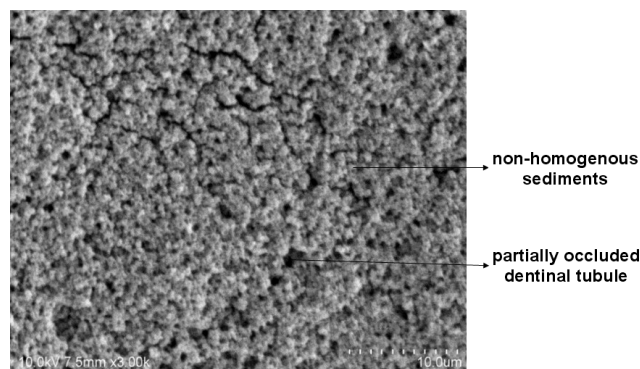


Fig. 4. SEM micrograph after the application of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) at $\times 3,000$ magnification, showing mineral deposits partially occluding the dentinal tubules, with non-homogeneous sediment deposits observed on the dentinal surface

Discussion

Decades of research have shifted the focus of caries management toward the early detection of lesions and the use of non-invasive treatment approaches.¹³ Ideally, the natural buffering capacity of saliva would help reverse early tooth demineralization. When these natural protective mechanisms are insufficient to compensate for mineral loss, several approaches can be employed to arrest or reverse lesion progression.¹⁴ Remineralization is one such approach, involving the replenishment of the lost minerals through the availability of calcium and phosphate ions, often aided by remineralizing agents. This process helps restore the mineral content of dental hard tissues and maintain the optimal Ca/P ratio of hydroxyapatite, approx. 1.67, thereby contributing to the strengthening of the tooth lattice network.¹⁵ Fluoride varnishes have been widely used for the professional application of topical fluoride for more than 25 years, and were developed to prolong the contact time between fluoride and the tooth surface.¹⁶

Currently, SDF has attracted considerable attention from both researchers and dental practitioners. It has been proposed that SDF can simultaneously inhibit the progression of the existing carious lesions and reduce the development of new carious lesions.¹⁷ Silver diamine fluoride has demonstrated antimicrobial activity, including the inhibition of cariogenic microorganisms and the disruption of dental plaque biofilm, and may also promote calcium uptake and mineral deposition.¹⁸ SDF-treated carious lesions have been reported to exhibit significantly greater surface microhardness and increased resistance to acid-induced demineralization.¹⁹ A 38% concentration of SDF is commonly used for caries management and contains approx. 44,800 ppm fluoride.⁴ However, lower concentrations, including 30% and 12%, have also been investigated. A review by Gao et al. reported that increasing the SDF concentration from 12% to 38% may enhance its effectiveness in arresting caries.¹⁷

Casein phosphopeptide–amorphous calcium phosphate is another widely studied and established non-fluoridated remineralizing agent. CPP-ACP is a nanocomplex that was patented in the United States in 1991; CPP-ACP acts by stabilizing calcium and phosphate ions in the presence of fluoride, maintaining them in a bioavailable form.²⁰ According to Reynolds et al., CPP-ACP has demonstrated greater potential for the remineralization of subsurface enamel lesions as compared to other forms of calcium phosphate.²¹

Dentin remineralization is more challenging than enamel remineralization because of differences in their composition and structure. The dentin contains a substantially higher proportion of organic matrix, primarily type I collagen, as well as a tubular microstructure.²² Dentin mineralization is regulated by non-collagenous proteins, and involves the deposition of calcium and phosphate ions within and around the collagen fibrils, followed by the formation and growth of hydroxyapatite crystals.²³ Fluoride is an important ion in the formation of fluoridated apatite, which is more resistant to acid dissolution and cariogenic challenges than hydroxyapatite.²⁴

Hence, CPP-ACPF was compared with SDF to evaluate their remineralization potential on demineralized dentin. Scanning electron microscopy combined with energy-dispersive X-ray analysis (SEM-EDX) is a useful micro-analytical technique for evaluating surface morphology and elemental composition. The SEM provides detailed, high-resolution images by scanning a focused electron beam across the specimen surface and detecting secondary and backscattered electron signals. When combined with EDX, SEM can provide the qualitative identification and quantitative assessment of the elemental composition of specific areas of the specimen. Therefore, SEM-EDX was used in the present study to evaluate changes in surface morphology and mineral composition following the application of the test agents.

In the present study, a single application of SDF resulted in improvement in the mean Ca/P ratio and significantly

higher fluoride uptake as compared to CPP-ACPF. Richards reported significantly greater remineralization following the application of SDF under net demineralizing conditions.²⁵ Similarly, Yadav et al. demonstrated the greatest changes in the Ca/P ratio following remineralization in the SDF group as compared to the other treatment groups.²⁶

Sinha et al. reported that the treatment of deep carious lesions with SDF increased the concentrations of calcium, phosphate and fluoride ions in caries-affected dentin.²⁷ Yilmaz et al. suggested that the superior remineralizing effect of SDF may be attributed to its high fluoride concentration and the alkaline nature of the solution.²⁸ Chibinski et al. reported that, after 12 months, the caries-arresting effect of SDF was 66% greater than that of other active materials and 154% greater than that of placebo; overall, SDF demonstrated an 89% greater caries-arresting effect as compared to the other treatment groups.²⁹

The CPP-ACPF samples showed improvement in the mean Ca/P ratio and increased fluoride uptake in comparison with the demineralized dentin samples. Cross et al. reported similar findings, suggesting that the localization of CPP-ACPF at the surface layer facilitates the sustained release of calcium, phosphate and fluoride ions while preventing their rapid precipitation as fluorapatite, thereby promoting mineralization within the demineralized lesion.³⁰ Hedge et al. reported that CPP-ACPF demonstrated a marginally greater remineralization potential as compared to the other agents evaluated.³¹

The addition of fluoride (0.2% NaF) to CPP-ACP has been reported to produce a marginally greater degree of remineralization than CPP-ACP alone, possibly through the formation of fluorapatite in the presence of calcium and phosphate ions.³² Yadav et al. reported that CPP-ACPF demonstrated greater remineralization than fluoride-free toothpaste, fluoride-containing toothpaste, and CPP-ACP alone.²⁶ This effect may be attributed to the incorporation of fluoride into the CPP-ACP complex, resulting in a synergistic effect that enhances remineralization in comparison with CPP-ACP alone, which is consistent with the findings of the present study. Kamath et al. also observed an increase in the mean fluoride content from the post-demineralization stage to the post-remineralization stage following treatment with CPP-ACPF in the enamel.¹

The variation in the Ca/P ratio and fluoride content values may be attributed to differences in the inorganic composition of the specimens, as well as the absence of the complex oral environment and the controlled experimental conditions. In vivo conditions present more dynamic and multifactorial challenges to the tested materials. Since the existing literature provides evidence supporting the beneficial effects of SDF and CPP-ACPF, further studies should evaluate these agents under conditions that more closely mimic the oral environment. Such investigations may provide a better understanding of their remineralization potential and clinical effectiveness in the management of demineralized dental hard tissues.

Conclusions

A single application of both SDF and CPP-ACPF demonstrated potential to promote mineral uptake in demineralized dentin, with comparable effects on calcium and phosphate incorporation. However, SDF showed a greater capacity for fluoride uptake and produced a denser, more compact surface morphology, whereas CPP-ACPF resulted in a more porous mineral deposition pattern. Overall, both agents demonstrated remineralization potential, although their effects on the mineral composition and surface characteristics of demineralized dentin differed.

Ethics approval and consent to participate

The study protocol was approved by the institutional ethics committee at Vishnu Dental College, Bhimavaram, India (IECVDC/2021/PG01/PPD/IVT/30).

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

- Kamath P, Nayak R, Kamath SU, Pai D. A comparative evaluation of the remineralization potential of three commercially available remineralizing agents on white spot lesions in primary teeth: An in vitro study. *J Indian Soc Pedod Prev Dent*. 2017;35(3):229–237. doi:10.4103/JISPPD.JISPPD_242_16
- Patil N, Choudhari S, Kulkarni S, Joshi SR. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. *J Conserv Dent*. 2013;16(2):116–120. doi:10.4103/0972-0707.108185
- Yamaga R, Yokomizo I. Arrestment of caries of deciduous teeth with diamine silver fluoride. *Dent Outlook*. 1969;33:1007–1013.
- Mei ML, Chu CH, Lo ECM, Samaranayake LP. Fluoride and silver concentrations of silver diamine fluoride solutions for dental use. *Int J Paediatr Dent*. 2013;23(4):279–285. doi:10.1111/ipd.12005
- Akyildiz M, Sönmez IS. Comparison of remineralising potential of nano silver fluoride, silver diamine fluoride and sodium fluoride varnish on artificial caries: An in vitro study. *Oral Health Prev Dent*. 2019;17(5):469–477. doi:10.3290/j.ohpd.a42739
- Zhao IS, Gao SS, Hiraishi N, et al. Mechanisms of silver diamine fluoride on arresting caries: A literature review. *Int Dent J*. 2018;68(2):67–76. doi:10.1111/idj.12320
- Agob JN, Aref NS, Saeid Al-Wakeel EE. Effect of casein phosphopeptide–amorphous calcium phosphate on fluoride release and micro-shear bond strength of resin-modified glass ionomer cement in caries-affected dentin. *Restor Dent Endod*. 2018;43(4):e45. doi:10.5395/rde.2018.43.e45
- Nakajima M, Kunawarote S, Prasansuttiporn T, Tagami J. Bonding to caries-affected dentin. *Jpn Dent Sci Rev*. 2011;47(2):102–114. doi:10.1016/j.jdsr.2011.03.002
- Cross KJ, Huq NL, Stanton DP, Sum M, Reynolds EC. NMR studies of a novel calcium, phosphate and fluoride delivery vehicle – alpha(S1)-casein (59–79) by stabilized amorphous calcium fluoride phosphate nanocomplexes. *Biomaterials*. 2004;25(20):5061–5069. doi:10.1016/j.biomaterials.2004.01.045
- Kumar N, Kumari R. Historical background and clinical implications of silver diamine fluoride in dentistry: A narrative review. *BIJCRID*. 2022;1(1):49–53. doi:10.54646/bijcrd.010
- Cabalén MB, Molina GF, Bono A, Burrow MF. Nonrestorative caries treatment: A systematic review update. *Int Dent J*. 2022;72(6):746–764. doi:10.1016/j.identj.2022.06.022
- Kohn WG, Collins AS, Cleveland JL, et al.; Centers for Disease Control and Prevention (CDC). Guidelines for infection control in dental health-care settings – 2003. *MMWR*. 2003;52(RR17):1–61. <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5217a1.htm>. Accessed November 1, 2021.
- Thimmaiah C, Shetty P, Shetty SB, Natarajan S, Thomas VA. Comparative analysis of the remineralization potential of CPP-ACP with fluoride, tri-calcium phosphate and nano hydroxyapatite using SEM/EDX – an in vitro study. *J Clin Exp Dent*. 2019;11(12):e1120–e1126. doi:10.4317/jced.55941
- Balakrishnan A, Jonathan R, Benin P, Kuumar A. Evaluation to determine the caries remineralization potential of three dentifrices: An in vitro study. *J Conserv Dent*. 2013;16(4):375–379. doi:10.4103/0972-0707.114347
- Raghu TN, Ananthakrishna S. Remineralization potential of calcium sucrose phosphate on demineralized enamel: Results of an in vitro study. *J Int Oral Health*. 2016;8(6):704–708. doi:10.2047/jioh-08-06-12
- Virupaxi SG, Roshan NM, Poornima P, Nagaveni NB, Neena IE, Bharath KP. Comparative evaluation of longevity of fluoride release from three different fluoride varnishes – an in vitro study. *J Clin Diagn Res*. 2016;10(8):ZC33–ZC36. doi:10.7860/JCDR/2016/19209.8242
- Gao SS, Zhao IS, Hiraishi N, et al. Clinical trials of silver diamine fluoride in arresting caries among children: A systematic review. *JDR Clin Trans Res*. 2016;1(3):201–210. doi:10.1177/2380084416661474
- Wu L, Yang F. The effects of three fluoride-containing agents on the remineralization of deciduous teeth in vitro [in Chinese]. *J Pract Stomatol*. 2002;18:347–349.
- Li YJ. Effect of a silver ammonia fluoride solution on the prevention and inhibition of caries [in Chinese]. *Zhonghua Kou Qiang Ke Za Zhi*. 1984;19(2):97–100. PMID:6596183.
- Kucukyilmaz E, Savas S. Evaluation of shear bond strength, penetration ability, microleakage and remineralisation capacity of glass ionomer-based fissure sealants. *Eur J Paediatr Dent*. 2016;17:17–23. PMID:26949234.
- Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res*. 2003;82(3):206–211. doi:10.1177/154405910308200311
- Cao CY, Mei ML, Li QL, Lo ECM, Chu CH. Methods for biomimetic remineralization of human dentine: A systematic review. *Int J Mol Sci*. 2015;16(3):4615–4627. doi:10.3390/ijms16034615
- Abou Neel EA, Aljabo A, Strange A, et al. Demineralization–remineralization dynamics in teeth and bone. *Int J Nanomed*. 2016;11:4743–4763. doi:10.2147/IJN.S107624
- Alhussain AM, Alhaddad AA, Ghazwi MM, Farooq I. Remineralization of artificial carious lesions using a novel fluoride incorporated bioactive glass dentifrice. *Dent Med Probl*. 2018;55(4):379–382. doi:10.17219/dmp/97311
- Richards D. The effectiveness of silver diamine fluoride in arresting caries. *Evid Based Dent*. 2017;18(3):70. doi:10.1038/sj.ebd.6401250
- Yadav RK, Bharti D, Tikku AP, et al. Comparative evaluation of remineralizing effect of fluoride and nonfluoride agents on artificially induced caries using different advanced imaging techniques. *J Conserv Dent*. 2022;25(1):26–31. doi:10.4103/jcd.jcd_426_21

27. Sinha N, Gupta A, Logani A, Shah N. Remineralizing efficacy of silver diamine fluoride and glass ionomer type VII for their proposed use as indirect pulp capping materials – Part II (A clinical study). *J Conserv Dent*. 2011;14(3):233–236. doi:10.4103/0972-0707.85796
28. Yilmaz N, Ocak M, Ökte Z. Remineralization of primary molar dentine with silver diamine fluoride and sodium fluoride: An in vitro study. *Cumhuriyet Dent J*. 2020;23(4):340–347. doi:10.7126/cumudj.796823
29. Chibinski AC, Wambier LM, Feltrin J, Loguercio AD, Wambier DS, Reis A. Silver diamine fluoride has efficacy in controlling caries progression in primary teeth: A systematic review and meta-analysis. *Caries Res*. 2017;51(5):527–541. doi:10.1159/000478668
30. Cross KJ, Huq NL, Reynolds EC. Casein phosphopeptides in oral health – chemistry and clinical applications. *Curr Pharm Des*. 2007;13(8):793–800. doi:10.2174/138161207780363086
31. Hegde MN, Gafoor F, Devadiga D. Resistance to demineralization of remineralizing agents on dentin – an atomic absorption spectrophotometric study. *J Pierre Fauchard Acad (India)*. 2014;28(4):110–113. doi:10.1016/j.jpfa.2014.12.001
32. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization – an in vitro study using scanning electron microscope and DIAGNOdent. *Indian J Dent Res*. 2011;22(1):77–82. doi:10.4103/0970-9290.80001

Effect of the contamination and cleaning methods on the tensile bond strength of the polyetheretherketone restorative material cemented with dual-cure resin cement

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Abstract

Background. These days, computer-aided design/computer-aided manufacturing (CAD/CAM) resin-based materials are being used daily in clinical practice for restorations in prosthetic dentistry. In comparison with ceramic materials, they are characterized by better stress distribution and decreased abrasion of the enamel of the opposing teeth. Consequently, they have been applied as alternative materials to ceramics in various dental restorations.

Objectives. The contamination of the indirect restorative material, which occurs at the clinical and dental laboratory stages, might deteriorate the bonding strength. The ideal surface treatment of the novel polyetheretherketone (PEEK) material for decontamination is yet unknown. The present study was conducted to evaluate the tensile bond strength (TBS) between PEEK and dual-cure self-adhesive resin cement, and determine the effect of contaminants, like temporary cement, artificial saliva and a fit checker, as well as the cleaning methods, like ultrasonic cleaning, phosphoric acid etching and universal cleaning paste (Ivoclean), on the bond.

Material and methods. Eighty PEEK disks were milled, having the final dimensions of 12 mm × 4 mm. The specimens were air-abraded with 50-micrometer aluminum oxide particles at a pressure of 2.8 bar for 15 s at a fixed distance of 10 mm, and then divided into 4 groups according to the contaminant used: temporary cement; artificial saliva; a fit checker; and a control group with no contamination. Furthermore, the first 3 groups were subsequently subdivided into 3 subgroups each according to the cleaning method applied: ultrasonic cleaning; phosphoric acid etching; and universal cleaning paste Ivoclean. The bonding of the specimens was done using dual-cure self-adhesive resin cement. The TBS of the different groups and subgroups was then measured at a crosshead speed of 2 mm/min in a universal testing machine (UTM), using a special test configuration.

Results. There was a significant interaction between both tested variables (the contamination and cleaning methods) ($p < 0.001$). The samples contaminated with artificial saliva showed a significantly higher TBS value than the samples subjected to other contaminants ($p = 0.005$). For the samples contaminated with temporary cement and a fit checker, there were significant differences between the different cleaning methods, with ultrasonic cleaning providing the highest TBS values, followed by phosphoric acid etching, and finally Ivoclean ($p < 0.001$).

Conclusions. Under the conditions of the present study, temporary cement and a fit checker adversely affected the TBS of PEEK, and ultrasonic cleaning was most effective for decontamination.

Keywords: contamination, PEEK, bonding, cleaning, tensile bond strength

Highlights

- The tensile bond strength (TBS) of the polyetheretherketone (PEEK) restorations cemented with resin was most significantly reduced by contamination from temporary cement and fit checker materials.
- Artificial saliva contamination had the least significant effect on the TBS of the resin-cemented PEEK restorations.
- To effectively remove all 3 types of contaminants, the use of an ultrasonic cleaner proved to be the most efficient method.

Introduction

These days, computer-aided design/computer-aided manufacturing (CAD/CAM) resin-based materials are being used daily in clinical practice for restorations in prosthetic dentistry.¹ In comparison with ceramic materials, they are characterized by better stress distribution and decreased abrasion of the enamel of the opposing teeth. Consequently, they have been applied as alternative materials to ceramics in various dental restorations.²

The novel high-performance composite polyetheretherketone (PEEK) is a polymer derived from the main group of polyaryletherketones (PAEKs), initially used in orthopedic and spinal implants. It is considered a biocompatible material, and it is chemically stable with regard to nearly all organic and inorganic chemicals.^{3,4} With a stiffness comparable to that of bone, PEEK has been used for fixation plates.⁵ It is mainly composed of an aromatic backbone molecular chain with ketone and ether functional groups interconnected to it.

The earlier literature on PEEK has shown that it has better chemical, thermal, biological, and mechanical properties – a high strength-to-weight ratio, a modulus of elasticity similar to that of bone and dentin, in addition to its zero corrosion rate and extremely low water absorption – in comparison to many restorative materials used today.⁶

Polyetheretherketone is used in dentistry for implants, provisional abutments and implant-supported bars. Recently, it has been found suitable for fixed dental prostheses (FDP) and resin-bonded fixed dental prostheses (RBFDP) as an interim treatment option, due to its high resiliency and a low modulus of elasticity. These properties may reduce stress concentration at the cementation interface and prevent debonding.⁷ Due to its low translucency and white opaque color, PEEK is not suitable for monolithic, esthetic dental restorations, and a composite veneering material or cemented ceramic crowns over PEEK frameworks are required to achieve satisfactory esthetics.⁸ Also, PEEK has low surface energy, and many studies have analyzed different surface conditioning methods to increase its surface free energy, and thus optimize its bond strength.^{9–11} Kern and Lehmann, studying the influence of surface conditioning on bonding to PEEK, demonstrated that after air abrasion and applying

a resin varnish with a methacrylate group, promising and durable bonding was achieved.¹² Uhrenbacher et al. also stated that the type of conditioning had a significant effect on the retention strength of PEEK.¹³ A clinical implication of their study is that airborne-particle abrasion (with 50-micrometer alumina) or etching with sulfuric acid (for 60 s) and methyl methacrylate (MMA)-based adhesive systems can be used for conditioning. In a review by Skirbutis et al., regarding articles published between 2010 and 2017 on PEEK and its characteristics, it was concluded that the highest bonding values were obtained by conditioning with airborne-particle abrasion and sulfuric acid surface treatment, as well as for crowns pretreated with the Signum® universal bond system and the visio.link adhesive system.⁹

Contamination reduces the adaptation between the restorative material and the bonded surface, thus inhibiting the formation of a long-term, stable bond.^{14–16} Therefore, the cleaning of both surfaces, or either the substrate or the material, is essential to improve the strength of the bond and its durability.¹⁶ During the try-in procedure, bonding to ceramics, zirconia and resins can be compromised by contamination. Saliva, blood, a silicone indicator, and die stone have been identified to reduce the bond strength of resins to restorations.^{17,18} Since the efficiency of the cleaning methods in removing contaminants from the adherend surface varies by method and material, it is critical for the clinician to use the most effective cleaning method for the material used prior to bonding to achieve the best bond strength of the restoration. Studies have determined the effect of different cleaning protocols on zirconia and ceramics.^{19,20}

Polyetheretherketone has significant advantages to be used for dental applications. However, a major clinical disadvantage is the difficulty in establishing strong and durable adhesion to other dental materials. Since there are 2 bonded interfaces with resin cement – the tooth structure and the veneering esthetic crown material – current studies focus on enhancing the PEEK surface for reacting with resins to allow optimal bonding.²¹ Bonding to PEEK is considered a challenge due to the contaminants present in the oral cavity, like saliva, temporary cement or a fit checker, and also its low surface energy, poor wetting capabilities and resistance to surface modification.

Debonding is one of the major failures in prosthetic dentistry, especially for PEEK with its low surface energy, which causes problems regarding surface treatment and conditioning.⁹ For that reason, this study was conducted to evaluate the tensile bond strength (TBS) between PEEK and dual-cure self-adhesive resin cement, and determine the effect of contaminants, like temporary cement, artificial saliva and a fit checker, as well as the cleaning methods, like ultrasonic cleaning, phosphoric acid etching and universal cleaning paste (Ivoclean), on the bond. The null hypothesis was that there would be no effect of the contaminants and the cleaning methods on the TBS between PEEK and the resin cement.

Material and methods

Power analysis

A power analysis, using G*Power, v. 3.1.9.7 (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>), was performed in order to calculate the smallest sample size necessary to test the null hypothesis: no difference in TBS between the tested groups. The sample size (N) was established at 60 samples (i.e., 6 samples per subgroup), with an alpha level $\alpha = 0.05$, a beta level $\beta = 0.2$ (i.e., a power of 80%) and an effect size $f = 0.554$. The calculation was based on the findings from a previous study.²² The sample size was raised to 80 samples (i.e., 8 samples per subgroup) to accommodate for the anticipated testing failures.

Preparation of specimens

A total of 80 milled PEEK disks (KERA® starPEEK; Eisenbacher Dentalwaren ED, Wörth am Main, Germany), with dimensions of 12 mm in diameter and 4 mm in thickness, were used in this study. The disks were divided into 4 main groups according to the contaminant used: group T ($n = 24$) – temporary cement (RelyX™ Temp NE, 3M ESPE, Maplewood, USA); group S ($n = 24$) – artificial saliva (prepared at the Laboratory of Biochemistry at Ain Shams University, Cairo, Egypt, using 1 L of double-distilled H₂O, 1.68020 g NaHCO₃, 0.41397 g NaH₂PO₄·H₂O, and 0.11099 g CaCl₂); group F ($n = 24$) – a fit checker (Fit Checker; GC International, Tokyo, Japan); and group C ($n = 8$) – the control group without contamination. The first 3 groups were further divided into 3 subgroups ($n = 8$) according to the cleaning method applied: subgroup U – ultrasonic cleaning (ultrasonic cleaner CD 4862; Codyson, Shenzhen, China); subgroup P – phosphoric acid etching (N-Etch; Ivoclar Vivadent, Shaan, Liechtenstein); and subgroup I – universal cleaning paste Ivoclean (Ivoclar Vivadent), which is composed of zirconium oxide (10–15 wt%), water (65–80 wt%), polyethylene glycol (8–10 wt%), and sodium hydroxide (≤ 1 wt%).

All study procedures were carried out by the same operator and according to the manufacturers' recommendations. The milled 80 PEEK disks were air-abraded with 50-micrometer aluminum oxide particles at a pressure of 2.8 bar for 15 s at a fixed distance of 10 mm, and then cleaned in an ultrasonic bath of distilled water for 3 min to remove any residues from their surfaces. Afterward, the specimens were air-dried with compressed air for 15 s to remove any remaining surface liquid.

Contamination

For group T, the temporary cement was mixed on a glass slab in a 1:1 ratio and applied with a spatula to the surface of each disk. After placing a glass slab on the cement, pressure was applied to it. To standardize the thickness of the cement layer and allow enough time for the cement to set, a load of 2 kg was applied for 5 min, using a cementation device, specially designed for applying pressure during the cementation process. The disks contaminated with the temporary cement were stored in water at 37°C for 24 h. For group S, artificial saliva was applied, rubbed for 60 s with a micro-brush into the surface of each disk, and then left undisturbed for 10 s. For group F, a fit checker was applied using an Automix gun and Automix tips on the surface of each disk, which were then stored in water at 37°C for 5 min.

Cleaning

The contaminants were removed from the disk surfaces of the 3 groups. Group S specimens were air-water-sprayed for 20 s, and then dried. The contaminants in groups T and F were removed with the tip of a blunt instrument. Each of these groups was further divided into 3 subgroups according to the cleaning method. For subgroups U, 8 disks from each contaminant group were immersed in an ultrasonic cleaner filled with 99% isopropanol alcohol, and left for 3 min. Then, each disk was air-dried for 15 s. For subgroups P, 8 disks from each contaminant group were cleaned using 35% phosphoric acid. Phosphoric acid was applied to the surfaces of the specimens with a tube tip and left on the surface for 1 min. Each disk was then cleaned with a water spray for 15 s and air-dried for 15 s. For subgroups I, 8 disks from each contaminant group were cleaned with Ivoclean, applied to the surface with a micro-brush, and left for 1 min. Each disk was then water-sprayed for 15 s and air-dried for 15 s.

Conditioning

In accordance with the clinical implication provided in a previous study,¹³ all specimens from all groups were conditioned before cementation with a MMA-based universal primer, visio.link (Bredent Group, Senden, Germany). The primer was rubbed into the air-abraded surfaces, using

an applicator brush for 10 s. The surfaces of the specimens were then air-dried for 5 s for thinning the primer layer, and subsequently light-cured for 40 s, using a dental curing unit with an output of 1,200 mW/cm² before cementation.

Bonding the specimens

3D-printed plastic tubes (Formlab2; Formlabs Inc., Somerville, USA), with an inner diameter of 3.2 mm and a length of 10 mm, were fabricated and filled with a dual-cure composite resin (MultiCore Flow; Ivoclar Vivadent). Seven minutes after filling the tubes, they were bonded to the pretreated PEEK disks under a load of 2 kg with the use of dual-cure self-adhesive cement (RelyX™ Unicem; 3M ESPE). A separating agent was applied to the 1-millimeter plastic tube border around the dual-cure composite resin to avoid any interference with bonding. Excess cement was removed and air-blocking gel (Liquid Strip; Ivoclar Vivadent) was applied around the bonding margins to prevent the formation of an oxygen inhibition layer on the luting resin. Light polymerization of all specimens was performed from 2 opposing sites for 20 s at a light intensity of 1,200 mW/cm².

Tensile bond strength testing

All specimens were stored in distilled water at 37°C for 24 h. This experimental study was carried out in accordance with the International Organization for Standardization (ISO) guidance ISO/TR 11405:1994.²³ The specimens were loaded with a tensile force at a cross-head speed of 2 mm/min until failure with a universal testing machine (UTM) (Z010/TN2A; ZwickRoell, Ulm, Germany). The tensile bond strength was calculated with the following formula: fracture load / bonded area (N/mm²). Since the bonded area was constant in all specimens, TBS was automatically calculated by the SCM3000 testing software (MICROTEST, Madrid, Spain).

Failure mode analysis

The fractured interfaces of the specimens were examined under a digital microscope (Dino-Lite; AnMo Electronics

Corp., New Taipei City, Taiwan) at ×40 magnification to determine the mode of failure. The failure modes were categorized into the following 3 types²⁴: adhesive failure (type 1); cohesive failure in the luting resin or PEEK (type 2); and mixed failure, when one area exhibited cohesive failure, while other areas exhibited an adhesive fracture (type 3).

Statistical analysis

Numerical data was represented as mean and standard deviation ($M \pm SD$) values. The Shapiro–Wilk test was used to check for normality. The homogeneity of variance was tested using Levene's test. The data showed parametric distribution and variance homogeneity, and was analyzed using the two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The comparisons of simple main effects were done utilizing the error term of the two-way model, with the p -value adjustment using the Bonferroni correction. The intergroup comparisons – to compare different groups with the control group – were done using the one-way ANOVA followed by Dunnett's post hoc test. The significance level was set at $p < 0.05$ for all tests. Statistical analysis was performed with the R statistical analysis software for Windows, v. 4.1.3.²⁵

Results

The results of the two-way ANOVA for the TBS values showed that there was a significant interaction between both tested variables (the contamination and cleaning methods) ($p < 0.001$). The comparisons of simple main effects showed that for the samples cleaned with an ultrasonic cleaner and Ivoclean, there were no significant differences with regard to the effects of the different contaminants ($p > 0.05$). However, for the samples treated with phosphoric acid, the difference was statistically significant ($p = 0.005$), and the post hoc pairwise comparisons showed the samples contaminated with artificial saliva to have a significantly higher mean TBS value than the samples subjected to other contaminants ($p = 0.005$) (Table 1).

Table 1. Comparisons of simple main effects with regard to the tensile bond strength (TBS) [MPa]

Cleaning method	Contaminant			f -value	p -value
	temporary cement	artificial saliva	fit checker		
Ultrasound cleaning	27.27 $\pm$ 2.62 ^{Aa}	28.00 $\pm$ 1.66 ^{Aa}	27.60 $\pm$ 2.54 ^{Aa}	0.20	0.821
Phosphoric acid etching	23.90 $\pm$ 1.62 ^{Bb}	27.20 $\pm$ 1.80 ^{Aa}	24.20 $\pm$ 2.39 ^{Bb}	6.90	0.005*
Ivoclean	20.90 $\pm$ 1.45 ^{Ac}	20.77 $\pm$ 1.94 ^{Ab}	20.00 $\pm$ 2.75 ^{Ac}	0.42	0.661
f -value	21.03	38.63	17.63	–	–
p -value	<0.001*	<0.001*	<0.001*	–	–

Data presented as mean $\pm$ standard deviation ($M \pm SD$).

Means with different upper- and lowercase superscript letters within the same horizontal row and vertical column, respectively, are statistically significantly different; * statistically significant ($p < 0.05$).

For the samples contaminated with temporary cement and a fit checker, there were significant differences between the different cleaning methods, with ultrasonic cleaning providing the highest TBS values, followed by phosphoric acid etching, and finally Ivoclean, and with all pairwise comparisons being statistically significant ($p < 0.001$). For the samples contaminated with artificial saliva, the difference was also statistically significant ($p < 0.001$), but the pairwise comparisons showed only the samples cleaned with Ivoclean to have a significantly lower mean TBS value than other subgroups ($p < 0.001$) (Table 1).

The results of the intergroup comparisons showed the samples contaminated with temporary cement and a fit checker, and treated with phosphoric acid, as well as all samples cleaned with Ivoclean, to be statistically significantly different from the control group ($p < 0.001$) (Table 2). The mean values with 95% confidence intervals (CIs) for TBS are presented in Fig. 1.

Discussion

Contamination during the try-in procedure makes surface conditioning before the cementation of the restorative material difficult.^{17,26,27} In a previous study, artificial saliva, temporary cement and a silicone-disclosing medium were shown to decrease the bond strength of restorations.²⁸ In the present study, eugenol-free temporary cement was used, since eugenol has been shown to significantly decrease the bond strength of restorative materials to resin cement.²⁷ Also, artificial saliva was used, including mainly inorganic components, such as calcium (Ca) and phosphate, as well as proteins in the form of immunoglobulin and the salivary amylase enzyme, since the use of human saliva in experimental studies may lead to ethical concerns, or problems in the reproducibility and standardization of experiments due to human variation.²⁹

The cleaning methods used in this study were chosen according to their availability in the clinic as a chairside procedure and due to their presence in the literature as methods for cleaning contamination off the surface of the restorative material to be cemented. Previous studies reported different cleaning methods, both mechanical and chemical, to improve the bond strength of the contaminated surfaces.^{30,31} The use of phosphoric acid was reported by Aboush to be the most beneficial method to remove saliva from the contaminated porcelain veneer surface.¹⁵ Wattanasirmit and Charasseangpaisarn stated that the saliva and silicone-disclosing medium contaminating zirconia should be cleaned with phosphoric acid, Ivoclean or hydrofluoric acid for 20 s prior to cementation.³² In a study by Phark et al., combining the ultrasonic cleaner isopropanol bath and phosphoric acid proved to be an efficient method for cleaning the contaminated zirconia surfaces.³³ The bond strength of resin cement

Table 2. Intergroup comparisons with regard to the tensile bond strength (TBS)

Group	TBS [MPa]
Control	28.17 ± 1.93
Ultrasonic cleaning–temporary cement	27.27 ± 2.62
Ultrasonic cleaning–artificial saliva	28.00 ± 1.66
Ultrasonic cleaning–fit checker	27.60 ± 2.54
Phosphoric acid etching–temporary cement	23.90 ± 1.62*
Phosphoric acid etching–artificial saliva	27.20 ± 1.80
Phosphoric acid etching–fit checker	24.20 ± 2.39*
Ivoclean–temporary cement	20.90 ± 1.45*
Ivoclean–artificial saliva	20.77 ± 1.94*
Ivoclean–fit checker	20.00 ± 2.75*

Data presented as $M \pm SD$.

*significantly different from the control group ($p < 0.05$).

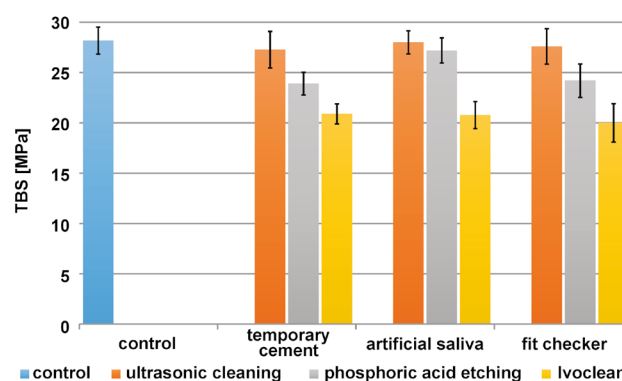


Fig. 1. Bar chart showing the mean and confidence interval values (error bars) for the tensile bond strength (TBS)

to the saliva-contaminated lithium disilicate ceramic etched with hydrofluoric acid was proven by Yoshida to be restored when cleaned with phosphoric acid and the Ivoclean gel.³⁴

The grouping system in this study was applied in accordance with other previous studies measuring the TBS of different materials.^{35,36} The bonding protocol used was based on a previous study by Kern and Lehmann, who concluded that durable bonding to PEEK could be achieved using a multifunctional methacrylate-containing resin varnish on the air-abraded PEEK surfaces.¹² The visio.link primer has a good wetting ability that allows good mechanical interlocking in the micropores of the surface. It contains MMA and a highly reactive triacrylate monomer, pentaerythritol triacrylate (PETIA), which can penetrate the resin matrix of the polymeric restoration material and create entanglements that function as mechanical connections. Furthermore, visio.link allows covalent bonding to methacrylate in polymeric restoration materials and resin composite cement, providing high crosslinking density at the interface and good mechanical properties.³⁷

The null hypothesis was rejected, since the contamination and cleaning methods had a significant effect on the TBS between PEEK and dual-cure self-adhesive resin cement.

The results of the current study showed that contamination with temporary cement and a fit checker had the greatest effect on the TBS of PEEK cemented with resin when cleaned with phosphoric acid and Ivoclean. The difference compared with the control group was significant. Residues may affect the wettability of the adherend surface with the self-adhesive resin cement, and act as a barrier that inhibits the interactions between the inorganic fillers of the self-adhesive resin cement and the organic monomers in the polymer matrix.^{27,38} Ultrasonic cleaning was the most effective cleaning method to remove all 3 contaminants. Güers et al. found that cleaning was efficient with combining mechanical and chemical cleaning methods, and that isopropanol was an excellent solvent and left almost no oil traces on the surface.²⁰ Other studies have found that an ultrasonic bath triggers the release of larger particles, as well as some sub-micron particles, significantly reducing the amount of contaminant left on the adherend surface.³⁹

Contamination with artificial saliva had the least significant effect on the TBS of PEEK cemented with resin, and the difference compared with the control group was not significant except for the combination with Ivoclean. This can be explained by the fact that PEEK is a polymer with which artificial saliva does not react chemically, as it has no outer oxide layer that can be infiltrated by saliva; it merely affects its hydrophilicity. Unlike zirconia, where artificial saliva significantly affects its TBS to resin cement due to the phospholipids present in saliva that bond to and occupy the outer oxide layer of zirconia, leaving little remaining oxide layer space that visio.link could bond to.²⁹

There was a slight interaction between artificial saliva and PEEK, which was easily diminished by phosphoric acid. It is possible that the acid penetrates the salivary film and etches the adherend surface underneath it.¹⁵ Regarding Ivoclean, its composition is specially designed to remove saliva contamination. The zirconium oxide particles in Ivoclean can strongly interact with the phosphate groups in the salivary film and remove the film from the adherent surface.²⁹

This study used a tensile test to evaluate the adhesive capacity of the material rather than, for instance, the stress created during clinical function. However, intraorally, indirect restorations are subjected to different forces, such as tensile, shear, compressive, oblique, and combinations of these types. This study evaluated only tensile forces. The interpretation of the current TBS results is that the different contaminants affected the bond strength differently, depending on the cleaning method.

The results of this research are in agreement with previous papers studying different restorative materials other

than PEEK under similar conditions. Temporary cement and a fit checker affected the TBS of PEEK more than artificial saliva, and the ultrasonic isopropanol cleaner bath had a better cleaning effect than phosphoric acid or Ivoclean. In this article, TBS was measured in vitro, without thermal or mechanical load cycling. The experiments involving thermocycling and mechanical loading provide a better evaluation of the bond strength, so we recommend more research on the long-term stability of the bond strength, using different aging protocols.

Conclusions

Within the limitations of this study, the following conclusions could be drawn:

Temporary cement and a fit checker adversely affected the TBS of PEEK, more than artificial saliva.

An ultrasonic cleaner effectively decontaminates the PEEK surface and enhances the TBS to resin cement.

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. Miyazaki T, Hotta Y, Kunii J, Kuriyama S, Tamaki Y. A review of dental CAD/CAM: Current status and future perspectives from 20 years of experience. *Dent Mater J*. 2009;28(1):44–56. doi:10.4012/dmj.28.44
2. Magne P, Schlichting LH, Maia HP, Baratieri LN. In vitro fatigue resistance of CAD/CAM composite resin and ceramic posterior occlusal veneers. *J Prosthet Dent*. 2010;104(3):149–157. doi:10.1016/S0022-3913(10)60111-4
3. Kurtz SM, Devine JN. PEEK biomaterials in trauma, orthopedic, and spinal implants. *Biomaterials*. 2010;28(32): 4845–4869. doi:10.1016/j.biomaterials.2007.07.013
4. Kurtz SM, ed. *PEEK Biomaterials Handbook*. Norwich, NY: Elsevier/William Andrew; 2011.
5. Skinner HB. Composite technology for total hip arthroplasty. *Clin Orthop Relat Res*. 1988;235:224–236. PMID:3416528.

6. Wiesli MG, Özcan M. High-performance polymers and their potential application as medical and oral implant materials: A review. *Implant Dent.* 2015;24(4):448–457. doi:10.1097/ID.0000000000000285
7. Zoidis P, Papatthanasiau I. Modified PEEK resin-bonded fixed dental prosthesis as an interim restoration after implant placement. *J Prosthet Dent.* 2016;116(5):637–641. doi:10.1016/j.prosdent.2016.04.024
8. Gama LT, Duque TM, Özcan M, Philippi AG, Mendonça Mezzomo LA, Vega Gonçalves TM. Adhesion to high-performance polymers applied in dentistry: A systematic review. *Dent Mater.* 2020;36(4):e93–e108. doi:10.1016/j.dental.2020.01.002
9. Skirbutis G, Dzingutė A, Masiliūnaitė V, Šulcaitė G, Žilinskas J. PEEK polymer's properties and its use in prosthodontics. A review. *Stomatologija.* 2018;20(2):54–58. PMID:30531169.
10. Henriques B, Fabris D, Mesquita-Guimarães J, et al. Influence of laser structuring of PEEK, PEEK-GF30 and PEEK-CF30 surfaces on the shear bond strength to a resin cement. *J Mech Behav Biomed Mater.* 2018;84:225–234. doi:10.1016/j.jmbbm.2018.05.008
11. Tsuka H, Morita K, Kato K, et al. Effect of laser groove treatment on shear bond strength of resin-based luting agent to polyetheretherketone (PEEK). *J Prosthodont Res.* 2019;63(1):52–57. doi:10.1016/j.jpor.2018.08.001
12. Kern M, Lehmann F. Influence of surface conditioning on bonding to polyetheretherketone (PEEK). *Dent Mater.* 2012;28(12):1280–1283. doi:10.1016/j.dental.2012.09.010
13. Uhrenbacher J, Schmidlin PR, Keul C, et al. The effect of surface modification on the retention strength of polyetheretherketone crowns adhesively bonded to dentin abutments. *J Prosthet Dent.* 2014;112(6):1489–1497. doi:10.1016/j.prosdent.2014.05.010
14. Duarte SJ, Lolato AL, Buainain de Freitas CR, Dinelli W. SEM analysis of internal adaptation of adhesive restorations after contamination with saliva. *J Adhes Dent.* 2005;7(1):51–56. PMID:15892364.
15. Aboush YE. Removing saliva contamination from porcelain veneers before bonding. *J Prosthet Dent.* 1998;80(6):649–653. doi:10.1016/s0022-3913(98)70050-2
16. Quaas AC, Yang B, Kern M. Panavia F 2.0 bonding to contaminated zirconia ceramic after different cleaning procedures. *Dent Mater.* 2007;23(4):506–512. doi:10.1016/j.dental.2006.03.008
17. Yang B, Lange-Jansen HC, Scharnberg M, et al. Influence of saliva contamination on zirconia ceramic bonding. *Dent Mater.* 2008;24(4):508–513. doi:10.1016/j.dental.2007.04.013
18. Eiriksson SO, Pereira PN, Swift EJ, Heymann HO, Sigurdsson A. Effects of blood contamination on resin–resin bond strength. *Dent Mater.* 2004;20(2):184–190. doi:10.1016/s0109-5641(03)00090-3
19. Szep S, Schmid C, Weigl P, Hahn L, Heidemann D. Effect of the silicone disclosing procedure on the shear bond strength of composite cements to ceramic restorations. *J Prosthet Dent.* 2003;89(1):60–65. doi:10.1067/mpr.2003.1
20. Güers P, Wille S, Strunskus T, Polonskyi O, Kern M. Durability of resin bonding to zirconia ceramic after contamination and the use of various cleaning methods. *Dent Mater.* 2019;35(10):1388–1396. doi:10.1016/j.dental.2019.07.027
21. Caglar I, Ates SM, Duymus ZY. An in vitro evaluation of the effect of various adhesives and surface treatments on bond strength of resin cement to polyetheretherketone. *J Prosthodont.* 2019;28(1):e342–e349. doi:10.1111/jopr.12791
22. Schmidlin PR, Stawarczyk B, Wieland M, Attin T, Hämmerle CH, Fischer J. Effect of different surface pre-treatments and luting materials on shear bond strength to PEEK. *Dent Mater.* 2010;26(6):553–559. doi:10.1016/j.dental.2010.02.003
23. International Organization for Standardization (ISO). Dental materials – guidance on testing of adhesion to tooth structure. ISO/TR 11405:1994.
24. Kharouf N, Rapp G, Mancino D, Hemmerlé J, Haikel Y, Reitzer F. Effect of etching the coronal dentin with the rubbing technique on the microtensile bond strength of a universal adhesive system. *Dent Med Probl.* 2019;56(4):343–348. doi:10.17219/dmp/111697
25. R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>. Accessed February 1, 2023.
26. Yang B, Scharnberg M, Wolfart S, et al. Influence of contamination on bonding to zirconia ceramic. *J Biomed Mater Res B Appl Biomater.* 2007;81(2):283–290. doi:10.1002/jbm.b.30664
27. Takimoto M, Ishii R, Iino M, et al. Influence of temporary cement contamination on the surface free energy and dentine bond strength of self-adhesive cements. *J Dent.* 2012;40(2):131–138. doi:10.1016/j.jdent.2011.11.012
28. Angkasi P, Burgess JO, Bottino MC, Lawson NC. Cleaning methods for zirconia following salivary contamination. *J Prosthodont.* 2016;25(5):375–379. doi:10.1111/jopr.12441
29. Kawaguchi-Uemura A, Mine A, Matsumoto M, et al. Adhesion procedure for CAD/CAM resin crown bonding: Reduction of bond strengths due to artificial saliva contamination. *J Prosthodont Res.* 2018;62(2):177–183. doi:10.1016/j.jpor.2017.08.006
30. Wolfart M, Lehmann F, Wolfart S, Kern M. Durability of the resin bond strength to zirconia ceramic after using different surface conditioning methods. *Dent Mater.* 2007;23(1):45–50. doi:10.1016/j.dental.2005.11.040
31. Özdemir H, Yanikoğlu N, Sağsöz N. Effect of MDP-based silane and different surface conditioner methods on bonding of resin cements to zirconium framework. *J Prosthodont.* 2019;28(1):79–84. doi:10.1111/jopr.12650
32. Wattanasirmit K, Charasseangpaisarn T. Effect of different cleansing agents and adhesive resins on bond strength of contaminated zirconia. *J Prosthodont Res.* 2019;63(3):271–276. doi:10.1016/j.jpor.2018.12.007
33. Phark JH, Duarte S Jr., Blatz M, Sadan A. An in vitro evaluation of the long-term resin bond to a new densely sintered high-purity zirconium-oxide ceramic surface. *J Prosthet Dent.* 2009;101(1):29–38. doi:10.1016/S0022-3913(08)60286-3
34. Yoshida K. Influence of cleaning methods on the bond strength of resin cement to saliva-contaminated lithium disilicate ceramic. *Clin Oral Investig.* 2019;24(6):2091–2097. doi:10.1007/s00784-019-03074-w
35. Stawarczyk B, Keul C, Beuer F, Roos M, Schmidlin PR. Tensile bond strength of veneering resins to PEEK: Impact of different adhesives. *Dent Mater J.* 2013;32(3):441–448. doi:10.4012/dmj.2013-011
36. Schwindling FS, Stober T, Rustemeier R, Schmitter M, Rues S. Retention behavior of double-crown attachments with zirconia primary and secondary crowns. *Dent Mater.* 2016;32(5):695–702. doi:10.1016/j.dental.2016.03.002
37. Bähr N, Keul C, Edelhoff D, et al. Effect of different adhesives combined with two resin composite cements on shear bond strength to polymeric CAD/CAM materials. *Dent Mater J.* 2013;32(3):492–501. doi:10.4012/dmj.2012-329
38. Sheth J, Jensen M, Tolliver D. Effect of surface treatment on etched porcelain bond strength to enamel. *Dent Mater.* 1988;4(6):328–337. doi:10.1016/s0109-5641(88)80045-9
39. Wanderley Lima RB, Barreto SC, Hajhamid B, de Souza GM, de Goes MF. Effect of cleaning protocol on silica deposition and silica-mediated bonding to Y-TZP. *Dent Mater.* 2019;35(11):1603–1613. doi:10.1016/j.dental.2019.08.099

Influence of various nanosilica coating techniques on the zirconia surface and bond strength to resin cement

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Abstract

Background. Zirconia restorations have attracted considerable attention because of their favorable mechanical and esthetic properties, which meet the requirements for both anterior and posterior restorations.

Objectives. This study aimed to evaluate the microshear bond strength (μ SBS) between resin cement and zirconia surfaces dip-coated with nanosilica and nanozirconia suspensions of different compositions.

Material and methods. An extra translucent (XT) zirconia disc composed of 5 mol% yttrium-stabilized zirconia was sectioned into 66 samples and divided into 6 groups: untreated zirconia as the control group; zirconia blasted with silica-coated alumina; and zirconia dip-coated with nanosilica–nanozirconia suspensions at volume ratios of 100:0, 75:25, 50:50, and 25:75. The coated surfaces were analyzed using energy-dispersive X-ray spectroscopy (EDS), field emission scanning electron microscopy (FESEM), atomic force microscopy (AFM), and X-ray diffraction (XRD). Resin cement cylinders were bonded to the treated zirconia surfaces, and μ SBS testing was performed. The differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with the significance level set at $p \leq 0.05$.

Results. The highest μ SBS was observed for the 50:50 nanosilica–nanozirconia group (51.63 ± 5.56 MPa), whereas the lowest values were recorded for untreated zirconia (17.38 ± 3.74 MPa). Zirconia blasted with commercially available silica-coated alumina showed μ SBS of 31.00 ± 6.54 MPa.

Conclusions. Dip coating with nanosilica–nanozirconia suspensions appears to be a feasible method for improving resin–zirconia bonding while preserving the zirconia structure. Among the tested compositions, the 50:50 nanosilica–nanozirconia suspension produced the highest μ SBS.

Keywords: microshear bond strength, nanosilica coating, nanozirconia, resin–ceramic bonding

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Highlights

- The study aimed to identify a practical and low-cost method of increasing the shear bond strength between resin and zirconia using readily available materials.
- The dip-coating technique effectively modifies zirconia surface characteristics by increasing surface roughness.
- Dip coating with equal amounts of zirconia and silica nanoparticles improves the shear bond strength of zirconia.

Introduction

Zirconia has become one of the most widely used ceramic materials in dentistry owing to its favorable mechanical properties, high fracture strength, esthetics, and biocompatibility.^{1,2} Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) is widely used as an alternative to metal-ceramic materials for anterior and posterior restorations. Zirconia is available in various brands and translucency levels, which exhibit different mechanical properties.^{3,4} Highly translucent zirconia restorations should generally be limited to abutments with a color similar to that of the planned definitive restoration, whereas less translucent zirconia may be used in areas where high translucency is not required; both have demonstrated clinical success.^{5–7}

However, achieving durable resin bonding to zirconia remains a major challenge because of its chemically inert surface and its resistance to roughening with hydrofluoric acid (HF), owing to the absence of silica, unlike other types of ceramic.^{3,4} Conventional methods for zirconia surface treatment have their own limitations and drawbacks, providing a rationale for investigating other approaches.^{5,6}

Zirconia surface treatments should provide adequate shear bond without adversely affecting the microstructure or integrity of the treated material.⁷ A widely used protocol for achieving durable bonding to zirconia consists of sandblasting followed by primer application. However, sandblasting may be considered a double-edged approach. Although it can enhance bond strength, it may adversely affect the mechanical properties and reliability of zirconia by causing surface damage, inducing tetragonal-to-monoclinic phase transformation, introducing surface flaws, altering surface topography, and changing surface chemistry through alumina contamination.^{7,8}

To achieve durable bonding between a ceramic material and resin cement, 2 fundamental requirements should be met: micromechanical interlocking and chemical bonding.⁹ The former can be achieved through appropriate surface treatment, including sandblasting and HF etching, while chemical bonding is promoted by primers that facilitate adhesion between hydrophobic resin cement and different ceramic computer-aided design/computer-aided manufacturing (CAD/CAM) materials. In silica-based ceramics, bonding protocols generally involve HF etching, which removes the glassy phase of the ceramic matrix and creates surface roughness (Sa) to enhance mechanical bonding, followed by the application of silane coupling

agent to promote chemical bonding to resin-based materials.^{10–12} For oxide ceramics such as zirconia, various surface-roughening methods can be used to enhance mechanical bonding, whereas primers containing 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) promote chemical bonding to the zirconia surface.

Resin bonding to zirconia can be improved by applying silica coatings to the zirconia surface,^{13,14} although currently available techniques have significant limitations. Tribochemical silica coating introduces silica onto the zirconia surface while simultaneously increasing Sa, thereby enhancing bonding.¹⁵ Particle bombardment may also induce phase transformation at the zirconia surface and adversely affect its mechanical properties.¹⁶ Another approach is sol–gel deposition, which uses silicon alkoxide precursors to produce smooth silica coatings. However, these uniform films may crack during high-temperature sintering, potentially compromising their performance.^{17,18} Other techniques, such as reactive sputtering and chemical vapor deposition, can also be used to deposit silica coatings but require expensive, specialized equipment that is not readily available in dental laboratories.^{19,20} More recently, research has focused on nanoscale silica coatings and composite coatings as potential approaches to improving bonding. Fusion sputtering uses an air–water jet to spray zirconia nanoparticles onto unsintered zirconia, creating a porous layer that provides micromechanical retention after sintering.¹⁵ However, controlling the thickness and homogeneity of this nanoparticulate layer is difficult. Similarly, applying nanosilica–zirconia composites with a microbrush followed by sintering can improve adhesion, but achieving consistent coating remains challenging.²¹

Silica coatings have demonstrated the potential to enhance resin bonding to zirconia; however, existing approaches have not yet achieved an optimal balance between strong and durable adhesion and preservation of the favorable surface and mechanical properties of zirconia. Further investigation of novel nanoscale surface modifications is therefore warranted to develop coatings capable of effectively conditioning the chemically inert zirconia surface while minimizing surface damage. Optimized nanoscale coatings may broaden the potential applications of adhesive bonding to zirconia restorations. Dip coating of zirconia with silica has recently emerged as a promising approach because it may improve resin bonding without damaging the zirconia surface while creating a glassy layer that can be chemically modified.^{22,23} Microshear bond

strength (μ SBS) testing is more reliable for clinical applications. Specimens are thought to follow an all-or-none principle, as if any defect introduced during sample preparation may result in immediate failure during testing, which is preferable to obtaining false-positive results. Therefore, the smaller specimen diameter reduces the likelihood of complex stress generation, resulting in a greater proportion of adhesive rather than mixed failures.²⁴

The purpose of this study was to evaluate the effects of coating zirconia with different concentrations of nanosilica on its surface characteristics and to compare these effects with those obtained using commercially available products recommended for zirconia surface treatment. The null hypothesis was that there would be no differences among zirconia specimens coated with different concentrations of nanosilica and those treated with silica-coated alumina blasting in terms of surface characteristics or μ SBS.

Material and methods

Extra translucent zirconia (VITA YZ[®] XT; VITA ZahnFabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany), silica and zirconia nanopowders (US Research Nanomaterials, Inc., Houston, USA), silica-coated alumina (CoSil; Veloplex International, London, UK), Z-Prime Plus (BISCO, Inc., Schaumburg, USA), and self-adhesive resin cement (G-CEM ONE[™]; GC Corporation, Tokyo, Japan) were used in the study.

Zirconia sample preparation

A zirconia disc (VITA YZ[®] XT), 100 mm in diameter and 18 mm in height, was cut under water cooling into 66 samples measuring 8 mm × 8 mm × 2.5 mm using a cutting machine (MTI Corporation, Richmond, USA). All specimens were polished using dual-speed grinder-polisher (MDP200; Anhui Future International Trading Co. Ltd., Bengbo, China) up to 1,200 grit and then cleaned in an ultrasonic bath (GT SONIC, Shenzhen, China) for 10 min. The specimens were subsequently dried in an oven at 100°C for 1 h.

The samples were grouped according to the composition of the dipping suspension and the proportions of its nano-oxide particles (SiO_2 and ZrO_2). Eleven specimens from each group were dipped simultaneously in the same suspension to ensure standardization (Table 1).

Preparation of zirconia dipping suspensions

Twenty milliliters of 99% methanol was mixed with the required amounts of nanosilica and nanozirconia for each group. The mixture was stirred using a magnetic stirrer for 30 min, after which the suspensions were sonicated in

Table 1. Zirconia dip-coating groups with different nanosilica and nanozirconia compositions

Group	Surface treatment/coating composition	SiO_2 [Vf%]	ZrO_2 [Vf%]
A	SiO_2	100	0
B	$\text{SiO}_2 + \text{ZrO}_2$	75	25
C	$\text{SiO}_2 + \text{ZrO}_2$	50	50
D	$\text{SiO}_2 + \text{ZrO}_2$	25	75
E	ZrO_2 blasted with CoSil	–	–
Control	untreated XT zirconia	–	–

an ultrasonic bath for 2 h. Subsequently, 1 mL of polyvinyl alcohol (PVA) was added as a binder, and the suspension was mixed again using the magnetic stirrer for 30 min. Finally, a high-power ultrasonic probe (Hielscher Ultrasonics GmbH, Teltow, Germany) was used for 10 min to obtain a homogenous suspension that remained stable for a longer period without settling. The dipping time was set at 6 min based on preliminary trials, in which a dipping time of 6 min provided the highest μ SBS.

Zirconia sample sintering

All zirconia specimens were sintered (VITA ZYRCOMAT[®] 6000 MS; VITA ZahnFabrik H. Rauter GmbH & Co. KG) for 120 min at 1,450°C according to the manufacturer's instructions (Fig. 1).

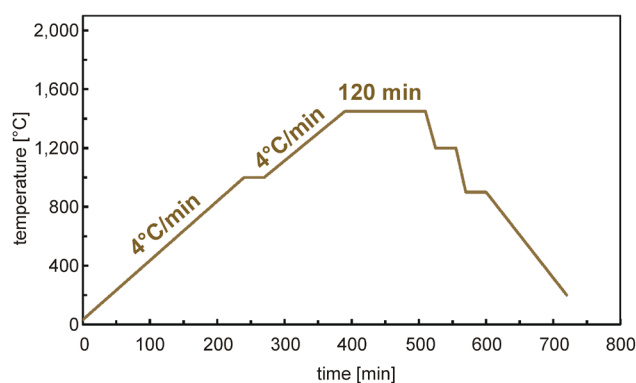


Fig. 1. Sintering program for extra translucent (XT) zirconia

Characterization

Three specimens were randomly selected from each group for characterization using energy dispersive X-ray spectroscopy (EDS) (Axia ChemiSEM System; Thermo Fisher Scientific, Eindhoven, Netherlands), field emission scanning electron microscopy (FESEM) (Inspect[™] F50; FEI Company, Hillsboro, USA), atomic force microscopy (AFM) (NaioAFM; Nanosurf, Liestal, Switzerland), and X-ray diffraction (XRD) (Shimadzu XRD-6000; Shimadzu Corporation, Kyoto, Japan).

Microshear bond strength testing

Microshear bond strength testing was used to quantify the interfacial adhesion between the resin cement and zirconia substrates after different surface treatments. This method applies an increasing shear load to a small cross-sectional bonding area until failure occurs, allowing a localized assessment of bond strength.¹⁹ Resin cement (G-CEM ONE™) was used to create cylindrical specimens that were polymerized and bonded to the primed zirconia surfaces. A universal testing machine fitted with a knife-edge shearing tool²⁵ was used to measure the μ SBS at a crosshead speed of 0.5 mm/min. The load at failure was recorded in newtons [N] and divided by the cylindrical bond area to calculate the μ SBS in megapascals [MPa]. This standardized microshear testing protocol allowed precise, localized measurement of bond strength at the adhesive interface between the resin cement and nanosilica-coated zirconia surface. The test provided a measure of interfacial bond strength and assessed the durability and fatigue resistance of the bonded restorations.

Statistical analysis

Data was statistically analyzed using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). The normality of the data was assessed using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was used to compare the groups, followed by Tukey's post hoc test. The significance level was set at $p \leq 0.05$.

Results

Microstructure analysis

Scanning electron microscopy provided high-resolution images of the zirconia surface morphology after different coating treatments. On untreated polished zirconia, FESEM revealed a smooth, flat surface with only minor scratches and grooves from the polishing process (Fig. 2). At high magnification, the zirconia grains were tightly packed and clearly visible, with grain boundaries in close contact and no apparent gaps or defects. In contrast, in group E, in which zirconia was sandblasted with CoSil using the tribochemical silica-coating method, Sa was markedly increased, obscuring the underlying zirconia grain structure (Fig. 3). The particle bombardment created an irregular, porous surface with peaks, valleys and protruding features. No smooth or flat areas remained after abrasive blasting with the silica-coated alumina particles.

Group A, in which zirconia was dip-coated with a 100% nanosilica suspension, showed an island-like coating that partially covered the zirconia surface (Fig. 4). Within this coating, the islands contained pores and cavities, and these nanostructures were perpendicularly oriented. The coating showed incomplete coverage and high porosity, facilitating micro-mechanical interlocking with resin cement, with some areas of the zirconia surface remaining exposed for chemical bonding interactions.

After nanozirconia was added to the dipping suspension (75% SiO₂ + 25% ZrO₂) in group B, the surface morphology changed from an island-like appearance to a more compact structure, with a decrease in the amount of exposed zirconia substrate (Fig. 5). The surface topography of the zirconia coating changed, as indicated by the absence of the scattered pattern observed in specimens coated with pure silica. In addition, the coating islands appeared to be more closely attached to the zirconia substrate, although some areas of the substrate remained uncovered. At high magnification ($\times 10,000$), nanozirconia particles were observed dispersed within the melted silica (fused silica).

As the proportion of zirconia nanoparticles in the dipping suspension increased to 50 Vf% in group C, the surface topography showed reduced spacing and closer approximation among islands, resulting in a decrease in the amount of exposed zirconia substrate (Fig. 6). At high magnification ($\times 10,000$), a greater number of zirconia particles were observed distributed within the melted silica, with porosities formed within the coating.

With a further increase in the zirconia content of the suspension to 75% Vf% in group D, no significant changes were observed at low magnification ($\times 150$), although a slight reduction in surface coating cracks was noted (Fig. 7). At high magnification ($\times 10,000$), the melted silica appeared to be densely loaded with nanozirconia particles, with porosities present within the coating.

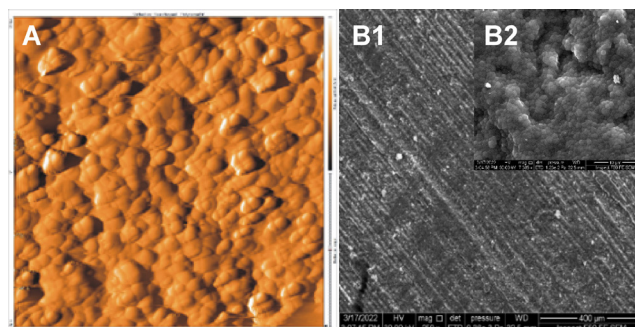


Fig. 2. Untreated extra translucent (XT) zirconia

A. Atomic force microscopy (AFM) image; B1. Field emission scanning electron microscopy (FESEM) image at $\times 250$ magnification; B2. FESEM image at $\times 10,000$ magnification.

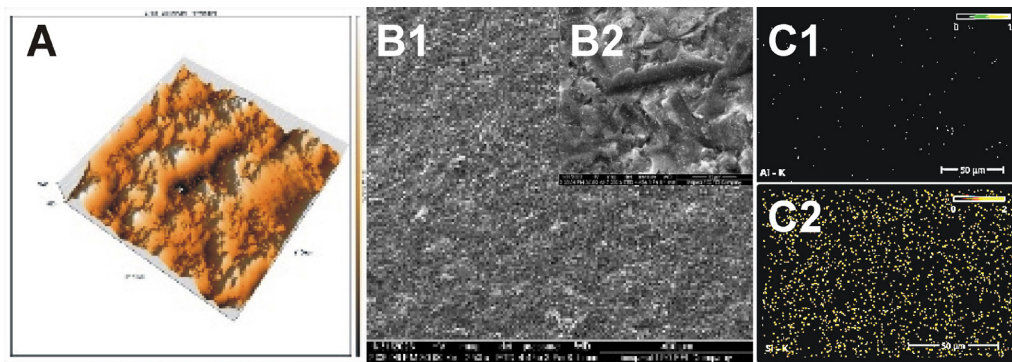


Fig. 3. Zirconia blasted with CoSi (group E)

A. AFM image; B1. FESEM image at $\times 150$ magnification; B2. FESEM image at $\times 10,000$ magnification; C1. Energy dispersive X-ray spectroscopy (EDS) mapping of alumina; C2. EDS mapping of silica.

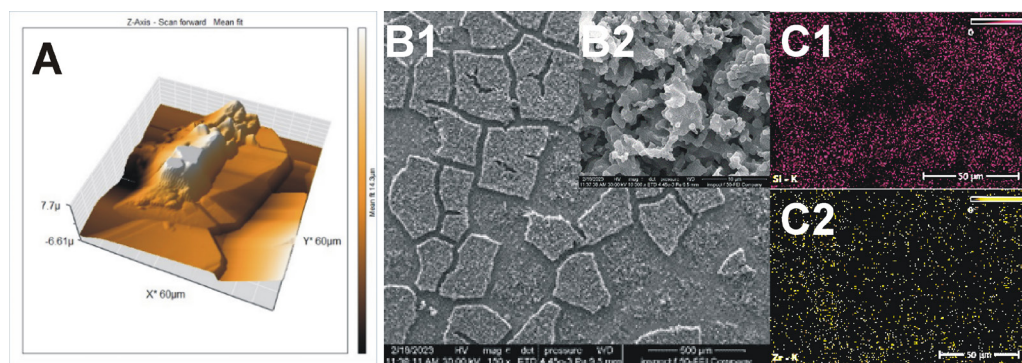


Fig. 4. Zirconia dip-coated with 100% SiO_2 (group A)

A. AFM image; B1. FESEM image at $\times 150$ magnification; B2. FESEM image at $\times 10,000$ magnification; C1. EDS mapping of silica; C2. EDS mapping of zirconia.

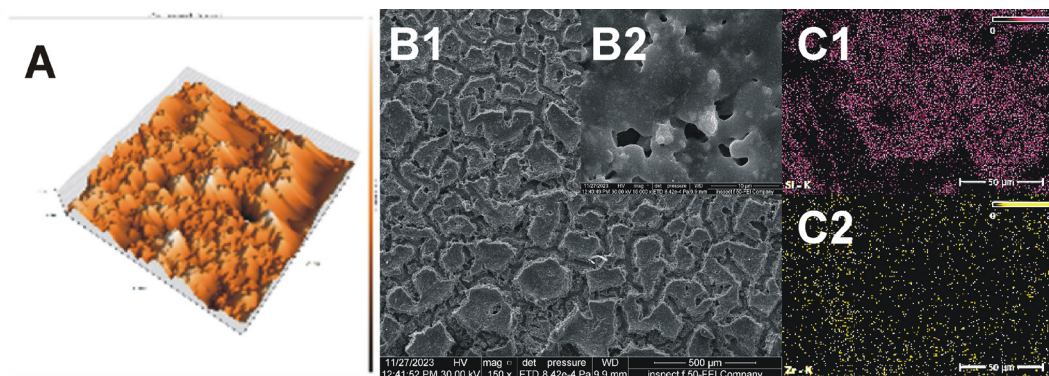


Fig. 5. Zirconia dip-coated with 75% SiO_2 + 25% ZrO_2 (group B)

A. AFM image; B1. FESEM image at $\times 150$ magnification; B2. FESEM image at $\times 10,000$ magnification; C1. EDS mapping of silica; C2. EDS mapping of zirconia.

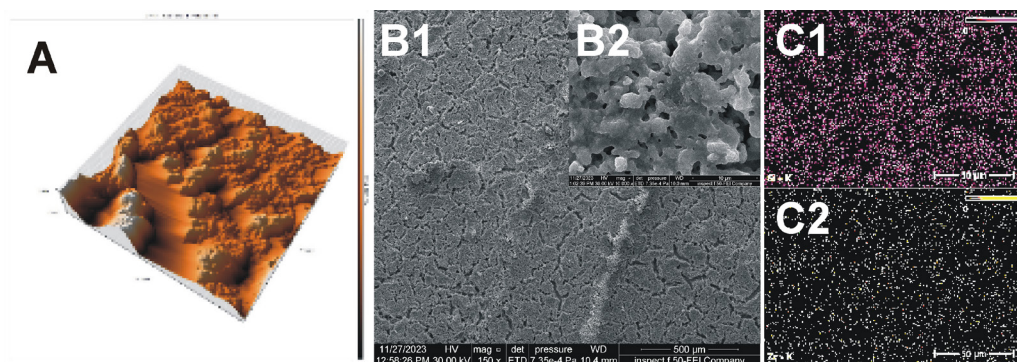


Fig. 6. Zirconia dip-coated with 50% SiO_2 + 50% ZrO_2 (group C)

A. AFM image; B1. FESEM image at $\times 150$ magnification; B2. FESEM image at $\times 10,000$ magnification; C1. EDS mapping of silica; C2. EDS mapping of zirconia.

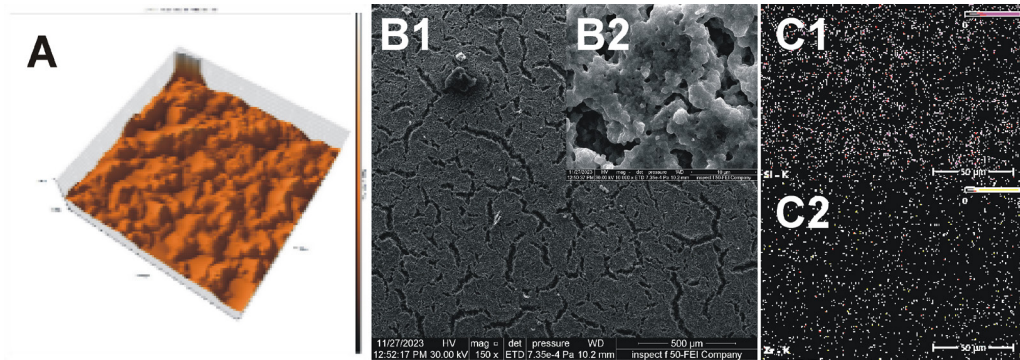


Fig. 7. Zirconia dip-coated with 25% SiO₂ + 75% ZrO₂ (group D)

A. AFM image; B1. FESEM image at $\times 150$ magnification; B2. FESEM image at $\times 10,000$ magnification; C1. EDS mapping of silica; C2. EDS mapping of zirconia.

Elemental analysis

The EDS spectra (Table 2) showed the weight percentages of oxygen, yttrium, hafnium, and zirconium, with an insignificant percentage of detectable silicon and aluminum on the surface of untreated zirconia. For group E, in which the zirconia surface was blasted with silica-coated alumina, EDS revealed the presence of silicon and aluminum in addition to the elements detected on the untreated zirconia surface. In group A, the amount of silicon was higher than that in group E. Groups B, C and D showed different proportions of silicon and zirconium. EDS mapping demonstrated the distribution of the nanoparticles within the coating of each group (Fig. 8).

XRD characterization

X-ray diffraction analysis provided critical insights on the crystallographic phases present in zirconia after different nanocoating treatments (Fig. 9). By measuring diffraction peak angles and intensities, the atomic lattice structure was characterized. For untreated polished zirconia controls, distinct XRD peaks were observed at 2θ values of 30.3° , 35.2° , 50.5° , 60.0° , and 62.8° , corresponding to the (101), (110), (112), (211), and (202) crystal planes of the metastable tetragonal zirconia phase.¹⁸

Table 2. Elemental composition of zirconia surfaces determined by energy dispersive X-ray spectroscopy (EDS)

Group	O	Al	Si	Zr	Y	Hf
A	50.2	1.1	9.8	38.7	–	–
B	33.4	0.0	16.8	44.0	5.8	–
C	24.7	0.0	8.6	59.8	6.9	–
D	23.1	0.0	4.6	66.5	5.8	–
E	34.7	2.2	0.6	62.3	–	–
Control	59.4	0.0	0.4	30.7	6.4	3.2

Data presented as percentage [%]. O – oxygen; Al – aluminum; Si – silicon; Zr – zirconium; Y – yttrium; Hf – hafnium.

The nanosilica–zirconia-coated samples showed identical peaks, indicating that the coatings did not disrupt the tetragonal lattice structure of zirconia. Preserving the tetragonal phase is crucial for maintaining optimal mechanical properties in zirconia restorations.²⁶ In contrast, tribochemical silica coating induced a phase transformation, with an additional peak at 28.2° representing the monoclinic phase.¹⁸ Particle bombardment cause sufficient surface damage to disrupt the tetragonal structure. Such a phase transformation may introduce surface flaws and residual stresses, potentially compromising the mechanical properties of the restoration.^{27,28}

The 100% nanosilica coatings also exhibited new peaks at 49.0° and 50.5° , which were assigned to the (012) and (110) planes within SiO₂.¹⁸ For the nanosilica–zirconia coatings, distinct peaks were observed at 30.1264° , 50.1339° and 60.0273° . The presence of characteristic peaks associated with the nanomaterials confirmed the composition of the composite coating on the zirconia surface. The XRD findings indicated that the inert zirconia phase and crystallinity were unaffected by dip coating. By preserving the optimal tetragonal structure, the nanocoatings help maintain the favorable mechanical properties of zirconia for dental applications.

Topographic analysis

The topography of the coated surfaces was evaluated using AFM. The surface roughness of the specimens, assessed over an average scanned area of $60\text{ nm} \times 60\text{ nm}$, ranged from 30 nm to 1,000 nm. A comparison of Sa among the groups is presented in Table 3. The average Sa values for groups A–E and the untreated group were 1,552 nm, 707 nm, 792.8 nm, 433 nm, 372 nm, and 193 nm, respectively. The Sa of group A was significantly greater than that of all other groups ($p < 0.001$).

The reconstructed three-dimensional (3D) surface roughness images representative of each group are shown in Fig. 2–7. Different surface topographies were observed among the groups.

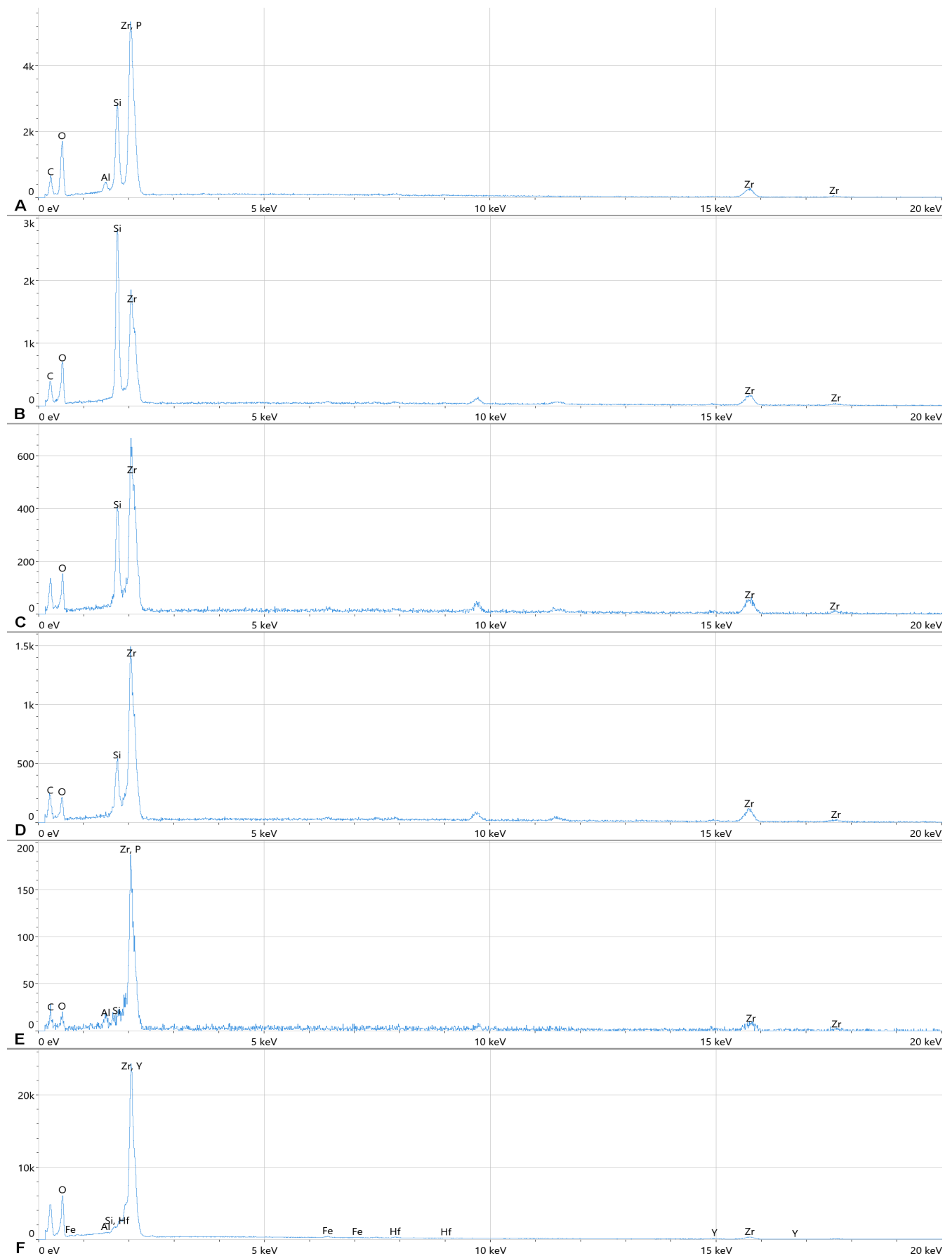


Fig. 8. Energy dispersive X-ray spectroscopy (EDS) spectra of zirconia surfaces showing their elemental composition

A. Group A; B. Group B; C. Group C; D. Group D; E. CoSil-blasted zirconia (group E); F. Untreated XT zirconia.

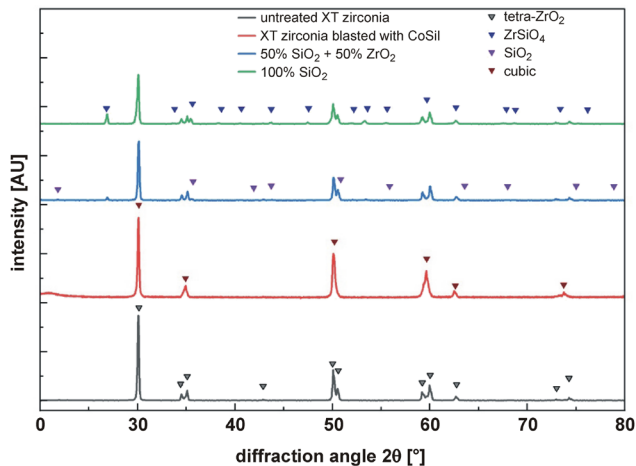


Fig. 9. X-ray diffraction (XRD) patterns of zirconia surfaces showing the crystallographic phases after different surface treatments

Table 3. Surface roughness (Sa) values of zirconia specimens after different surface treatments measured by atomic force microscopy (AFM)

Group	Sa [nm]
A	1,552
B	707
C	792.8
D	433
E	372
Control	193

Table 4. Microshear bond strength (μ SBS) of resin cement bonded to zirconia surfaces after different surface treatments

Group	μ SBS [MPa] <i>M</i> $\pm$ <i>SD</i>
A	37.41 $\pm$ 5.45
B	42.10 $\pm$ 6.92
C	51.63 $\pm$ 5.56
D	42.96 $\pm$ 8.96
E	31.00 $\pm$ 6.54
Control	17.38 $\pm$ 3.74

M – mean; *SD* – standard deviation.

Table 5. Failure modes of treated and untreated zirconia specimens

Group	Adhesive I	Adhesive II	Mixed (adhesive II/cohesive)	Cohesive	Total
A	8 (100.0%)	–	–	–	8 (100.0%)
B	5 (62.5%)	3 (37.5%)	–	–	8 (100.0%)
C	–	5 (62.5%)	3 (37.5%)	–	8 (100.0%)
D	–	8 (100.0%)	–	–	8 (100.0%)
E	–	6 (75%)	2 (25.0%)	–	8 (100.0%)
Control	–	6 (75%)	2 (25.0%)	–	8 (100.0%)

Microshear bond strength

The μ SBS values and standard deviations for the different surface treatments are shown in Table 4.

One-way ANOVA showed that the surface treatments significantly affected μ SBS ($p \leq 0.05$). The nanosilica and nanosilica–zirconia groups with different proportions of nanoparticles in the dipping suspensions exhibited significantly higher μ SBS values than the CoSil-treated and untreated zirconia groups ($p \leq 0.05$).

Modes of failure

After the μ SBS test, the failure modes were examined using a digital microscope at $\times 250$ magnification. Failure modes were categorized into 3 types, as follows (Fig. 10):

- adhesive I: failure between the zirconia substrate and the coating (ZrO_2 –coating interface);
- adhesive II: failure between the coating and the resin cement (coating–resin cement interface);
- mixed failure: a combination of adhesive II failure and cohesive failure.²⁸

The failure mode was evaluated for each group. In group A (100% SiO_2), failure occurred between the zirconia substrate and the coating, and was therefore classified as adhesive I failure. In group B (75% SiO_2 + 25% ZrO_2), 62.5% of failures occurred at the zirconia–coating interface, whereas 37% occurred at the coating–resin cement interface. In group C (50% SiO_2 + 50% ZrO_2), 62.5% of failures occurred at the coating–resin cement interface, whereas 37% of the specimens exhibited mixed failure. In group D (25% SiO_2 + 75% ZrO_2), 100% of failures were reported at the coating–resin cement interface. In group E (CoSil), 75% of failures were reported at the coating–resin cement interface and 25% were mixed failures, similarly to the control group (Table 5).

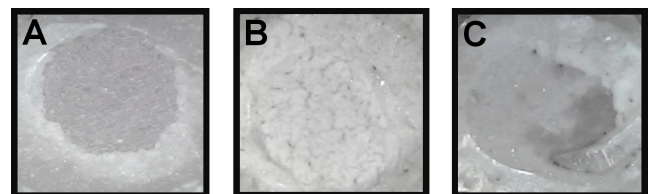


Fig. 10. Failure modes after microshear bond strength (μ SBS) testing
A. Adhesive I failure; B. Adhesive II failure; C. Mixed failure.

Discussion

The null hypothesis was rejected, since different surface topographies and morphologies were observed on the zirconia surface after dipping in different nanosuspensions and after blasting with silica-coated alumina. In addition, the blasting technique induced a tetragonal-to-monoclinic phase transformation, which may contribute to zirconia surface damage.

The measurement of bond strength at the zirconia–resin cement interface is complex. Microshear bond strength testing was utilized in this study to quantify the interfacial adhesion between resin cement and zirconia after dip-coating surface treatments. Compared with conventional macroscale shear testing, the microshear method provides more reliable and representative bond-strength results.^{29,30} Microshear testing is performed by bonding small cylindrical samples and applying an increasing shear force parallel to the interface.^{19,24} Failure occurs by debonding at the weakest point, which is often within the microscopic bonding complex. This provides a more representative measure of interfacial adhesive strength rather than the cohesive strength of the cement itself.²⁹ However, the small surface area can introduce challenges related to stress concentrations and non-uniform loading. Despite these limitations, microshear testing remains an accepted and useful technique for comparing bond strengths in localized regions.^{29,30}

In this study, all nanostructured coatings significantly enhanced zirconia bond strength compared with the polished untreated zirconia surface. The inert zirconia surface lacks retentive features or chemical reactivity with resin cements, resulting in low bond strength. These findings are consistent with those reported in the literature.^{6,31–33} By introducing nanoscale textures and porosities, as demonstrated by FESEM, the coatings increased the surface area and surface energy available for micromechanical retention and adsorption of resin monomers. The 50:50 silica–zirconia ratio provided an optimal balance between chemical and mechanical bonding with the resin cement.

Tribochemical silica coating with CoSil particles increased μ SBS compared with polished zirconia. The particle bombardment roughened the inert surface and enhanced micromechanical retention.³⁴ However, a tetragonal-to-monoclinic phase transformation was observed, indicating surface damage. Residual stresses associated with this transformation can compromise the restoration.^{8,27} In contrast, dip coating formed homogeneous nanolayers without disrupting the fragile zirconia surface. Porous silica–zirconia composite coatings increased bond strength while preserving the optimal tetragonal phase, as verified by XRD (Fig. 9).

Dip coating enables precise control over nanolayer properties by adjusting the coating composition and deposition parameters.³⁵ The approach modifies zirconia surfaces to enhance resin bonding without abrasive blasting or the use

of high temperatures. Further optimization of nanocoating composition, thickness, and sintering parameters could produce durable yet microretentive surfaces, expanding the potential applications of high-strength zirconia restorations.

The silica–zirconia-coated surfaces exhibited higher μ SBS ($p \leq 0.05$) compared with the other groups; this indicates that silica–zirconia coating may be an effective method for improving resin–zirconia adhesion. The observed increase in μ SBS could be attributed to increased micromechanical interlocking in addition to chemical bonding.³⁶ The FESEM images (Fig. 5–7) show numerous microgaps between the particles within the silica–zirconia coating. This observation supports the assumption that micromechanical interlocking between the coated zirconia surface and resin cement was enhanced.

The highest μ SBS among all groups was observed for the 50% SiO₂ + 50% ZrO₂ suspension (51.63 $\pm$ 5.6 MPa) which could be due to the high Sa in this group (792.8 nm), as shown by AFM analysis (Table 3). FESEM demonstrated that the coating formed a network with sharp edges (Fig. 5), which could be attributed to the sintering of nanosilica and nanozirconia on the zirconia surface.

Field emission scanning electron microscopy also showed that the coating provided complete coverage of the zirconia substrate. The increased average Sa may have contributed to enhanced micromechanical bonding, while the presence of zirconium at approx. 59.8 wt% on the coating surface could facilitate interaction with 10-MDP in the Z-Prime Plus and thereby promote chemical bonding. The combination of chemical and mechanical bonding may have contributed to the increased μ SBS, in agreement with previous findings.³⁷ This high bond strength was also reflected in the failure mode, which showed that the adhesive strength of the coating to the zirconia substrate was high, with no adhesive I failures. Failure occurred at the resin cement–coating interface (adhesive II) in 62.5% of specimens, while 37.5% showed mixed adhesive II/cohesive failure (Table 5, Fig. 10).

Dip coating with 100% SiO₂ resulted in higher μ SBS than tribochemical silica coating and untreated zirconia. The pure silica coating led to the formation of a patch-like coating pattern, leaving some areas of bare zirconia exposed. This surface pattern may have increased the available surface area and enhanced micromechanical bonding.³⁸ In addition, the exposed zirconia substrate could interact with 10-MDP from the primer, thereby enhancing chemical bonding. This finding agrees with previous studies demonstrating that 10-MDP affects the bonding effectiveness of conventional composite resin cement to zirconia ceramics.³⁹ However, this increase in bond strength appeared to be insufficient to ensure strong adhesion between the coating and zirconia substrate, as 100% of failures in this group occurred at the coating–zirconia interface and were classified as adhesive I failures (Table 5).

This simple, laboratory-friendly dip-coating technique shows promise for improving resin bonding to zirconia restorations.³³ By combining nanosilica and nanozirconia, bond strength was improved without surface damage. A key challenge was developing stable dispersions of the nanoparticle mixtures to enable homogeneous coatings.⁴⁰ Nanoparticles tend to agglomerate due to their high surface energy. Adequate particle dispersion and prolonged stabilization of the suspension were achieved by sonication using an ultrasonic probe.⁴¹ This allowed consistent dip coating with tunable nanolayer properties. With further optimization of deposition parameters and sintering protocols, this approach could be broadly implemented to tailor zirconia bonding surfaces.

These findings were obtained using only one type of zirconia (XT). Because different types of zirconia have different chemical compositions and physical properties, this technique should also be evaluated using other zirconia types (LT and HT zirconia), to determine whether it similarly improves μ SBS between resin cement and these materials.

The study had some limitations, particularly the lack of simulation of clinically relevant intraoral conditions. Aging procedures such as water storage, mechanical loading and thermocycling were not performed. Future studies incorporating thermocycling and mechanical cyclic loading may provide more clinically relevant results by better simulating intraoral conditions.

Conclusions

The new dip-coating method utilizing nanosilica–nanozirconia composite suspensions represents a promising strategy for enhancing resin adhesion to zirconia surfaces. This approach resulted in higher μ SBS than silica-coated alumina blasting while preserving the fragile zirconia structure. The ability to modify surface textures and porosities by adjusting the nanoparticle composition of the suspension further demonstrates the versatility and efficacy of this technique. Among the nanosuspensions evaluated, the 50:50 nanosilica–nanozirconia composition produced the most favorable overall results.

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. Mohammed D, Mudhaffar M. Effect of modified zirconium nano-fillers addition on some properties of heat cured acrylic denture base material. *J Bagh Coll Dent*. 2012;24(4):1–7.
2. Aljaferi AM, MAH B. Effect of addition $ZrO_2-Al_2O_3$ nanoparticles mixture on some properties and denture base adaptation of heat cured acrylic resin denture base material. *J Baghdad Coll Dent*. 2015;27(3):15–21. doi:10.12816/0015028
3. Kontonasi 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
4. Jurado CA, Villalobos-Tinoco J, Watanabe H, Sanchez-Hernandez R, Tsujimoto A. Novel translucent monolithic zirconia fixed restorations in the esthetic zone. *Clin Case Rep*. 2022;10(3):e05399. doi:10.1002/ccr3.5499
5. Xie H, Tay FR, Zhang F, Lu Y, Shen S, Chen C. Coupling of 10-methacryloyloxydecylidihydrogenphosphate to tetragonal zirconia: Effect of pH reaction conditions on coordinate bonding. *Dent Mater*. 2015;31(10):e218–e225. doi:10.1016/j.dental.2015.06.014
6. Farhan FA, Sulaiman E, Kutty MG. Effect of new zirconia surface coatings on the surface properties and bonding strength of veneering zirconia substrate. *Surf Coat Technol*. 2018;333:247–258. doi:10.1016/j.surfcoat.2017.10.030
7. Inokoshi M, Kameyama A, De Munck J, Minakuchi S, Van Meerbeek B. Durable bonding to mechanically and/or chemically pre-treated dental zirconia. *J Dent*. 2013;41(2):170–179. doi:10.1016/j.jdent.2012.10.017
8. Alammam A, Blatz MB. The resin bond to high-translucent zirconia – A systematic review. *J Esthet Restor Dent*. 2022;34(1):117–135. doi:10.1111/jerd.12876
9. Hmedat SJA, Ibraheem AF. An in vitro evaluation of fit of the crowns fabricated by zirconium oxide-based ceramic CAD/CAM systems, before and after porcelain firing cycles and after glaze cycles. *J Baghdad Coll Dent*. 2013;25(1):43–48. doi:10.12816/0014962
10. Salman TA, Khalaf HA. The influence of adding of modified ZrO_2-TiO_2 nanoparticles on certain physical and mechanical properties of heat polymerized acrylic resin. *J Baghdad Coll Dent*. 2015;27(3):33–39. doi:10.12816/0015032
11. Jassim SJ, Majeed MA. Effect of plasma surface treatment of three different CAD/CAM materials on the micro shear bond strength with resin cement (A comparative in vitro study). *Heliyon*. 2023;9(7):e17790. doi:10.1016/j.heliyon.2023.e17790
12. Kadhim OMA, Aljuboury MR. Shear bond strengths of composite resin used to repair lithium disilicate and feldspathic CAD/CAM ceramics treated by different lasers (a comparative in vitro study). *J Res Med Dent Sci*. 2023;11(06):15–24. <https://www.jrmds.in/articles/shear-bond-strengths-of-composite-resin-used-to-repair-lithium-disilicate-and-feldspathic-cadcam-ceramics-treated-by-dif.pdf>. Accessed February 23, 2024.
13. Su Z, Li M, Zhang L, et al. A novel porous silica-zirconia coating for improving bond performance of dental zirconia. *J Zhejiang Univ Sci B*. 2021;22(3):214–222. doi:10.1631/jzus.B2000448
14. Liang Z, Liu Y, Jiang Y, et al. Effect of hot etching with HF on the surface topography and bond strength of zirconia. *Front Mater*. 2022;9:1008704. doi:10.3389/fmats.2022.1008704
15. Shen D, Wang H, Shi Y, Su Z, Hannig M, Fu B. The effect of surface treatments on zirconia bond strength and durability. *J Funct Biomater*. 2023;14(2):89. doi:10.3390/jfb14020089
16. Klaisiri A, Krajangta N, Thamrongananskul N. The durability of zirconia/resin composite shear bond strength using different functional monomer of universal adhesives. *Eur J Dent*. 2022;16(4):756–760. doi:10.1055/s-0041-1736331

17. Hajjaj MS, Barboud HM, Almashabi HK, et al. Evaluation of different priming agents with conventional and bioactive self-adhesive resin cements on shear bond strength to zirconia. *Appl Sci*. 2023;13(14):8369. doi:10.3390/app13148369
18. Kumar R, Singh MD, Sharma V, et al. Effect of surface treatment of zirconia on the shear bond strength of resin cement: A systematic review and meta-analysis. *Cureus*. 2023;15(9):e45045. doi:10.7759/cureus.45045
19. Kern M, Wegner SM. Bonding to zirconia ceramic: Adhesion methods and their durability. *Dent Mater*. 1998;14(1):64–71. doi:10.1016/s0109-5641(98)00011-6
20. Lim MJ, Kim TG, Yu MK, Lee KW. Effects of different silica-based layer coatings on bond strength of Y-TZP to bovine dentin. *Dent Mater J*. 2020;39(1):154–160. doi:10.4012/dmj.2018-317
21. Nishigawa G, Maruo Y, Irie M, et al. Ultrasonic cleaning of silica-coated zirconia influences bond strength between zirconia and resin luting material. *Dent Mater J*. 2008;27(6):842–848. doi:10.4012/dmj.27.842
22. Guilardi LF, Pereira GKR, Giordani JC, Kleverlaan CJ, Valandro LF, Rippe MP. Effect of zirconia surface treatment, resin cement and aging on the load-bearing capacity under fatigue of thin simplified full-contour Y-TZP restorations. *J Mech Behav Biomed Mater*. 2019;97:21–29. doi:10.1016/j.jmbbm.2019.04.050
23. Kern M. Bonding to oxide ceramics – laboratory testing versus clinical outcome. *Dent Mater*. 2015;31(1):8–14. doi:10.1016/j.dental.2014.06.007
24. Nagaoka N, Yoshihara K, Tamada Y, Yoshida Y, Van Meerbeek B. Ultrastructure and bonding properties of tribochemical silica-coated zirconia. *Dent Mater J*. 2019;38(1):107–113. doi:10.4012/dmj.2017-397
25. Oliveira-Ogliari A, Collares FM, Feitosa VP, Sauro S, Ogliari FA, Moraes RR. Methacrylate bonding to zirconia by in situ silica nanoparticle surface deposition. *Dent Mater*. 2015;31(1):68–76. doi:10.1016/j.dental.2014.11.011
26. Harada A, Shishido S, Barkarmo S, et al. Mechanical and microstructural properties of ultra-translucent dental zirconia ceramic stabilized with 5 mol% yttria. *J Mech Behav Biomed Mater*. 2020;111:103974. doi:10.1016/j.jmbbm.2020.103974
27. Madani A, Nakhaei M, Karami P, Rajabzadeh G, Salehi S, Bagheri H. Sol-gel dip coating of yttria-stabilized tetragonal zirconia dental ceramic by aluminosilicate nanocomposite as a novel technique to improve the bonding of veneering porcelain. *Int J Nanomedicine*. 2016;11:3215–3223. doi:10.2147/IJN.S104885
28. Ismail AM, Bourauel C, ElBanna A, Eldin TS. Micro versus macro shear bond strength testing of dentin-composite interface using chisel and wireloop loading techniques. *Dent J (Basel)*. 2021;9(12):140. doi:10.3390/dj9120140
29. Chen C, Chen G, Xie H, Dai W, Zhang F. Nanosilica coating for bonding improvements to zirconia. *Int J Nanomedicine*. 2013;8:4053–4062. doi:10.2147/IJN.S52145
30. Şişmanoğlu S, Turunç-Oğuzman R. Microshear bond strength of contemporary self-adhesive resin cements to CAD/CAM restorative materials: Effect of surface treatment and aging. *J Adhes Sci Technol*. 2020;34(22):2484–2498. doi:10.1080/01694243.2020.1763543
31. Denry I, Kelly JR. Emerging ceramic-based materials for dentistry. *J Dent Res*. 2014;93(12):1235–1242. doi:10.1177/0022034514553627
32. Vichi A, Sedda M, Bonadeo G, et al. Effect of repeated firings on flexural strength of veneered zirconia. *Dent Mater*. 2015;31(8):e151–e156. doi:10.1016/j.dental.2015.04.012
33. Lung CYK, Liu D, Matinlinna JP. Silica coating of zirconia by silicon nitride hydrolysis on adhesion promotion of resin to zirconia. *Mater Sci Eng C Mater Biol Appl*. 2015;46:103–110. doi:10.1016/j.msec.2014.10.029
34. Aboushelib MN. Fusion sputtering for bonding to zirconia-based materials. *J Adhes Dent*. 2012;14(4):323–328. doi:10.3290/j.jad.a25684
35. Liu JF, Yang CC, Luo JL, Liu YC, Yan M, Ding SJ. Bond strength of self-adhesive resin cements to a high transparency zirconia crown and dentin. *J Dent Sci*. 2022;17(2):973–983. doi:10.1016/j.jds.2021.12.008
36. Amory ZS, Rashid M, Jaleel MA, Addie AJ. Evaluation of the microshear bond strength of resin cement to zirconia treated by different nanomaterials using the dip coating technique. 2024;2024(2). doi:10.5339/jemtac.2024.uncidc.6
37. Kim DW, Lee JJ, Kim KA, Seo JM. Influence of nano-structured alumina coating treatment on shear bond strength between zirconia ceramic and resin cement. *J Korean Acad Prosthodont*. 2016;54(4):354. doi:10.4047/jkap.2016.54.4.354
38. Matinlinna JP, Lung CYK, Tsoi JKH. Silane adhesion mechanism in dental applications and surface treatments: A review. *Dent Mater*. 2018;34(1):13–28. doi:10.1016/j.dental.2017.09.002
39. Mendes F, Zanini MM, Favarão J, et al. Bonding strength of luting cement to zirconia-based ceramic under different surface treatments. *Eur J Dent*. 2019;13(2):222–228. doi:10.1055/s-0039-1696076
40. Fernandes VVB, Oliani MG, Nogueira L, Silva JMF, Araújo RM. Analysis and comparison of different bond strength tests. *JSM Dent*. 2016;4(5):1076. <https://www.jscimedcentral.com/public/assets/articles/dentistry-4-1076.pdf>. Accessed February 23, 2024.
41. Liu H, Hou X, Li X, Jiang H, Tian Z, Ali MKA. Effect of mixing temperature, ultrasonication duration and nanoparticles/surfactant concentration on the dispersion performance of Al₂O₃ nanolubricants. *Research Square*. Preprint posted online October 5, 2020. doi:10.21203/rs.3.rs-84466/v1

Bond strength of resin composite to caries-affected dentin after remineralization by different remineralizing agents: An in vitro study

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Abstract

Background. Following selective caries removal, the efficacy of bonding to the dentin substrate is questionable due to alterations in mineral content and the quality of remaining dentin, especially in deep proximal cavities.

Objectives. The aim of the study was to evaluate the microtensile bond strength of resin composite to artificially demineralized dentin and sound dentin compared to demineralized dentin that underwent different remineralization protocols. Additionally, the study sought to evaluate the effect of the 2 bonding modes (total-etch/self-etch) on the applied remineralizing agent, and to analyze the failure mode.

Material and methods. The study utilized 30 sound proximal molar surfaces. They were randomly divided into 5 groups based on the remineralization protocols, as follows: sound dentin as positive control (G1); artificially demineralized dentin but without surface treatment as negative control (G2); and artificially demineralized dentin surface groups with surface treatments (sodium fluoride (NaF) solution (G3), nano-hydroxyapatite (nHAp) (G4), and a combination of remineralizing agents (G5)). Different modes of application of the bonding agent (total-etch (B1) and self-etch (B2)) were implemented. Then, a nanohybrid composite was applied and light cured. The microtensile bond strength test was performed, and the failure mode was evaluated.

Results. The application of the total-etch mode resulted in a statistically significant difference between microtensile bond strength values of remineralization protocols. The NaF and nHAp groups (G3 and G4) exhibited the highest values. A statistically significant difference was observed between the microtensile bond strength values of remineralization protocols in the self-etch mode. The positive control, NaF, nHAp, and combined groups (G1, G3, G4, and G5) showed the highest values. The negative control group (G2) in both bonding modes demonstrated the lowest microtensile bond strength.

Conclusions. All remineralization protocols applied to demineralized dentin demonstrated an improvement in bond strength that was equivalent to sound dentin. All remineralized dentin surfaces exhibited a favorable failure mode in comparison to the negative control group.

Keywords: deep demineralized dentin, microtensile bond strength, dentin remineralization, eggshell nano-hydroxyapatite, selective caries removal

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Highlights

- The bonding effectiveness of resin composites to proximally located, demineralized dentin is unreliable.
- All tested remineralizing agents enhanced bond strength to levels comparable with sound dentin.
- The nano-hydroxyapatite (nHAp) paste and sodium fluoride (NaF) solution improved the bond strength to caries-affected dentin when used with a universal adhesive in both bonding modes.
- The combination of nHAP and NaF did not provide any additional benefit over their individual application.
- The remineralization of caries-affected dentin improves the hybrid layer quality and favors the mode of failure.

Introduction

In modern restorative dentistry, a conservative approach is the prevailing treatment modality for dental caries. The complete removal of caries employed in the treatment of deep lesions is currently regarded as an over-treatment due to the risk of unnecessary and unwarranted removal of sound tooth structure, which can result in a high risk of pulp exposure. Therefore, conservative, selective caries removal is recommended for deep lesions.¹ This approach entails the removal of caries from the peripheral and lateral walls of the lesion until sound enamel and dentin margins are reached. For the deepest part of the lesion, the outer softened infected carious dentin is removed, while the inner affected firm layer is left behind. During the process of cavity restoration, demineralized tissue poses a challenge to adhesion when utilized as the bonding substrate, resulting in reduced bond strength values.² The adhesion to the peripheral sound enamel and dentin is crucial for ensuring the proper sealing of the margins and, consequently, halting the progression of the carious lesion. This is further supported by clinical trials that demonstrated a higher survival rate for teeth restored through the selective caries removal technique.³

As demonstrated in previous literature, the carious process causes a significant reduction in mineral content, especially in deep dentin layers. This is due to a significant increase in porosity and alterations in dentin collagen structure and distribution.⁴ Consequently, there is a decline in the mechanical properties of dentin, including microhardness, stiffness, microtensile strength, and modulus of elasticity. This reduction affects the quality of the hybrid layer and leads to questionable bond strength and failures under occlusal forces, which has an impact on the overall prognosis of the restoration.^{5,6} A biomimetic concept was introduced to regain and restore the normal conditions of the caries-affected dentin substrate and its mineral content through remineralization.^{7,8} The application of remineralizing agents prior to bonding has been shown to increase bond strength by restoring the inorganic lost components and reducing the water content and porosity. Consequently, the usage of remineralizing agents on deep caries-affected dentin has the potential to facilitate

the recovery of its mechanical properties.⁹ This approach may be advantageous in cases of subgingival deep cavities where obtaining a sound margin would be challenging.

Different types of dental remineralizing agents were evaluated in literature.^{10,11} Sodium fluoride (NaF) solution was first introduced in 1941 by Lulkomsky and is considered the gold standard for desensitization and remineralization.^{12–14} In recent years, there has been significant progress in the field of dentistry with the introduction of nano-hydroxyapatite (nHAp), which is expected to be the future of remineralization.^{15,16} Nano-hydroxyapatite particles derived from eggshell powder have shown a biomimetic remineralizing effect on enamel surfaces for the treatment of white spot lesions, as well as on exposed dentin for the treatment of erosions.¹⁷ Derived from calcium-rich natural sources such as chicken eggshells, nHAp crystals contain a high percentage of calcium carbonate and, to a lesser extent, calcium phosphate, magnesium carbonate and organic matter.¹⁸ The high stability and bioactivity of nHAp can potentially provide calcium and phosphate in an adequate concentration to enable repair and promote remineralization. In addition to its antimicrobial properties, a dual action strategy involving mineralization and antimicrobial activity is anticipated from the nHAp.^{19,20}

Recently, a novel approach has been introduced that utilizes the eggshell-derived nHAp as a remineralizing agent on caries-affected deep dentin substrate. The objective of this approach is to facilitate the restoration of the dentin's lost inorganic mineral.^{19,21,22} The underlying principle is to achieve a biomimetic tooth restoration complex under functional loading, with the preservation of deep dentin, especially in deep proximal lesions and deep proximal margins that require deep margin elevation. In other studies, the effect of nHAp when incorporated with varying percentages of NaF was examined in an effort to ascertain the optimal remineralization outcome.^{23,24}

In the domain of adhesion, universal adhesives are regarded as a state-of-the-art development due to their incorporation of 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which has been shown to facilitate chemical bonding along with the micromechanical adhesion shown in previous systems.²⁵

A review of the literature regarding the bonding to proximal deep caries-affected dentin, its remineralization prior to deep margin elevation, restoration with composite resin, and its effect on bond strength revealed a paucity of research in this area. Thus, conducting a study to evaluate the microtensile bond strength of resin composite restoration bonded using a universal adhesive to proximal artificially demineralized dentin after treatment with NaF and/or nHAp might be of value.

The purpose of this in vitro study was to examine the microtensile bond strength of resin composite restorations to proximal artificially demineralized dentin and sound dentin in comparison to demineralized dentin following remineralization with 2 agents (NaF solution and eggshell nHAp) either individually or in combination using a universal adhesive system in the total-etch or self-etch mode.

Material and methods

Sample size calculation

The microtensile bond strength was used as the primary outcome, and the mean values were determined based on the results of the study by Barbosa-Martins et al.²⁶ The mean values for microtensile bond strength of 5 groups were 43.32, 21.96, 33.43, 45.25, and 46.42 MPa. The standard deviation (*SD*) within the group was assumed to be 12 MPa, and the effect size (*f*) was 0.773. Using an alpha level of 5% and a beta level of 20%, that is, establishing a power of 80%, the minimum estimated sample size was determined to be 6 specimens (rods) per subgroup. The sample size calculation was performed using G*Power v. 3.1.9.2.

Preparation of dentin samples

Proximal surfaces of 30 sound molars were used in accordance with the guidelines established by the Academy of Dental Materials (ADM).²⁷ Molars of appropriate size (10 mm or larger mesiodistally) were obtained from the National Institute of Diabetes and Endocrinology, Cairo, Egypt. The teeth were extracted mainly from diabetic patients due to periodontal indications, and the subjects were informed that their teeth would be utilized for research purposes. The teeth were cleaned to remove any soft debris and fixed in acrylic cylinders to allow easy cutting. The occlusal enamel was ground using a water-cooled Sectioning IsoCut® Wafering Blade affixed to an IsoMet 4000 linear saw (Buehler Ltd., Lake Bluff, USA) under copious water coolant (Cool 2 water-soluble anticorrosive cooling lubricant; Buehler Ltd.) to expose the proximal dentin–enamel junction. Subsequently, the proximal surfaces were cut 3 mm from the outer proximal enamel surface, allowing for the examination of deep

proximal dentin. In this study, each tooth was utilized as a comparator for the bonding mode. The teeth were decoronated perpendicular to the long axis, the roots were discarded, and the coronal dentin samples were used for the study (Fig. 1).

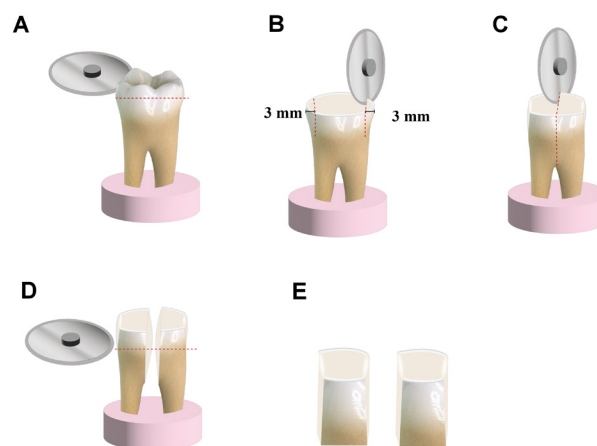


Fig. 1. Teeth-cutting process and the preparation of dentin samples

A. Grounding of occlusal enamel; B. Exposure of deep proximal dentin; C. Splitting the teeth at midline; D. Teeth decoronation; E. Coronal dentin samples.

Grouping and randomization

A total of 30 dentin samples (mesial and distal) were fixed in self-cured acrylic resin cylinders. Each sample was coded to ensure that each tooth represented its comparator regarding the bonding mode. Then, the samples were stored in distilled water for 48 h. Subsequently, they were randomly divided into 5 groups according to the remineralization protocols. Group 1 (G1) was the positive control group, in which dentin was left sound without undergoing demineralization or any surface treatment. Group 2 (G2) was the negative control group, in which the dentin surfaces were demineralized and left untreated. In group 3 (G3), the surfaces were demineralized and treated with 2% NaF solution as a remineralizing protocol. In group 4 (G4), the surfaces were demineralized and treated with eggshell nHAp as a remineralizing protocol, and in group 5 (G5), the surfaces were demineralized and treated by a combination of 2% NaF solution and eggshell nHAp. Grouping was performed to ensure that every tooth (mesial and distal halves) received the same remineralization protocol and to overcome the variability in the composition, the heterogeneous nature of dentin, and tooth age.²⁷ Subsequently, each group was subdivided into 2 groups using the previous coding to ensure that each tooth served as its own comparator regarding the application mode of the bonding agent. In this case, B1 represented the total-etch mode, while B2 represented the self-etch mode (Fig. 2,3).

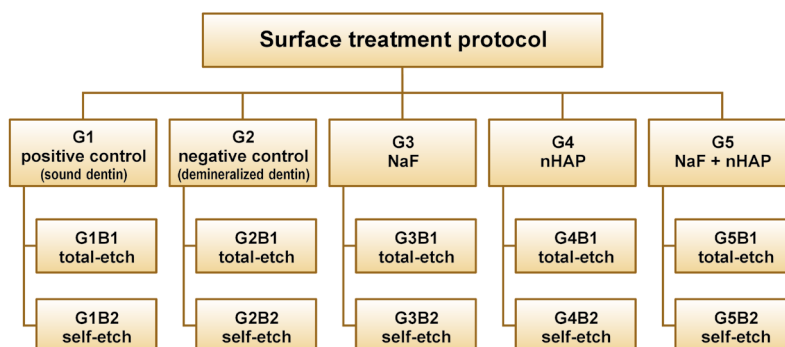


Fig. 2. Grouping of the study samples

NaF – sodium fluoride; nHAp – nano-hydroxyapatite.

Dentin surface treatment protocols

As illustrated in Fig. 3, artificial demineralization was performed in all experimental groups, with the exception of G1. The demineralization process was conducted using a gel composed of 5 mL of 6% carboxymethylcellulose acid gel (0.1 M lactic acid titrated to pH 5.0 in potassium hydroxide (KOH) solution) at 37°C. The deep proximal dentin surfaces were covered in gel for 48 h without renewal. This model has been reported to provide demineralized dentin analogous to that of caries-affected dentin.²⁶ After 48 h, the samples were rinsed with distilled water and air-dried. The following surface treatments were performed in G3, G4 and G5:

- G3: chemically prepared 2% NaF solution (where 5 g of NaF solid (product number 30139; BDH Chemicals Ltd, Poole, UK) was dissolved in 250 mL of water in a polyethylene bottle until complete dissolution, using a magnetic stirrer) was applied using a 1-mm polyethylene

plastic pipette. The solution was left on the surfaces for 1 min and blot dried using a cotton swab;

- G4: eggshell nHAp was chemically prepared following the method described by Azis et al.²⁸ The eggshell nHAp powder was dissolved in distilled water (1.8 g of nHAp mixed with 0.3 mL of distilled water), applied using a spatula, and left on the surfaces for 1 min. Thereafter, the surfaces were rinsed with distilled water and blot dried using a cotton swab;
- G5: combination of 1.8 g of eggshell nHAp dissolved in 0.3 mL of 2% NaF solution was applied using a spatula and left on the surfaces for 1 min. Then, it was rinsed with distilled water and blot dried.

Composite resin build-up

In the total-etch groups, the pretreated dentin surfaces were etched with 37% phosphoric acid semi-gel (Meta Etchant; Meta Biomed, Cheongju-si, Republic of Korea)

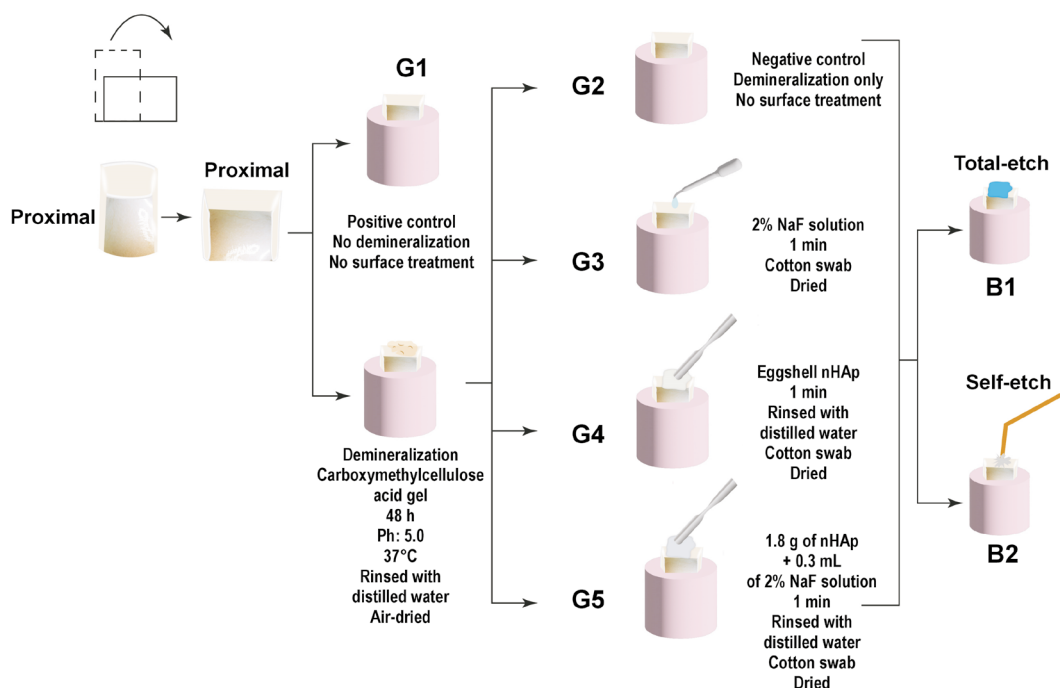


Fig. 3. Methodology employed in the treatment of dentin surfaces

for 15 s, rinsed for 10 s, and blot dried. In the total-etch and self-etch groups, the Single Bond Universal Adhesive (3M™ ESPE, Seefeld, Germany) was applied, rubbed on the surface for 20 s, left to air dry for 5 s, and light cured for 20 s using an LED Light Curing unit (Woodpecker X-Cure; Guilin Woodpecker Medical Instrument Co., Ltd, Guilin, China) with an intensity of 1200 mW/cm². A Shade A3 (Body) nanocomposite (3M™ Filtek™ Z350 XT; 3M™ ESPE) was used in the fabrication of a 4-mm composite block on the deep dentin surfaces. The application of each 1-mm increment was executed using gold-plated plastic instrument and subjected to light curing for 20 s. The samples were then kept in artificial saliva for 24 h before sectioning (Fig. 4).

Microtensile testing

A metal housing with 2 screws was used to firmly attach the acrylic blocks and ensure standard cutting in buccolingual and mesio-distal directions (90° to each other). As presented in Fig. 4, the 30 samples were transferred to the IsoMet 4000 linear saw (Buehler Ltd.) to allow sectioning of each surface longitudinally in horizontal cuts. Then, vertical cuts were made using a Sectioning IsoCut® Wafering Blade to create rods of similar cross-sectional area (1 mm × 1 mm) and similar remaining dentin thickness. Rods were obtained for each group, with specimens that had pre-test failures excluded from the analysis ($n = 9$). A total of 9 rods per subgroup and 90 rods for 10 subgroups were expected, yet the negative control group exhibited pre-test failure, resulting in the collection of 72 rods for 8 subgroups. These rods were stored in distilled water. Each beam was then affixed to Gerald Eli's jig, glued on the jig by its ends using a cyanoacrylate-based glue (705 Universal Fast Adhesive; Akfix, Istanbul, Turkey), a minimum 1 mm from the adhesive interface

to allow microtensile testing. The Universal Testing Machine (Model 3345; Instron, High Wycombe, UK) was used to apply microtensile forces to the 72 beams until fracture. Digital software was employed for test control and information acquisition. A tensile load of 500 N was applied to the specimen at a crosshead speed of 0.5 mm/min until the occurrence of bonding failure. The bond strength was calculated in MPa (Bluehill Lite software; Instron, Norwood, USA).

Examination of failure mode

Each fractured beam was examined under a stereomicroscope (MA 100N; Nikon, Tokyo, Japan) at ×30 magnification to ascertain failure modes (Fig. 5).

Statistical analysis

The normality of the numerical data was investigated through the examination of its distribution and the implementation of normality tests, namely the Kolmogorov–Smirnov and Shapiro–Wilk tests. The microtensile bond strength data exhibited a non-normal (non-parametric) distribution. The data was presented as median (*Me*), range, mean (*M*), and *SD* values. The Kruskal–Wallis test was used to compare the remineralization protocols. Dunn's test was used for pairwise comparisons when the Kruskal–Wallis test yielded a significant result. The Mann–Whitney *U* test was employed to compare between the 2 bonding modes. The failure mode data was presented as frequencies and percentages. Fisher's exact test was used for the comparisons between the groups. The significance level was set at $p \leq 0.05$. The statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA).

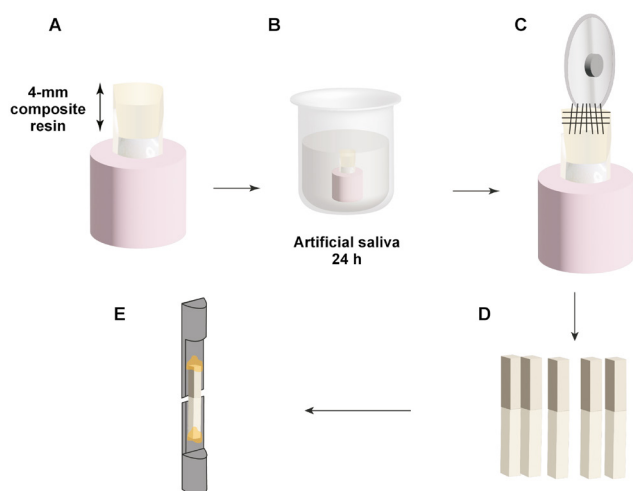


Fig. 4. Composite build-up and rod creation for microtensile testing

A. Composite build-up; B. Storage of samples in artificial saliva; C. Horizontal and vertical sectioning of samples; D. Rods of similar cross-sectional area; E. Microtensile testing.

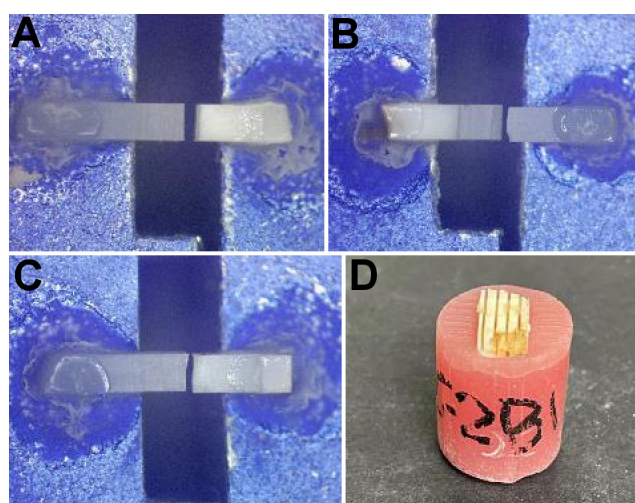


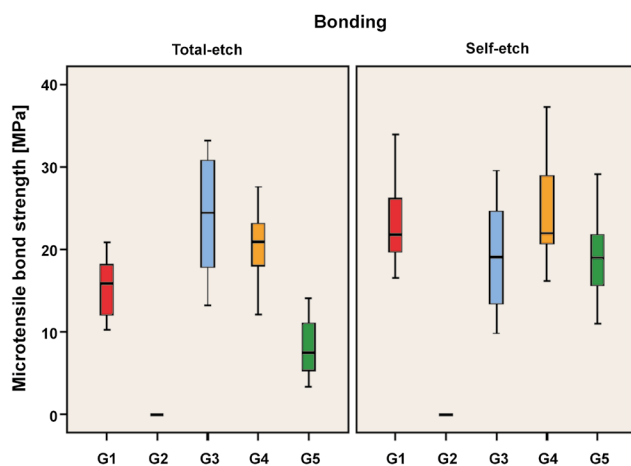
Fig. 5. Examples of failure modes

A. Adhesive failure; B. Cohesive restoration failure; C. Mixed failure; D. Pre-test (immediate) failure.

Table 1. Descriptive statistics and the comparison between the microtensile bond strength values of different remineralization protocols and the 2 bonding modes

Remineralization protocol	Microtensile bond strength [MPa]				Mann–Whitney <i>U</i> test	
	total-etch (B1) (<i>n</i> = 9)		self-etch (B2) (<i>n</i> = 9)			
	<i>Me</i> (range)	<i>M</i> (<i>SD</i>)	<i>Me</i> (range)	<i>M</i> (<i>SD</i>)	<i>p</i> -value	effect size (<i>d</i>)
Positive control (G1)	15.9 (10.3–20.9) ^B	15.6 (3.9)	21.8 (16.6–34.0) ^A	23.8 (6.1)	0.007*	1.643
Negative control (G2)	0.0 (0.0–0.0) ^D	0.0 (0.0)	0.0 (0.0–0.0) ^B	0.0 (0.0)	1.000	0.000
NaF (G3)	24.4 (13.2–33.2) ^A	24.1 (7.6)	19.1 (9.8–29.6) ^A	19.8 (7.1)	0.200	0.633
nHAp (G4)	20.9 (12.1–27.6) ^A	20.3 (4.8)	22.0 (16.2–37.3) ^A	24.8 (7.2)	0.233	0.586
NaF + nHAp (G5)	7.5 (3.4–14.1) ^C	8.5 (3.9)	19.0 (11.0–29.1) ^A	19.1 (6.1)	0.002*	2.193
Kruskal–Wallis test	<i>p</i> -value	<0.001*	<0.001*		–	–
	effect size (<i>η</i> ²)	0.789	0.721		–	–

* statistically significant ($p \leq 0.05$); *Me* – median; *M* – mean; *SD* – standard deviation; NaF – sodium fluoride; nHAp – nano-hydroxyapatite. Different superscript letters in the same column show statistical differences between the remineralization protocols.

**Fig. 6.** Box plots representing median and range values for microtensile bond strength in different groups

Results

Comparison of remineralization protocols

As demonstrated in Table 1 and Fig. 6, the utilization of the total-etch mode resulted in a statistically significant difference between the microtensile bond strength values of remineralization protocols ($p < 0.001$, effect size = 0.789). Pairwise comparisons revealed that there was no statistically significant difference between the NaF and nHAp groups (G3 and G4); both showed the highest statistically significant values. The positive control group (G1) exhibited statistically significant reduction in microtensile bond strength. The combined NaF and nHAp group (G5) demonstrated a similar trend.

The application of the self-etch mode resulted in a statistically significant difference between the microtensile bond strength values of remineralization protocols ($p < 0.001$, effect size = 0.721). Pairwise comparisons revealed that there was no statistically significant difference between the positive control, NaF, nHAp, and combined NaF and nHAp groups (G1, G3, G4, and G5); all showed

the highest statistically significant values. The negative control group (G2) in both bonding modes exhibited the lowest microtensile bond strength.

Comparison of bonding modes

As demonstrated in Table 1 and Fig. 6, the total-etch positive control group (G1B1) exhibited statistically significant lower microtensile bond strength values compared to the self-etch positive control group (G1B2) ($p = 0.007$, effect size = 1.643).

With regard to the negative control, NaF and nHAp groups, there was no statistically significant difference between the microtensile bond strength values of the 2 bonding modes (B1 and B2) ($p = 1.000$, effect size = 0.000; $p = 0.200$, effect size = 0.633; $p = 0.233$, effect size = 0.586, respectively). In the case of the combined NaF and nHAp group, the total-etch group (G5B1) exhibited a statistically significant decrease in microtensile bond strength compared to the self-etch group (G5B2) ($p = 0.002$, effect size = 2.193).

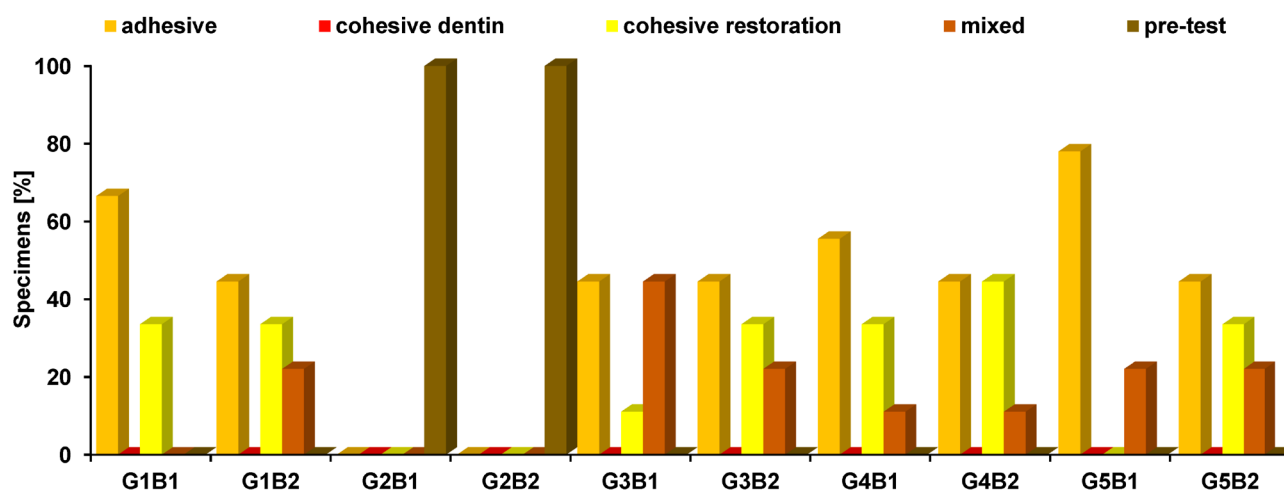
Failure mode analysis

The mode of failure was observed for each beam ($n = 9$ per subgroup, $9 \times 10 = 90$, excluding those with pre-test failure, so a total of 72 beams were evaluated) to detect the type of failure as presented in Table 2 and Fig. 7. A statistically significant difference was identified between the modes of failure among the different groups ($p < 0.001$, effect size = 0.615). The G5B1 group demonstrated the highest prevalence of adhesive failure. Groups G2B1 and G2B2 exhibited no instances of adhesive failure. None of the groups showed signs of cohesive dentin failure. The highest prevalence of cohesive restoration failure was observed in the G4B2 group. Groups G2B1, G2B2 and G5B1 demonstrated no cohesive restoration failure. The G3B1 group exhibited the highest prevalence of mixed failure. Groups G1B1, G2B1 and G2B2 showed no instances

Table 2. Descriptive statistics and the comparison between failure modes of the experimental groups

Group	Failure mode					Fisher's exact test	
	adhesive	cohesive dentin	cohesive restoration	mixed	pre-test	p-value	effect size (v)
G1B1	6 (66.5%)	–	3 (33.5%)	–	–	<0.001*	0.615
G1B2	4 (44.5%)	–	3 (33.5%)	2 (22.0%)	–		
G2B1	–	–	–	–	9 (100.0%)		
G2B2	–	–	–	–	9 (100.0%)		
G3B1	4 (44.5%)	–	1 (11.0%)	4 (44.5%)	–		
G3B2	4 (44.5%)	–	3 (33.5%)	2 (22.0%)	–		
G4B1	5 (55.5%)	–	3 (33.5%)	1 (11.0%)	–		
G4B2	4 (44.5%)	–	4 (44.5%)	1 (11.0%)	–		
G5B1	7 (78.0%)	–	–	2 (22.0%)	–		
G5B2	4 (44.5%)	–	3 (33.5%)	2 (22.0%)	–		

* statistically significant ($p \leq 0.05$).

**Fig. 7.** Bar chart illustrating failure modes across different groups

of mixed failure. All specimens in the G2B1 and G2B2 groups exhibited pre-test failure, and these were the only groups that demonstrated this mode of failure.

Discussion

The complete caries removal approach for deeply cavi- tated lesions is no longer considered mandatory and is even regarded as an aggressive protocol. In fact, there is growing evidence supporting selective caries removal as the line of treatment for deep carious lesions.²⁹ Theo- retically, it is argued that a completely sealed cavity with sound peripheral enamel and dentin margins is necessary for successful bonding and proper restorative procedures, which will allow the remaining carious lesion to arrest. This concept is frequently referred to as “seal is the deal”.³⁰ However, other in vitro studies have shown reduced frac- ture resistance of incompletely excavated carious tissue and lower bonding strength values of resins to carious dentin.³¹ Furthermore, a histobacteriological study of human

subjects conducted by Ricucci et al. has demonstrated that affected carious dentin contains residual bacteria that may induce subclinical chronic pulpal inflammation when used as a bonding substrate.³²

Caries lesions cause a significant decrease in mineral content, especially in deep dentin layers. Concurrently, there is a significant increase in porosity and water con- tent.⁴ These alterations affect the durability of the bond at the resin–dentin interface, causing a reduction in bond strength over time and an increase in leakage.³³ Thus, the adhesion of restorative materials to caries-affected dentin has been proven to result in a bond strength that is lower than that observed for sound dentin, regardless of the adhesive system employed.³⁴ The low bonding efficacy of resin to caries-affected dentin contributes to the deterioration of the adhesive interface of the restoration.⁵ Previous researchers stated that the clinical management of cases involving caries-affected dentin can be addressed by leveraging the caries-free margins of the restoration, thereby facilitating the bonding to sound enamel and den- tin margins.^{2,35} However, in cases where deep subgingival

cariou lesions are present, the attainment of sound cervical margins may not be feasible, necessitating the consideration of surgical crown lengthening, a treatment modality regarded as invasive.³⁶ In addition, other researchers have found that caries-affected dentin left behind may cause biological problems that affect the pulp health and may lead to chronic pulpal inflammation or even silent pulp death.³²

As demonstrated in previous studies, the application of a liner prior to restoring a tooth with resin composite improves the quality of deep caries-affected dentin and promotes its self-remineralization.^{37,38} Yet, other clinical studies and systematic reviews have shown no difference between the application of a liner and its absence.³⁹ In contrast, this might affect the fracture resistance and overall survival of the restoration due to the absence of direct dentin bonding.⁴⁰ To overcome these problems and still follow the conservative selective caries removal approach, another concept has evolved. The question has been raised as to whether it would be advisable to remineralize caries-affected deep dentin prior to bonding and restoring the cavity. This concept aimed to overcome the challenges associated with bonding to caries-affected dentin when used as a bonding substrate, improve the quality of the hybrid layer, provide biomimetic protection of the pulp, and enable a conservative treatment for deep class II lesions by facilitating deep margin elevation after remineralization. The remineralization step should be clinically applicable, with ease of manipulation and short application time.

Biological mineralization of dental hard tissues is a progressive dehydration process. As the mineral content increases, the water content of the collagen matrix decreases.⁷ In dentin bonding, resin adhesive is incapable of dehydrating the collagen matrix sufficiently, leaving behind water that will allow hydrolysis of the hybrid layer components, especially when dealing with deep dentin layers and caries-affected layers.^{41,42} Biomimetic remineralization mimics the progressive dehydration of natural biomineralization by replacing matrix water with apatite crystallites.⁴³ In the hybrid layer, replacing water with minerals would increase the mechanical properties and inhibit water-related hydrolysis. *In vitro* studies indicate that biomimetic remineralization has considerable potential for the restoration of hybrid layers or caries-like dentin, facilitating calcium binding.⁴³ These studies have also demonstrated the preservation of the mechanical properties of dentin, thereby enhancing the quality of hybrid layers.⁴⁴ The development of clinically applicable materials that contain and release the critical components of the process (at least calcium and phosphate sources) within a clinically applicable time frame poses significant challenges and remains a subject of ongoing research.¹³

Furthermore, as previously stated, the bonding protocol is considered critical. Therefore, the selection of an appropriate adhesive system and bonding mode

may affect the overall outcome. It is anticipated that the total-etch mode may promote the removal of superficial particles that have previously undergone remineralization, thereby impacting the bonding process.³⁵ In contrast, the self-etch adhesive system is expected to improve bonding to the remineralized surfaces, particularly when using universal adhesives, composed of 10-MDP, which has been shown to provide unique bonding not only with micromechanical bonds but also by bonding ionically to calcium and hydroxyapatite in dentin.^{25,45}

The microtensile test is a method used to evaluate the adhesive properties of resin to dentin. It facilitates the evaluation of regional bond strengths of various portions of the dentin surface.⁴⁶ The modified testing method employs stick-shaped specimens with a cross-sectional area of 1 mm × 1 mm (non-trimming technique) to enable the testing of large variations in bond strength values that occur due to the formation of serial sections.⁴⁷ Although microshear could also be used to evaluate the bond strength, it offers a less discriminating evaluation of the adhesive performance. In comparison, microtensile testing provides a more effective means of evaluating dentin adhesion due to its more homogenous stress distribution.⁴⁸

The current study examined the results of remineralization protocols, revealing that the total-etch groups exhibited a significant difference in the microtensile bond strength values of the NaF and nHAp groups (G3 and G4). Both groups demonstrated the highest microtensile bond strength values, followed by the positive control group (G1) and the combined NaF and nHAp group (G5). The negative control group (G2) showed the lowest microtensile bond strength.

In this study, when NaF (G3) or nHAp (G4) were used in conjunction with the total-etch mode, the bond strength results exhibited a marked increase in comparison to those observed when bonding to sound dentin. This finding aligns with the results of previous studies, which demonstrated that the application of remineralizing agents led to enhanced bond strength values. Okuyama et al. claimed that the application of NaF improves the bond strength to dentin.¹² In addition, the findings of Abdallah et al.²¹ and Roushdy et al.²² suggest that nHAp improves the microtensile bond strength to dentin. However, the findings of these studies did not demonstrate a significantly higher bond strength compared to sound dentin, as observed in the current study. This could be attributed to the correlation between the depth of dentin and an increase in the number and width of dentinal tubules, as well as a decrease in peritubular dentin, which has a high influence on the bond strength. The reduced bond strength is also due to the inadequate mineral ratio present for bonding, particularly when using the total-etch mode with universal adhesives.¹³ The remineralization of the deep layer, closure of the dentinal tubules and increase in mineral content could promote the bonding when using universal adhesives.

The present study demonstrated that the dissolution of nHAp in NaF in the combined group (G5) resulted in lower microtensile bond strength values when compared to the use of each type separately. This finding contradicts the results of studies by Kunam et al.²³ and Rabee et al.,³⁵ which showed higher bond strength when nHAp and NaF were used in conjunction. This phenomenon can be explained as such: the combination of NaF and nHAp results in the formation of fluorohydroxyapatite crystals of increased size, accompanied by enhanced superficial closure and diminished penetration depth. Consequently, it is anticipated that the total-etch mode will facilitate the removal of remineralizing crystals, thereby exhibiting a lesser effect in comparison to the NaF (G3) and nHAp (G4) groups. Furthermore, previous studies applied the remineralizing agents daily for a duration of 7 min. In the current study, a single application for 1 min was employed to enhance its clinical applicability.

In the current study, when the self-etch mode was used, there was no statistically significant difference between the microtensile bond strength values of the positive control, NaF, nHAp and combined groups. This can be attributed to the presence of minerals on the dentin surface, which enhances bonding when using universal adhesives, particularly due to their ionic bonding capabilities to calcium and hydroxyapatite.²⁵ The occurrence of micro-interfaces and nano-gaps is expected to be reduced due to the presence of the hydroxyapatite-coated collagen fibrils (nanointeraction zone).⁴⁹ In the self-etch mode, there was no difference between sound dentin and the different remineralization protocols because the smear layer was not removed completely, and the dentinal tubules were not widely open, in contrast to the total-etch mode.³⁴ For the negative control group (G2), pre-test failures occurred in all the samples, using the total-etch or self-etch protocols. This finding contradicts the conclusions of Nakajima et al., who reported that bonding to caries-affected dentin could be obtained, but with reduced microtensile bonding results and dentin cohesive mode of failure.⁵

With regard to the different bonding strategies, the current study demonstrated that the positive control group (G1) exhibited significantly lower microtensile bond strength values when the total-etch mode (G1B1) was employed in comparison to the self-etch mode (G1B2). This finding aligns with the study by Pegado et al., which demonstrated that one-step adhesives exhibited superior efficacy in achieving optimal bonding when utilized on deep dentin compared to total-etch adhesives.⁵⁰ This enhanced bonding capability can be attributed to the presence of functional monomers (such as 10-MDP) in water-based adhesives.⁵⁰ As previously mentioned, this phenomenon may be due to the increase in the number and width of dentinal tubules and the decrease in peritubular dentin in deep dentin layers. The utilization of the total-etch mode results in the complete removal of the

smear layer with a reduction in the mineral ratio necessary for bonding. This alteration could impact the bond strength to deep dentin.¹³

With regard to the NaF (G3) and nHAp (G4) groups, no statistically significant difference was observed in the microtensile bond strength values of the 2 bonding modes. This outcome could be dependent on the consistency of the remineralizing agent, the size of the particles, and the penetration depth. In the NaF group (G3), the remineralizing agent was administered in the form of a solution (liquid) with high penetration capabilities. In the nHAp group (G4), the agent was applied in the form of a paste with small crystal size (nanocrystals) that exhibited good penetration capabilities. Therefore, the etchant applied in the total-etch mode did not have a detrimental effect on the remineralized surface, and both bonding modes showed analogous results.

In the case of the combined NaF and nHAp group (G5), the total-etch mode (G5B1) exhibited significantly lower microtensile bond strength in comparison to the self-etch mode (G5B2). This phenomenon can be due to the dissolution of nHAp in the NaF solution, which results in the formation of fluorohydroxyapatite crystals of greater size, accompanied by more superficial closure and less penetration depth. Consequently, it is anticipated that the total-etch mode will exhibit a higher degree of remineralizing crystal removal during the etchant step when compared with the self-etch mode.

In the present study, the mode of failure was observed for each beam to identify the type of failure, as it is indicative of the quality of the hybrid layer.⁴³ The highest prevalence of adhesive failure was observed in the combined NaF and nHAp group in the total-etch mode (G5B1), which may be attributed to the high crystal size and shallow penetration, as previously explained. None of the groups exhibited cohesive failure within the dentin, a failure mode commonly reported in the literature when bonding to caries-affected dentin. This finding suggests that the quality of the hybrid layer and the mechanical properties of the caries-affected dentin substrate have been enhanced after remineralization.^{33,43,44} The highest prevalence of cohesive restoration failure was observed in the nHAp group that utilized the self-etch mode (G4B2), indicating that the quality of the hybrid layer has been improved and that a high adhesive bond has been established.²¹ The highest prevalence of mixed failure was observed in the NaF group that employed the total-etch mode (G3B1). All specimens of demineralized dentin constituting the negative control groups (G2B1 and G2B2) exhibited pre-test failure. These groups were the only ones to demonstrate such a mode of failure, suggesting that the hybrid layer's quality and the adhesive bond were substandard.

The null hypothesis was rejected based on the results of the current study, which demonstrated a difference in the microtensile bond strength of composite restoration bonded to demineralized and remineralized dentin

substrates. The null hypothesis stating that the mode of adhesion (total-etch or self-etch) will not affect the applied remineralizing agent was partially rejected. Additionally, the remineralization step affected the mode of failure.

Limitations

During the preparation of the eggshell nHAp paste, a more diluted consistency might have improved the penetration capacity of the paste and yielded optimal results. Additionally, packable composite resin was applied to the remineralized surfaces without the usage of a flowable composite. The application of a layer of flowable composite resin may have enhanced the adaptability and improved the bond strength. In addition, the application time might have had an influence on the results. The duration of application in the present study was set at 1 min. The impact of varying application times could be further investigated. The variability in deep dentin quality and tooth age may also be a contributing factor, suggesting the necessity for a larger sample size in subsequent studies. The assessment of other dentin mechanical properties after remineralization could also be of value. The findings of this study are limited, as only the immediate effect was evaluated. Long-term studies are recommended to assess the durability of the observed effects.

Clinical recommendations

The findings of the present study indicate that the efficacy of a selective caries removal protocol is contingent upon the presence of sound enamel and dentin margins. In instances where it is not possible to obtain sound margins for the purpose of bonding, such as in cases of deeply seated Class II lesions with subgingival margins, dentin surface treatment with a remineralizing agent prior to bonding may facilitate the recovery of the dentin mechanical properties, thereby rendering them analogous to those of sound dentin. A conservative approach to restorative dentistry involves remineralizing deeply seated caries-affected dentin margins prior to bonding. This method facilitates the elevation of deep margins, particularly in scenarios where obtaining sound margins is challenging.

Conclusions

The utilization of remineralizing agents, such as the eggshell nHAp paste and NaF solution, has been demonstrated to improve bond strength to caries-affected dentin when using universal adhesive in the total-etch and self-etch modes. The combination of NaF and nHAp is only compatible with the self-etch mode, while its use with total-etch adhesives results in a detrimental effect. The bonding ability to caries-affected demineralized dentin is

unreliable. The remineralization of caries-affected dentin improves the hybrid layer quality and favors the mode of failure.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of Misr International University, Cairo, Egypt (approval No. MIU-IRB-2122-151).

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. Giacaman RA, Muñoz-Sandoval C, Neuhaus KW, Fontana M, Chalas R. Evidence-based strategies for the minimally invasive treatment of carious lesions: Review of the literature. *Adv Clin Exp Med*. 2018;27(7):1009–1016. doi:10.17219/acem/77022
2. Alleman DS, Magne P. A systematic approach to deep caries removal end points: The peripheral seal concept in adhesive dentistry. *Quintessence Int*. 2012;43(3):197–208. PMID:22299120.
3. Hoefler V, Nagaoka H, Miller CS. Long-term survival and vitality outcomes of permanent teeth following deep caries treatment with step-wise and partial-carries-removal: A systematic review. *J Dent*. 2016;54:25–32. doi:10.1016/j.jdent.2016.09.009
4. Perdigão J, Reis A, Loguercio AD. Dentin adhesion and MMPs: A comprehensive review. *J Esthet Restor Dent*. 2013;25(4):219–241. doi:10.1111/jerd.12016
5. Nakajima M, Kunawarote S, Prasansuttiporn T, Tagami J. Bonding to caries-affected dentin. *Jpn Dent Sci Rev*. 2011;47(2):102–114. doi:10.1016/j.jdsr.2011.03.002
6. Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. *Dent Mater J*. 2013;32(1):138–143. doi:10.4012/dmj.2012-243
7. Liu Y, Tjäderhane L, Breschi L, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J Dent Res*. 2011;90(8):953–968. doi:10.1177/0022034510391799
8. Anani H, Elasser D, Niazy M, Jamil W, Elsharkawy D. Evaluation of the remineralization and antibacterial effect of natural versus synthetic materials on deep carious dentin: A randomized controlled trial. *Dent Med Probl*. 2023;60(1):87–97. doi:10.17219/dmp/147075
9. Tay FR, Pashley DH. Biomimetic remineralization of resin-bonded acid-etched dentin. *J Dent Res*. 2009;88(8):719–724. doi:10.1177/0022034509341826

10. Xu Z, Neoh KG, Lin CC, Kishen A. Biomimetic deposition of calcium phosphate minerals on the surface of partially demineralized dentine modified with phosphorylated chitosan. *J Biomed Mater Res B Appl Biomater*. 2011;98(1):150–159. doi:10.1002/jbm.b.31844
11. Basak SS, Dokumacioglu E. Evaluation of the effects of different mouthrinses on dental remineralization. *Dent Med Probl*. 2023;60(2):219–225. doi:10.17219/dmp/134290
12. Okuyama K, Komatsu H, Yamamoto H, et al. Fluorine analysis of human dentin surrounding resin composite after fluoride application by μ -PIGE/PIXE analysis. *Nucl Instrum Methods Phys Res B*. 2011;269(20):2269–2273. doi:10.1016/j.nimb.2011.02.063
13. Tjäderhane L. Dentin bonding: Can we make it last? *Oper Dent*. 2015;40(1):4–18. doi:10.2341/14-095-BL
14. Brackett MG, Agee KA, Brackett WW, et al. Effect of sodium fluoride on the endogenous MMP activity of dentin matrices. *J Nat Sci*. 2015;1(6):e118. PMID:26052548.
15. Bhandari NL, Bista S, Gautam TR, et al. An overview of synthesis based biomedical applications of hydroxyapatite nanomaterials. *J Nepal Chem Soc*. 2021;42(1):64–74. doi:10.3126/jncs.v42i1.35333
16. Amin M, Mehta R, Duseja S, Desai K. Evaluation of the efficacy of commercially available nano-hydroxyapatite paste as a desensitizing agent. *Adv Hum Biol*. 2015;5(1):34–38. https://journals.lww.com/adhb/Abstract/2015/05010/Evaluation_of_the_Efficacy_of_Commercially.6.aspx. Accessed February 23, 2024.
17. Onwubu SC, Mhlungu S, Mdluli PS. In vitro evaluation of nanohydroxyapatite synthesized from eggshell waste in occluding dentin tubules. *J Appl Biomater Funct Mater*. 2019;17(2):2280800019851764. doi:10.1177/2280800019851764
18. Hui P, Meena SL, Singh G, Agarawal RD, Prakash S. Synthesis of hydroxyapatite bio-ceramic powder by hydrothermal method. *JMMCE*. 2010;09(8):683–692. doi:10.4236/jmmce.2010.98049
19. Gronwald B, Kozłowska L, Kijak K, et al. Nanoparticles in dentistry—current literature review. *Coatings*. 2023;13(1):102. doi:10.3390/coatings13010102
20. Imran E, Cooper PR, Ratnayake J, Ekambaram M, Mei ML. Potential beneficial effects of hydroxyapatite nanoparticles on caries lesions in vitro—a review of the literature. *Dent J (Basel)*. 2023;11(2). doi:10.3390/dj11020040
21. Abdallah R, Farouk H, Essa ME. Surface topography and thickness of hybrid layer assessment after dentin pretreatment with egg shell powder as desensitizing agent. *Egypt Dent J*. 2018;64(4):3845–3853. doi:10.21608/edj.2018.79359
22. Roushdy R, Niaz M, Mohamed H. Effect of egg shell and white tea dentin pretreatment on microtensile bond strength of universal adhesive system. *Al-Azhar J Dent*. 2022;9(3):399–409. doi:10.21608/adjg.2022.75825.1364
23. Kunam D, Manimaran S, Sampath V, Sekar M. Evaluation of dentinal tubule occlusion and depth of penetration of nano-hydroxyapatite derived from chicken eggshell powder with and without addition of sodium fluoride: An in vitro study. *J Conserv Dent*. 2016;19(3):239–244. doi:10.4103/0972-0707.181940
24. Comar LP, Souza BM, Gracindo LF, Buzalaf MAR, Magalhães AC. Impact of experimental nano-HAP pastes on bovine enamel and dentin submitted to a pH cycling model. *Braz Dent J*. 2013;24(3):273–278. doi:10.1590/0103-6440201302175
25. Cuevas-Suárez CE, de Oliveira da Rosa WL, Lund RG, da Silva AF, Piva E. Bonding performance of universal adhesives: An updated systematic review and meta-analysis. *J Adhes Dent*. 2019;21(1):7–26. doi:10.3290/j.jad.a41975
26. Barbosa-Martins LF, de Sousa JP, Alves LA, Davies RPW, Puppini-Rontanti RM. Biomimetic mineralizing agents recover the micro tensile bond strength of demineralized dentin. *Materials (Basel)*. 2018;11(9):1733. doi:10.3390/ma11091733
27. Armstrong S, Breschi L, Özcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μ TBS) approach. *Dent Mater*. 2017;33(2):133–143. doi:10.1016/j.dental.2016.11.015
28. Aziz Y, Adrian M, Alfarisi CD, Khairat, Sri RM. Synthesis of hydroxyapatite nanoparticles from egg shells by sol-gel method. *Mater Sci Eng*. 2018;345(1):012040. doi:10.1088/1757-899X/345/1/012040
29. Dhar V, Pilcher L, Fontana M, et al. Evidence-based clinical practice guideline on restorative treatments for caries lesions: A report from the American Dental Association. *J Am Dent Assoc*. 2023;154(7):551–566.e51. doi:10.1016/j.adaj.2023.04.011
30. Thompson V, Craig RG, Curro Fredrick A, Green WS, Ship JA. Treatment of deep carious lesions by complete excavation or partial removal: A critical review. *J Am Dent Assoc*. 2008;139(6):705–712. doi:10.14219/jada.archive.2008.0252
31. Hevinga MA, Opdam NJ, Frencken JE, Truin GJ, Huysmans MCDNJM. Does incomplete caries removal reduce strength of restored teeth? *J Dent Res*. 2010;89(11):1270–1275. doi:10.1177/0022034510377790
32. Ricucci D, Siqueira JF, Rôças IN, Lipski M, Shibata A, Tay FR. Pulp and dentine responses to selective caries excavation: A histological and histobacteriological human study. *J Dent*. 2020;100:103430. doi:10.1016/j.jdent.2020.103430
33. Erhardt MCG, Toledano M, Osorio R, Pimenta LA. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: Effects of long-term water exposure. *Dent Mater*. 2008;24(6):786–798. doi:10.1016/j.dental.2007.09.007
34. Shibata S, Vieira LCC, Baratieri LN, et al. Evaluation of microtensile bond strength of self-etching adhesives on normal and caries-affected dentin. *Dent Mater J*. 2016;35(2):166–173. doi:10.4012/dmj.2014-330
35. Rabee M, Nomaan K, Abdelhady A. Effect of remineralizing agents on bond strength of resin composite to dentin: An in-vitro study. *Al-Azhar J Dent Sci*. 2020;23(1):15–21. doi:10.21608/ajdsm.2020.108457
36. Samartzis TK, Papalexopoulos D, Ntovas P, Rahiotis C, Blatz MB. Deep margin elevation: A literature review. *Dent J (Basel)*. 2022;10(3):48. doi:10.3390/dj10030048
37. Weiner RS, Weiner LK, Kugel G. Teaching the use of bases and liners: A survey of North American dental schools. *J Am Dent Assoc*. 1996;127(11):1640–1645. doi:10.14219/jada.archive.1996.0100
38. Lynch CD, Frazier KB, McConnell RJ, Blum IR, Wilson NHF. State-of-the-art techniques in operative dentistry: Contemporary teaching of posterior composites in UK and Irish dental schools. *Br Dent J*. 2010;209(3):129–136. doi:10.1038/sj.bdj.2010.674
39. Blum IR, Wilson NHF. Consequences of no more linings under composite restorations. *Br Dent J*. 2019;226(10):749–752. doi:10.1038/s41415-019-0270-2
40. Opdam NJM, VanBeek V, VanBeek W, et al. Long term clinical performance of ‘open sandwich’ and ‘total-etch’ Class II composite resin restorations showing proximal deterioration of glass-ionomer cement. *Dent Mater*. 2023;39(9):800–806. doi:10.1016/j.dental.2023.07.001
41. Pashley DH, Tay FR, Breschi L, et al. State of the art etch-and-rinse adhesives. *Dent Mater*. 2011;27(1):1–16. doi:10.1016/j.dental.2010.10.016
42. Tjäderhane L, Nascimento FD, Breschi L, et al. Strategies to prevent hydrolytic degradation of the hybrid layer – A review. *Dent Mater*. 2013;29(10):999–1011. doi:10.1016/j.dental.2013.07.016
43. Niu LN, Zhang W, Pashley DH, et al. Biomimetic remineralization of dentin. *Dent Mater*. 2014;30(1):77–96. doi:10.1016/j.dental.2013.07.013
44. Gu L, Huffman BP, Arola DD, et al. Changes in stiffness of resin-infiltrated demineralized dentin after remineralization by a bottom-up biomimetic approach. *Acta Biomater*. 2010;6(4):1453–1461. doi:10.1016/j.actbio.2009.10.052
45. Sharifian A, Esmaeili B, Gholinia H, Ezoji F. Microtensile bond strength of different bonding agents to superficial and deep dentin in etch-and-rinse and self-etch modes. *Front Dent*. 2023;20:9. doi:10.18502/fid.v20i9.12608
46. Sano H, Shono T, Sonoda H, et al. Relationship between surface area for adhesion and tensile bond strength – evaluation of a micro-tensile bond test. *Dent Mater*. 1994;10(4):236–240. doi:10.1016/0109-5641(94)90067-1
47. Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH. Regional measurement of resin–dentin bonding as an array. *J Dent Res*. 1999;78(2):699–705. doi:10.1177/00220345990780021001
48. Münchow EA, Bossardi M, Priebe TC, et al. Microtensile versus microshear bond strength between dental adhesives and the dentin substrate. *Int J Adhes Adhes*. 2013;46:95–99. doi:10.1016/j.jadhadh.2013.06.005
49. Koshiro K, Sidhu SK, Inoue S, Ikeda T, Sano H. New concept of resin–dentin interfacial adhesion: The nanointeraction zone. *J Biomed Mater Res B Appl Biomater*. 2006;77(2):401–408. doi:10.1002/jbm.b.30450
50. Pegado REF, Botelho do Amaral FL, Flório FM, Basting RT. Effect of different bonding strategies on adhesion to deep and superficial permanent dentin. *Eur J Dent*. 2010;4(2):110–117. PMID:20396440.

Peri-implant strain analysis: An in vitro comparison between screw- and cement-retained posterior base metal restorations

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Abstract

Background. Research is required to characterize peri-implant strain distribution associated with different restorative designs.

Objectives. This in vitro qualitative photoelastic stress analysis aimed to compare the influence of retention modality (screw-retained or cement-retained) on strain development around implants supporting different 3-unit posterior restorative designs.

Material and methods. Six prosthetic restorative designs were evaluated according to retention modality and prosthetic configuration: 3 single crowns, cement-retained and screw-retained (SC-c and SC-s, respectively); 3 splinted crowns (SP-c and SP-s); and simple 3-unit bridges (SB-c and SB-s). All prosthetic units were morphologically identical. A photoelastic resin block incorporating 3 implants was mounted on a loading device that applied vertical loads of 200 N simultaneously at 3 identical loading points on each restoration to simulate chewing, occlusal clenching and deglutition. Fringe lines were photographically recorded and qualitatively analyzed after placement of the restorations and during loading.

Results. Screw-retained restoration with splinted crowns (SP-s) was the only design to exhibit strain in the unloaded state following placement, representing preload strain. Loading-induced strains were observed in SC-c and SC-s, with lower strain levels for SC-c than for SC-s, and in SP-c and SP-s, with lower strain levels for SP-c than for SP-s. Both configurations also exhibited crestal strains. The simple bridge (SB) designs revealed purely axial strains, with no crestal strains, regardless of retention modality.

Conclusions. Retention modality appears to influence peri-implant strain development in mesiodistal and occlusogingival dimensions for 3-unit single crown and splinted crown designs. Screw-retained 3-unit splinted prostheses may generate preload strains around the unloaded implant supporting the restoration. Simple 3-unit bridge designs demonstrated the most favorable strain pattern, regardless of the retention modality.

Keywords: preload, strain, photoelastic analysis, cement retention, screw retention

Cite as

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Highlights

- This study compares peri-implant strain patterns between cement-retained and screw-retained prostheses using 3-unit posterior implant-supported restorative designs.
- Axial loading generated peri-implant strains that were evaluated using photoelastic analysis based on peak strain and crestal location.
- Three screw-retained splinted crowns are the only design associated with preload strain.
- Cementation produced lower peak strains than screw retention for 3 splinted crowns and 3 single crowns.
- Simple 3-unit bridge showed the most favorable strain pattern, with no crestal strain and comparable strain magnitude for both retention modalities.

Introduction

High success and survival rates have demonstrated favorable clinical performance of posterior partial implant-supported restorations fabricated using various prosthetic materials, implant dimensions and combinations of grafting materials.^{1–4,5}

Research continues to focus on the circumstances and etiologies of technical and biological complications associated with implant-supported restorations.⁶ Crestal bone loss around implants is a key feature in the definition of peri-implantitis.⁷ Most etiological factors are related to the presence of oral biofilm, which initiates the infectious and inflammatory processes involved in peri-implantitis and may subsequently result in bone loss.^{8–10} Surgical procedures involving exposure of osseous tissue may themselves induce postoperative crestal bone loss.¹¹ Occlusal factors have also been considered to be associated with the onset and progression of crestal bone loss, both historically and in contemporary research.^{12–15} Theoretical as well as more substantial explanations for peri-implant crestal bone loss have been proposed, linking this pathology to functional overload.^{15,16} The role of occlusion in the initiation and progression of crestal bone loss therefore remains relevant, particularly in light of clinical studies associating peri-implant pathology with occlusal overload, although not exclusively.^{14,17}

Our research group has previously investigated the impact of occlusal loading on buccolingual strain recorded within 3-unit prostheses.¹⁸ Altering the restorative configuration was found to significantly influence strain development within the implant neck.^{18,19} Changing the direction of force also had a significant mechanical effect at the same location.¹⁸ Stress is transferred through the implants and restorative components and is expressed as strain according to the different moduli of elasticity of the various elements before reaching the surrounding bone.²⁰ The application of multiple simultaneous contacts to all restorative units represents an attempt to simulate parafunctional activity, such as static clenching, as well as routine functions, including chewing and swallowing.^{21–23}

Apart from prosthetic design, the composition of the crown material has also been documented in vitro as a factor influencing crestal strain under load.²⁴

One of the key components of restorative design is the retention mechanism, which is currently divided into screw retention and cement retention.²⁵ Both are legitimate treatment approaches with proven long-term survival rates, provided that clinicians consider the known limitations associated with each retention form.^{26,27} These include concerns regarding excess cement, which may contribute to peri-implant disease, and the higher probability of screw loosening and porcelain chipping associated with screw-retained prostheses.^{27,28} Limited retrievability of cement-retained restorations has also been regarded as a well-known drawback. However, recent evidence suggests that newer cement-retained designs may facilitate retrieval through improved retrieval techniques.^{29,30}

Although screw-retained restorations have gained considerable popularity concomitantly with the increasing use of digital impression procedures,^{31,32} several clinical studies and reviews have suggested a possible cause-and-effect relationship between crestal bone loss and screw retention mechanisms.^{33,34} Other reports have found no influence of the retention mode on crestal bone response.³⁵ Conversely, laboratory studies have indicated that welding techniques, framework material and mode of engagement may reduce stress development within the crestal zone.^{36,37} Such clinical and in vitro evidence may reflect differences in the strain imposed on the peri-implant crestal bone. Nevertheless, to the best of our knowledge, no direct observations relating peri-implant strain to retention mechanisms have been reported.

Scientific evidence in implant dentistry should establish whether, despite its rising popularity, screw retention induces excessive peri-implant crestal strain beyond known biomechanical limits, above which pathological bone remodeling may occur.¹⁵

It is therefore important to investigate potential mechanisms and hypotheses linking the retention mechanism and prosthetic design to crestal bone loss through peri-implant strain.

Strain developing within an implant may also be transferred to the surrounding bone. Photoelastic analysis is a highly illustrative method for realistic simulation and visualization of such strain.³⁸ The resulting peri-implant strain can be evaluated using photoelastic resin

surrounding the implants and simulating bone, in which the resulting fringe patterns can be visually inspected. Such a model provides a clear representation of strain development around implants supporting a fixed restoration along the implant surface, although the analysis remains confined to the sagittal plane. Analysis of the fringe lines can reveal the theoretical biomechanical response of bone to a load applied at the level of the entire implant and even to smaller components within an implant.^{38,39} Larger-scale photoelastic analyses have been employed to examine multi-unit restorations.⁴⁰ Therefore, photoelasticity may demonstrate a relationship between the biomechanical state of the implants under loading or at rest, and crestal strain, expressed in the mesiodistal and coronal dimensions.

The purpose of this *in vitro* study was to use photoelastic analysis to evaluate peri-implant strain elicited by the installation and loading of various 3-unit posterior fixed prostheses and to compare the effects of the retention mechanism and prosthetic design.

Material and methods

This study employed photoelastic strain analysis and was performed in the laboratories of Vishay Precision Group Corporation (Vishay Measurements Group, Raleigh, USA). It aimed to compare 3 posterior designs of cement-retained restorations with their screw-retained counterparts under simulated occlusal loading and unloaded installation conditions. The study was designed as observational qualitative research and conducted in accordance with the Standards for Reporting Qualitative Research (SRQR).⁴¹

A photoelastic model (PLM-4B; Vishay Measurements Group) was constructed. The model had a modulus of elasticity resembling that of human bone (2.8 GPa), and dimensions of 15.8 mm × 35.4 mm × 32 mm (Cat. No. S-116H).

The fringe order characteristics of the photoelastic block were provided by the manufacturer and are presented in Table 1.

Three holes were drilled into the block according to the standard surgical drilling protocol. Three implants measuring 3.75 mm in diameter and 13 mm in length were inserted (M.I.S. Implants Technologies Ltd., Bar Lev Industrial Area, Israel).

The drilling procedure was performed using drills of consecutively increasing diameters to minimize residual stress. Stress induced in the photoelastic model was relieved by placing it on a Teflon surface for 120 min at 70°C. The model was subsequently allowed to cool inside the closed oven. Stress relief was confirmed using a circular polariscope to verify the absence of residual strain. The implants were positioned linearly, with a distance of 6 mm between adjacent implants, to facilitate fringe registration. The constructed photoelastic block and the implants embedded within it remained unchanged throughout the experiment.

Transfer copings were screwed into place and interconnected with acrylic resin (DuraLay; Reliance Dental Manufacturing Co., Alsip, USA) for stabilization and precision. The open-tray technique with a polyether-based material (Impregum; 3M ESPE, Seefeld, Germany) was employed to take impressions at the implant level, utilizing a custom-made acrylic resin tray. After insertion of the analogues, a working cast was poured. Cement-retained or screw-retained abutments were mounted onto the analogues according to the retention modality and the design of the restorations they carried, using a controlled torque of 20 Ncm. An example of such a prosthesis-free and strain-free model is presented in Fig. 1.

Table 1. Isochromatic fringe characteristics of the PLM-4B photoelastic material (Vishay Measurements Group, Raleigh, USA)

Color	Approximate relative retardation		Fringe order <i>n</i>
	[nm]	[×10 ⁻⁶]	
Black	0	0.0	0.00
Pale yellow	345	14.0	0.60
Dull red	520	20.0	0.90
Red/blue transition	575	22.7	1.00
Blue-green	700	28.0	1.22
Yellow	800	32.0	1.39
Rose red	1,050	42.0	1.82
Red/green transition	1,150	45.4	2.00
Green	1,350	53.0	2.35
Yellow	1,440	57.0	2.50
Red	1,520	60.0	2.65
Red/green transition	1,730	68.0	3.00
Green	1,800	71.0	3.10

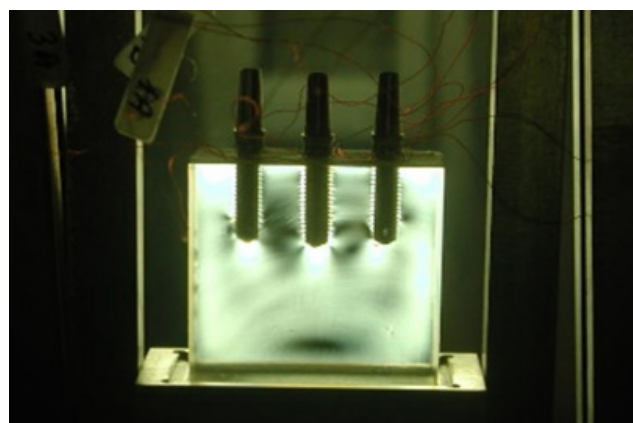


Fig. 1. Unrestored (uncrowned) photoelastic model showing no strain and displaying a black-and-white scale within the photoelastic block

Cast base metal fixed prostheses were fabricated using a Co–Cr–Mo alloy (Remanium GM3800; Dentauro, Ispringen, Germany). A silicone index was employed to provide identical morphology and dimensions for all 6 configurations tested. The 6 configurations were divided into 2 groups: 3 cement-retained configurations and 3 screw-retained configurations. Cement-retained restorations comprised 3 single crowns, 3 splinted crowns and a simple bridge (SC-c, SP-c and SB-c, respectively), as did the screw-retained restorations (SC-s, SP-s and SB-s, respectively). All restorations were fabricated by a single dental laboratory.

After completion of all restorations, the abutments were affixed to the implants with the same torque of 20 Ncm, according to each configuration. Prior to each loading session, the different cement-retained restorations were retained on the abutments with temporary cement (Temp-Bond NE; Kerr Dental, Brea, USA). Screw-retained restorations were tightened onto the abutments using a torque of 35 Ncm. Abutments were selectively removed when testing SB restorations. Remaining unloaded implants in the photoelastic block did not interfere with recording the fringe order, as shown in a previous study.⁴²

A custom-made load application device was created to apply a vertical load of 200 N to each of the 6 restorations. The load was transmitted to the restoration through loading pins, with 3 pins applying the load simultaneously. Each vertical loading session lasted 40 s, and images were captured 20 s after loading began using a digital camera. This procedure was repeated twice to verify the uniformity of the results. The photoelastic model was left to rest for 5 min between loading sessions to allow for stress relief. A white halogen light source was used with the aid of a Fresnel lens positioned between the light source and the tested assembly. Photographic recordings during the 5-min recess without loading verified the absence of residual stress in the photoelastic resin. The loading sessions then resumed.

Images were captured (Nikon D80; Nikon Corp., Tokyo, Japan) and further analyzed using image-processing software (Adobe Photoshop CS3; Adobe Inc., San Jose, USA). The images were classified according to the number of fringes and their locations in each sample using the manufacturer's reference table (Table 1). Fringe number and fringe color indicated strain intensity. All images were evaluated by the same operator (JN).

Results

Visual representation of the strains is presented in Fig. 1–8. Figure 1 shows the block's baseline strain-free state, to be compared with the subsequent installation and loading strains. Figures 2–7 show the loading strains for the 6 configurations: Fig. 2 and Fig. 3 compare the loading strains of SC-s and SC-c, respectively; Fig. 4

and Fig. 5 compare the loading strains of SB-s and SB-c, respectively; and Fig. 6 and Fig. 7 compare the loading strains of SP-s and SP-c, respectively. No strains were registered during the installation of the prostheses (zero

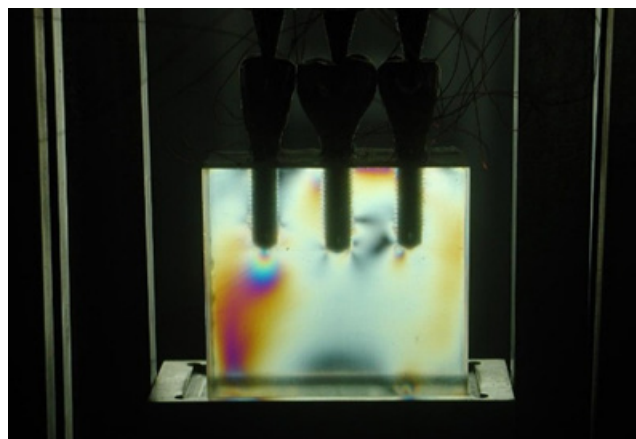


Fig. 2. Loaded screw-retained restoration with 3 single crowns (SC-s)

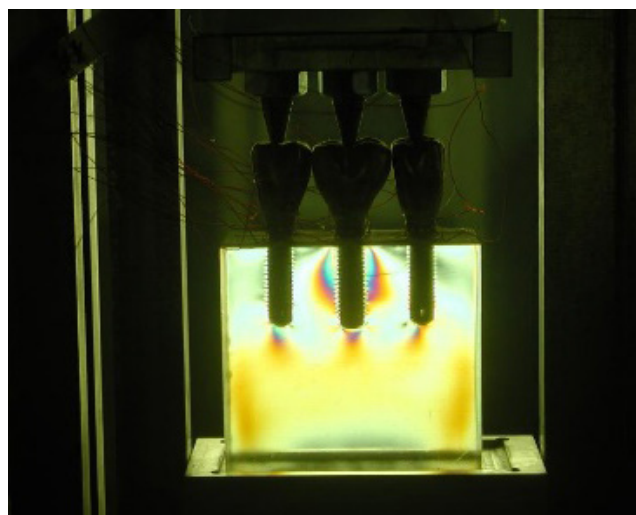


Fig. 3. Loaded cement-retained restoration with 3 single crowns (SC-c)

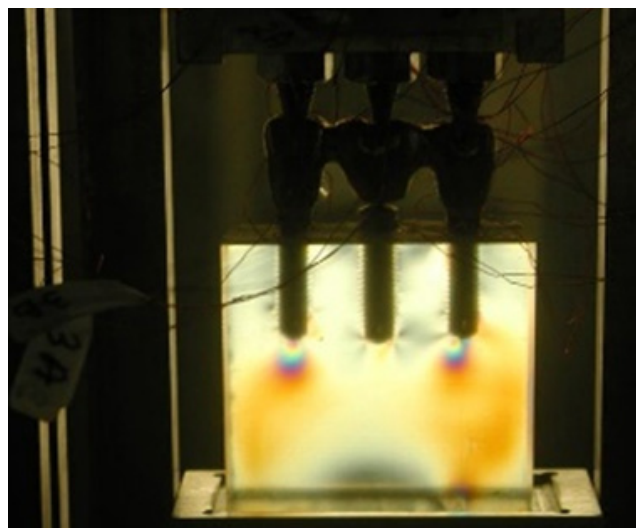


Fig. 4. Loaded screw-retained restoration with a single 3-unit bridge (SB-s)

load application) except for SP-s, which demonstrated strain fringes as presented in Fig. 8. Fringes were concentrated between the right and middle implants, as well as on the opposite side of the right implant, reaching the

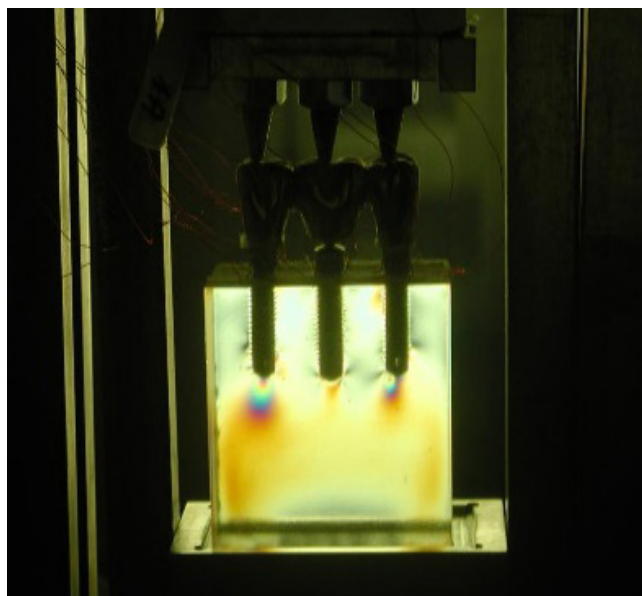


Fig. 5. Loaded cement-retained restoration with a single 3-unit bridge (SB-c)

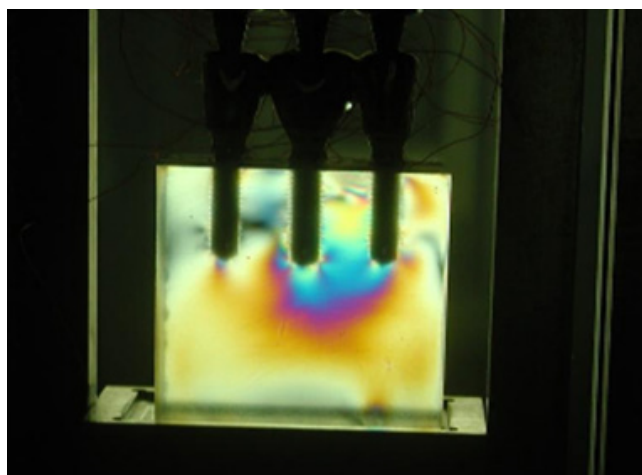


Fig. 6. Loaded screw-retained restoration with 3 splinted crowns (SP-s)

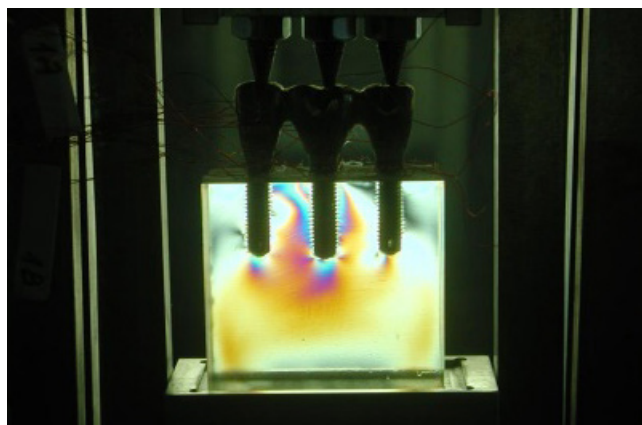


Fig. 7. Loaded cement-retained restoration with 3 splinted crowns (SP-c)

crestal bone on both sites. Such a clear representation of strain in a seemingly stress-free/load-free state demonstrates the preload status of the restorations. Figure 9 shows SP-c in its strain-free status during installation, which serves as a representation of all installation conditions with SC and SB configurations for both retention modalities.

Fringes concentrated at the implant apex alone indicate pure vertical or axial loading. Fringes on the proximal aspects reflect non-axial loading with bending strains, while fringes on the proximal and apical aspects represent combined compression and bending stresses.

The strains were divided into installation strains, defined as readings registered during the fixation of the cast restorations, and loading strains, indicating strain development during loading sessions.

By visual comparison between the 6 unloaded assemblies and the prosthesis-free model (Fig. 1), mounting the screw-retained splinted prosthesis (SP-s) was the only circumstance that produced detectable strain before loading, demonstrating peri-implant preload strain.

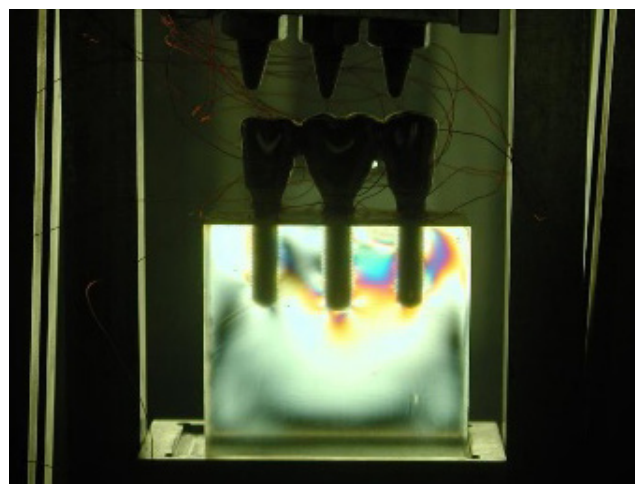


Fig. 8. Unloaded screw-retained restoration with 3 splinted crowns (SP-s)

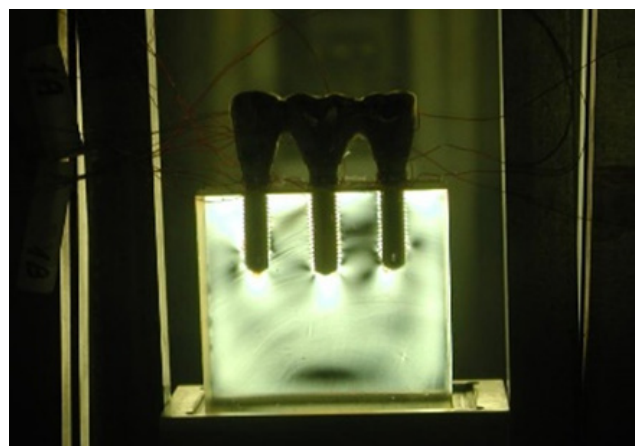


Fig. 9. Unloaded cement-retained restoration with 3 splinted crowns (SP-c)

Note the similarity to the black-and-white scale pattern observed within the photoelastic block in Fig. 1.

Quantification of the strain magnitude was achieved using 19 points of interest for each configuration, including 15 peri-implant locations and 4 crestal locations. An example of such an analysis is shown in Fig. 10

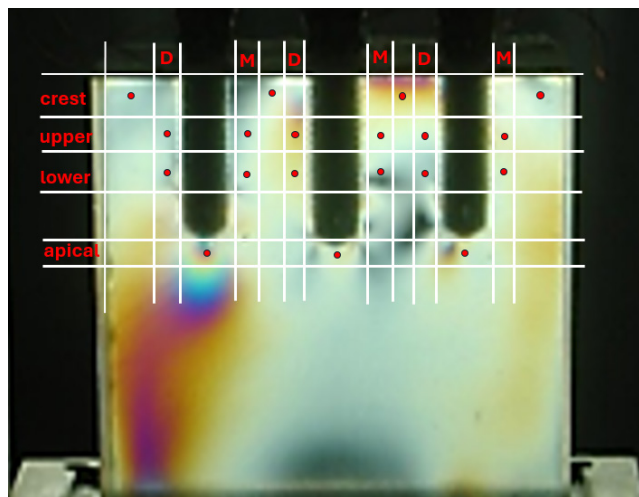


Fig. 10. Example of quantitative analysis of the SC-s configuration using 19 points of interest: 15 peri-implant points (1 apical, 2 distal and 2 mesial points for each implant) and 4 crestal points (2 flanking and 2 interimplant points)

D – distal; M – mesial.

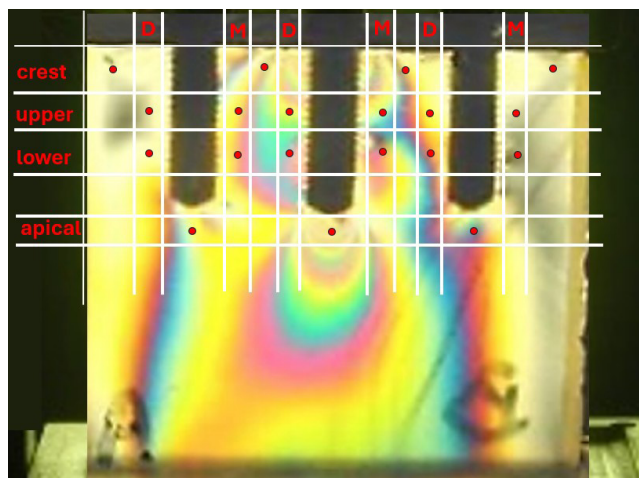


Fig. 11. Example of quantitative analysis of the SB-s configuration using 19 points of interest: 15 peri-implant points (1 apical, 2 distal and 2 mesial points for each implant) and 4 crestal points (2 flanking and 2 interimplant points)

and Fig. 11. Table 2 presents the fringe values recorded during the installation of SP-s, describing its preload strain. Table 3 shows the strain magnitude induced by simultaneous vertical loading of all 6 prosthetic assemblies. Table 4 illustrates the differences in the summed fringe orders between the various configurations at 3 levels: apical; crestal; and interproximal.

When examining the loaded assemblies, several features of strain distribution were observed. Cement-retained restorations with single crowns showed off-axis strain development, particularly along both sides of the middle implant, diminishing toward the crestal zone, with apical strains indicating axial stress around all implants. Screw-retained restorations with single crowns showed no uniform strain distribution, unlike SC-c. Crestal strain was evident on the right side of the middle implant, at the top of the crest, and considerable apical strain was evident around the left implant, which exhibited the highest apical strain among all SC assemblies (Table 3). Crestal strain was higher in SC-s than in SC-c, while apical strain was higher in SC-c than in SC-s (Table 4).

Cement-retained restorations with splinted crowns manifested axial and non-axial strains around the middle implant, as observed with SC-c, but with higher intensity than in SC-c. Screw-retained restorations with single crowns revealed much higher strain intensities than SP-c, mainly around the middle and right implants. Crestal strains were also noticeable adjacent to the necks of these 2 implants. According to the sum of the strain readings, higher strains were registered in SP-s than in SP-c (Table 4).

Simple bridge assemblies showed only apical strain concentrations around all flanking implants with both retention modalities. No significant change was observed between the retention modalities (Table 4). The unloaded middle implants in SB-c and SB-s exhibited apical strains as a result of the large stress fields originating from the loaded flanking implants. These potentially misleading strains were deliberately omitted from Table 3 and Table 4. Simple bridge assemblies showed no crestal or interproximal strain with either retention modality.

Table 2. Fringe order values recorded during the installation (preload) of screw-retained restoration with 3 splinted crowns (SP-s) at the implant level

Type of restoration	Left implant <i>n</i>					Middle implant <i>n</i>					Right implant <i>n</i>				
	distal		apical	mesial		distal		apical	mesial		distal		apical	mesial	
	U	L		U	L	U	L		U	L	U	L		U	L
SP-s	0.0	0.3	0.0	1.2	0.5	0.0	0.5	0.9	1.2	0.9	1.0	1.0	0.6	0.6	1.1
Crest (SP-s)	0.0	–	–	0.0	–	–	–	–	0.6	–	–	–	–	1.0	–

Measurements were obtained at 5 points around each implant (2 lower points: mesial and distal; 2 upper points: mesial and distal; and 1 apical point) and at 4 points located in the crestal areas (2 flanking crestal areas and 2 interimplant crestal areas); U – upper; L – lower; Crest – crestal zone; SP-s – screw-retained restoration with 3 splinted crowns.

Table 3. Fringe order values recorded during loading of 6 prosthetic configurations at the implant level

Type of restoration	Left implant <i>n</i>					Middle implant <i>n</i>					Right implant <i>n</i>				
	distal		apical	mesial		distal		apical	mesial		distal		apical	mesial	
	U	L		U	L	U	L		U	L	U	L		U	L
SC-c	0.0	0.0	0.9	0.0	0.0	1.3	0.4	0.9	1.3	0.4	0.0	0.0	0.9	0.0	0.0
Crest (SC-c)	0.0		–	–		0.0	–	–	–		0.3	–	–		0.3
SC-s	0.0	0.7	1.6	0.0	0.4	0.8	0.3	0.0	0.3	0.0	0.4	0.0	0.0	0.3	0.1
Crest (SC-s)	0.0		–	–		0.5	–	–	–		0.7	–	–		0.0
SB-c	0.0	0.0	1.2	0.0	0.0	–	–	–	–	–	0.0	0.0	1.3	0.0	0.0
Crest (SB-c)	0.0		–	–		0.0	–	–	–		0.0	–	–		0.0
SB-s	0.0	0.0	1.2	0.0	0.0	–	–	–	–	–	0.0	0.0	1.3	0.0	0.0
Crest (SB-s)	0.0		–	–		0.0	–	–	–		0.0	–	–		0.0
SP-c	0.0	0.0	1.2	0.6	0.0	1.2	1.1	1.2	1.2	0.8	0.0	0.0	0.7	0.0	0.0
Crest (SP-c)	0.0		–	–		1.2	–	–	–		0.5	–	–		0.0
SP-s	0.0	0.0	0.8	0.0	0.0	1.2	1.1	1.2	0.8	1.2	0.8	1.2	1.2	0.4	0.8
Crest (SP-s)	0.0		–	–		1.1	–	–	–		0.7	–	–		0.7

Measurements were obtained at 5 points around each implant (2 lower points: mesial and distal; 2 upper points: mesial and distal; and 1 apical point) and at 4 points located in the crestal areas (2 flanking crestal areas and 2 interimplant crestal areas); SC-c – cement-retained restoration with 3 single crowns; SC-s – screw-retained restoration with 3 single crowns; SB-c – cement-retained restoration with a single 3-unit bridge; SB-s – cement-retained restoration with a single 3-unit bridge; SP-c – cement-retained restoration with 3 splinted crowns.

Table 4. Comparison of total strain among 6 prosthetic configurations according to their location within the photoelastic block

Type of restoration	Crestal <i>n</i>	Interproximal <i>n</i>	Apical <i>n</i>
SC-c	0.6	3.4	2.7
SC-s	1.2	3.3	1.6
SB-c	0.0	0.0	2.5
SB-s	0.0	0.0	2.5
SP-c	1.7	3.9	3.1
SP-s	2.5	5.5	3.2

Discussion

Qualitative analysis was used in the present study, as in previous photoelastic studies.^{43–46} The assessment distinguished fringe locations as apical, interproximal (either mesial or distal) and crestal. An apical location should indicate favorable stress concentration, implying a force direction parallel to the long axis of the implant. An off-axis force direction is considered less favorable and might result in biological (peri-implant) and technical (prosthetic) complications.⁴⁷

There are few comparable photoelastic reports using multiple simultaneous occlusal contacts, which represent functional activities such as swallowing, chewing and static clenching, as employed in this study. The vast majority of comparable reports have used a single loading point, as mentioned in 2 previous reviews.^{38,48} Furthermore, our study employed a force of 200 N, a more representative value of human functional loading.⁴⁹ Therefore, only a limited number of studies are available for comparison with

the present findings. The photoelastic analysis showed no horizontal strains in the implant-supported SB configuration, compared with the remaining 2 configurations, regardless of the SB retention modality. Apart from favorable stress distribution, no crestal strains were observed in SB designs. These findings confirm those of a previous finite element analysis by Latifi et al., which attributed more favorable load distribution to configurations in which the supporting units were more widely distributed and less concentrated.⁵⁰ Another computerized model study by Silveira et al. reported findings after testing virtual 3-unit screw-retained fixed implant-supported prostheses, comparing 2-unit screw-retained bridge with a 3-unit screw-retained splint. The strain table at the bone level revealed findings similar to those of the present study, demonstrating an almost symmetrical strain distribution between the 2 flanking implants in the bridge and a more irregular strain pattern with higher intensity in the screw-retained splint.⁵¹

In the study by Bittencourt et al., a simple bridge, either cement-retained or screw-retained, was tested employing the same loading protocol as in our study, but with a load of 100 N. A single mesial contact point did not influence the favorable apical strain distribution around the apices of the 2 supporting implants.⁵² No differences were noted with regard to the retention modality when assessing simple bridge configurations, as observed in our research.

Clinical evidence also corroborates this theory. The results of a retrospective clinical study by Ravidà et al., encompassing 145 three-unit restorations identical to the 3 restoration designs tested in our study, found that the bridge configuration represented the most favorable therapeutic approach for posterior partial edentulism.⁵³

The results of the current *in vitro* study support these clinical outcomes, together with the findings of Latifi et al.,⁵⁰ Silveira et al.⁵¹ and Bittencourt et al.⁵²

The results differed with regard to single crowns and splinted crowns. Naves et al. investigated photoelastically 3 configurations of 3 cement-retained crowns under 3 simultaneous vertical occlusal contacts, using a lower load (30 N) than that applied in our study.⁵⁴ Their loading protocol was simultaneous, but there was no independent stress calibration for each of the 3 loading pins, and a mesial contact point participated in load distribution. The splinted and separate configurations exhibited favorable strains adjacent to the implant apices and almost no crestal strains. The fringe distribution patterns of the cemented crowns, either with contact points, contact surfaces or splinting, were similar to those observed in our study and, as in this study, were similar regardless of the splinting effect. Pimentel et al. also examined multiple-unit cement-retained prostheses under axial loads of 100 N.⁴⁶ The main difference was the use of a 5-unit interim cast superstructure framework that dispersed the load from a single middle point to all 5 units instead of the multiple point contact employed in our research. Their observations also revealed fringes concentrated around all 5 implant apices, with some extending coronally toward the crest, away from the single loading point, as expected.⁴⁶ Their qualitative stress analysis indicated greater strain in the loaded screw-retained restorations than in the cement-retained restorations, consistent with the findings of our study.

Crestal strains indicate a potential cause of crestal bone resorption due to occlusal overload. Identification of such a phenomenon is important because it may help identify peri-implant sites at risk of deterioration of the osseointegration process.⁵⁵

Such strain may be modified by the crown material itself, because different crown materials may produce considerably different levels of strain in the surrounding bone under the same load, as previously demonstrated by El Mahdy et al.²⁴ Restorative design might also influence crestal strain, as reported by Tonin et al.³⁶ The authors recorded high crestal strains using both single loading points and multiple loading points, as in our research, applied to 2 types of screw-retained restorations. Both loading protocols yielded high crestal strain due to the presence of a distal cantilever, which may serve as an example of the deleterious effects of unfavorable stress and strain.³⁶

In the present study, installation of the screw-retained splinted restoration, before the loading sessions commenced, revealed residual strains within the photoelastic block, representing preload. This phenomenon was not observed in any other prosthetic assembly during the preload stage and has been documented in previous *in vitro* studies in association with unloaded screw-retained restorations. The clamping effect created within the prosthetic complex as a result of prosthetic screw tightening

is believed to initiate this phenomenon.^{56,57} Due to screw tightening, the crown may be minimally deflected from the implant axis and, during loading, may generate a bending moment associated with this deflection. In a splint, there is a summation of the total misfit of the component parts, and consequently, the sum of the resulting moments may increase. During loading of a screw-retained restoration, these moments may further increase, resulting in greater stresses within the implants and supporting structures.

With regard to SC restorations, we observed a similar strain pattern between SC-c and SP-c around the middle implant. This may indicate the importance of the interproximal contact point, which may produce a "splinting" effect among separate units through load distribution and arch continuity. Previous photoelastic research has emphasized the importance of contact point tightness in relation to installation strains and, through qualitative analysis, suggested a direct relationship between these 2 factors.⁵⁸ Since no installation strains were detected with SC prostheses in our research, clinical examination by passing dental floss through the interproximal contact point may be considered a simple and practical method for obtaining adequately adjusted, stress-free restorations without apparent preload.

When the abutments were selectively removed according to the preplanned configuration under examination, the implants remained embedded in the photoelastic block. Under load, fringes were detected around implants in the SB configurations (Fig. 4,5). These fringes were not considered relevant and were not included in the recorded measurements because they resulted from the "breakwater effect" created by the unloaded implants when positioned within the stress field of the photoelastic block.³⁴ It is well documented that a photoelastic stress field can extend a considerable distance from the initially loaded implant, to a point where a neighboring implant may be exposed to part of the propagated strain field.³⁹ Such a factor may be misleading when strain fringes are counted and therefore highlights the major advantage of qualitative analysis.

The limitations of this study are considerable. Masticatory forces are known to include additional significant non-axial force components,⁵⁹ which may intensify the differences between the restorative designs. Although strain was evident in certain *in vitro* situations, this does not directly reflect true bone behavior *in vivo*.

Future research may focus on strain evaluation and development in the buccolingual dimension in order to construct a more accurate spatial representation of peri-implant strain. This may facilitate more appropriate planning of implant placement, contributing to better occlusal load dissipation within the bone, which may create favorable conditions for preventing peri-implant disease.²⁰ Additionally, other forms of screw-retained restorations, such as UCLA restorations, multi-unit screw-retaining abutments or screw-retrievable crowns,²³ should also be compared with cement-retained restorations.

Conclusions

Within the limitations of this in vitro study and based on its outcomes, the installation of screw-retained 3-unit splinted restorations on screw-type straight abutments resulted in mesiodistal and occlusogingival peri-implant preload strain within the surrounding medium, including the crestal region. When loading was applied simultaneously in an axial direction, 2 additional conclusions could be drawn: cement-retained restorations exhibited less peri-implant strain in 3-unit adjacent single crowns and 3-unit splinted crowns than their screw-retained counterparts; simple 3-unit bridge assemblies exhibited similar axial strain patterns around the implants, with no crestal strains, regardless of the retention modality.

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. Pjetursson BE, Sailer I, Merino-Higuera E, Spies BC, Burkhardt F, Karasan D. Systematic review evaluating the influence of the prosthetic material and prosthetic design on the clinical outcomes of implant-supported multi-unit fixed dental prosthesis in the posterior area. *Clin Oral Implant Res*. 2023;34:86–103. doi:10.1111/clr.14103
2. Alshiddi IF. Survival rate and clinico-radiographic parameters around narrow-diameter dental implants for fixed dental prostheses in the posterior regions: A systematic review. *Dent Med Probl*. 2023;60(2):345–353. doi:10.17219/dmp/140757
3. Fernandes G, Costa B, Trindade HF, Castilho RM, Fernandes J. Comparative analysis between extra-short implants (≤ 6 mm) and 6 mm-longer implants: A meta-analysis of randomized controlled trial. *Aust Dent J*. 2022;67(3):194–211. doi:10.1111/adj.12900
4. Fernandes GVO, Ferreira NRN, Heboyan A, Nassani LM, Pereira RMA, Fernandes JCH. Clinical assessment of short (> 6 mm and ≤ 8.5 mm) implants in posterior sites with an average follow-up of 74 months: A retrospective study. *Int J Oral Maxillofac Implants*. 2023;38(5):915–926. doi:10.11607/jomi.10197
5. Dominiak S, Karuga-Kuźniewska E, Popecki P, Kubasiewicz-Ross P. PRF versus xenograft in sinus augmentation in case of HA-coating implant placement: A 36-month retrospective study. *Adv Clin Exp Med*. 2021;30(6):633–640. doi:10.17219/acem/134202
6. Chochlidakis K, Fraser D, Lampraki E, Einarsdottir ER, Barmak AB, Papaspyrodakos P. Prosthesis survival rates and prosthetic complications of implant-supported fixed dental prostheses in partially edentulous patients. *J Prosthodont*. 2020;29(6):479–488. doi:10.1111/jopr.13185
7. Gehrke SA, Scarano A, Cortellari GC, Fernandes GVO, Mesquita AMM, Bianchini MA. Marginal bone level and biomechanical behavior of titanium-indexed abutment base of conical connection used for single ceramic crowns on morse-taper implant: A clinical retrospective study. *J Funct Biomater*. 2023;14(3):128. doi:10.3390/jfb14030128
8. Szymańska J, Szpak P. Marginal bone loss around dental implants with conical and hexagonal implant-abutment interface: A literature review. *Dent Med Probl*. 2017;54(3):279–284. doi:10.17219/dmp/74143
9. Dos Santos Martins BG, Fernandes JCH, Martins AG, de Moraes Castilho R, de Oliveira Fernandes GV. Surgical and nonsurgical treatment protocols for peri-implantitis: An overview of systematic reviews. *Int J Oral Maxillofac Implants*. 2022;37(4):660–676. doi:10.11607/jomi.9659
10. Salvi GE, Cosgarea R, Sculean A. Prevalence and mechanisms of peri-implant diseases. *J Dent Res*. 2017;96(1):31–37. doi:10.1177/0022034516667484
11. Martins SCR, da Costa Marques M, Gomes Vidal M, et al. Is the facial bone wall critical to achieving esthetic outcomes in immediate implant placement with immediate restoration? A systematic review. *Adv Clin Exp Med*. 2024;33(9):979–997. doi:10.17219/acem/173573
12. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (II). Etiopathogenesis. *Eur J Oral Sci*. 1998;106(3):721–764. doi:10.1046/j.0909-8836.t01-6.-x
13. Sadowsky SJ. Occlusal overload with dental implants: A review. *Int J Implant Dent*. 2019;5(1):29. doi:10.1186/s40729-019-0180-8
14. Di Fiore A, Montagner M, Sivoletta S, Stellini E, Yilmaz B, Brunello G. Peri-implant bone loss and overload: A systematic review focusing on occlusal analysis through digital and analogic methods. *J Clin Med*. 2022;11(16):4812. doi:10.3390/jcm11164812
15. Naveau A, Shinmyouzu K, Moore C, Avivi-Arber L, Jokerst J, Koka S. Etiology and measurement of peri-implant crestal bone loss (CBL). *J Clin Med*. 2019;8(2):166. doi:10.3390/jcm8020166
16. Skalak R. Biomechanical considerations in osseointegrated prostheses. *J Prosthet Dent*. 1983;49(6):843–868. doi:10.1016/0022-3913(83)90361-x
17. Salvi GE, Stähli A, Imber JC, Sculean A, Rocuzzo A. Physiopathology of peri-implant diseases. *Clin Implant Dent Relat Res*. 2023;25(4):629–639. doi:10.1111/cid.13167
18. Gelfan O, Nissan J, Shely A, et al. Analysis of strain distribution in common clinical designs of posterior implant-supported fixed partial restorations: Comparison between six configurations. *J Funct Biomater*. 2024;15(2):47. doi:10.3390/jfb15020047
19. Nissan J, Ghelfan O, Gross M, Chaushu G. Analysis of load transfer and stress distribution by splinted and unsplinted implant-supported fixed cemented restorations. *J Oral Rehabil*. 2010;37(9):658–662. doi:10.1111/j.1365-2842.2010.02096.x
20. Fernandes GVO, 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 Probl*. 2024;61(5):637–640. doi:10.17219/dmp/187215
21. Okeson JP, ed. *Management of Temporomandibular Disorders and Occlusion*. 8th ed. St. Louis, USA: Mosby; 2019:31–35.
22. Obara R, Komiyama O, Iida T, De Laat A, Kawara M. Influence of the thickness of silicone registration material as a means for occlusal contact examination – an explorative study with different tooth clenching intensities. *J Oral Rehabil*. 2013;40(11):834–843. doi:10.1111/joor.12088
23. Pameijer JH, Brion M, Glickman I, Roeber FW. Intraoral occlusal telemetry. IV. Tooth contact during swallowing. *J Prosthet Dent*. 1970;24(4):396–400. doi:10.1016/0022-3913(70)90080-6
24. El-Mahdy M, Aboelfadl A, Ahmed F, El-Banna A, Wahsh M. Strain gauge analysis and fracture resistance of implant-supported PEKK hybrid abutments restored with two crown materials: An in vitro study. *Dent Med Probl*. 2023;60(3):497–503. doi:10.17219/dmp/170311

25. Gehrke SA, Scarano A, Cortellari GC, Fernandes GVO, Watinaga SE, Bianchini MA. Evaluation of behavior of castable versus machined solid abutments for morse taper implant connection: A clinical retrospective study. *Medicina (Kaunas)*. 2023;59(7):1250. doi:10.3390/medicina59071250
26. Wittneben JG, Millen C, Brägger U. Clinical performance of screw- versus cement-retained fixed implant-supported reconstructions – a systematic review. *Int J Oral Maxillofac Implants*. 2014;29 Suppl:84–98. doi:10.11607/jomi.2014suppl.g2.1
27. Schoenbaum TR, Klokkevold PR. Prosthetic Considerations: Partially Edentulous. In: Newman MG, Klokkevold PR, Elangovan S, Kapilla Y. *Newman and Carranza's Clinical Periodontology and Implantology*. 14th ed. St. Louis, MI: Elsevier; 2024:890–904.
28. Nissan J, Narobai D, Gross O, Ghelfan O, Chaushu G. Long-term outcome of cemented versus screw-retained implant-supported partial restorations. *Int J Oral Maxillofac Implants*. 2011;26(5):1102–1107. PMID:22010095.
29. Kamar Affendi NH, Ahmad R, Tong Wah L, Abdul Hamid NF, Abdul Hakim AY. Screw-retrievable cement-retained implant restorations: A scoping review of fracture strength and clinical performance. *Dent Med Probl*. 2024;61(2):257–268. doi:10.17219/dmp/155811
30. Nissan J, Snir D, Rosner O, Kolerman R, Chaushu L, Chaushu G. The reliability of retrievable cemented implant supported prosthesis. *J Prosthet Dent*. 2016;115(5):587–591. doi:10.1016/j.prosdent.2015.10.013
31. Derksen W, Tahmaseb A, Wismeijer D. Randomized clinical trial comparing clinical adjustment times of CAD/CAM screw-retained posterior crowns on Ti-Base abutments created with digital or conventional impressions. One-year follow-up. *Clin Oral Implants Res*. 2021;32(8):962–970. doi:10.1111/clr.13790
32. Derksen W, Wismeijer D. Three-year follow-up of a randomized clinical trial on screw-retained monolithic zirconia restorations on Ti-Base abutments based on digital or conventional impression techniques. *Int J Prosthodont*. 2023;36(4):410–415. doi:10.11607/ijp.7891
33. Teixeira W, do Nascimento C, Pereira RMA, et al. Bacterial leakage evaluation through DNA-DNA checkerboard hybridization technique in morse taper implant–abutment connections: An in vitro study. *Int J Oral Maxillofac Implants*. 2023;38(2):313–320. doi:10.11607/jomi.9886
34. Strauss FJ, Hämmerle CHF, Thoma DS. Short communication: Cemented implant reconstructions are associated with less marginal bone loss than screw-retained reconstructions at 3 and 5 years of loading. *Clin Oral Implant Res*. 2021;32(5):651–656. doi:10.1111/clr.13737
35. Dos Reis INR, Fukuoka GL, Nagay BE, Pannuti CM, Spin-Neto R, da Silva EVF. Incidence of peri-implant disease associated with cement- and screw-retained implant-supported prostheses: A systematic review and meta-analysis. *J Prosthet Dent*. 2023;134(1):100–108. doi:10.1016/j.prosdent.2023.08.030
36. Tonin BSH, Peixoto RF, Fu J, De Mattos MdGC, 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
37. Alqahtani AR, Desai SR, Patel JR, et al. Investigating the impact of diameters and thread designs on the Biomechanics of short implants placed in D4 bone: A 3D finite element analysis. *BMC Oral Health*. 2023;23(1):686. doi:10.1186/s12903-023-03370-8
38. Pesqueira AA, Goiato MC, Filho HG, et al. Use of stress analysis methods to evaluate the biomechanics of oral rehabilitation with implants. *J Oral Implantol*. 2014;40(2):217–228. doi:10.1563/AAID-JOI-D-11-00066
39. Abarno S, Gehrke AF, Dedavid BA, Gehrke SA. Stress distribution around dental implants, generated by six different ceramic materials for unitary restoration: An experimental photoelastic study. *Dent Med Probl*. 2021;58(4):453–461. doi:10.17219/dmp/135997
40. Geramizadeh M, Katoozian H, Amid R, Kakhodazadeh M. Comparison of finite element results with photoelastic stress analysis around dental implants with different threads. *Dent Med Probl*. 2018;55(1):17–22. doi:10.17219/dmp/85077
41. O'Brien BC, Harris IB, Beckman TB, Reed DA, Cook DA. Standards for reporting qualitative research: A synthesis of recommendations. *Acad Med*. 2014;89(9):1245–1251. doi:10.1097/ACM.0000000000000388
42. Lee JI, Lee Y, Kim YL, Cho HW. Effect of implant number and distribution on load transfer in implant-supported partial fixed dental prostheses for the anterior maxilla: A photoelastic stress analysis study. *J Prosthet Dent*. 2016;115(2):161–169. doi:10.1016/j.prosdent.2015.08.021
43. French AA, Bowles CQ, Parham PL, Eick JD, Killoy WJ, Cobb CM. Comparison of peri-implant stresses transmitted by four commercially available osseointegrated implants. *Int J Periodontics Restorative Dent*. 1989;9(3):221–230. PMID:2639875.
44. Clelland NL, Gilat A, McGlumphy EA, Brantley WA. A photoelastic and strain gauge analysis of angled abutments for an implant system. *Int J Oral Maxillofac Implants*. 1993;8(5):541–548. PMID:8112794.
45. Da Silva EF, Pellizzer EP, Quinelli Mazaro JV, Garcia Junior IR. Influence of the connector and implant design on the implant–tooth-connected prostheses. *Clin Implant Dent Relat Res*. 2009;12(3):254–262. doi:10.1111/j.1708-8208.2009.00161.x
46. Pimentel AC, Manzi MR, Polo CI, Sendyk CL, Naclério-Homem MdG, Sendyk WR. Photoelastic analysis on different retention methods of implant-supported prosthesis. *J Oral Implantol*. 2015;41(3):258–263. doi:10.1563/AAID-JOI-D-12-00200
47. Resnick RR. *Misch's Contemporary Implant Dentistry*. 4th ed. St. Louis, MI: Elsevier; 2021:140–143.
48. Marín-Miranda M, Wintergerst AM, Moreno-Vargas YA, Juárez-López MLA, Tavera-Ruiz C. Photoelasticity for stress concentration analysis in dentistry and medicine. *Materials (Basel)*. 2022;15(19):6819. doi:10.3390/ma15196819
49. Morneburg TR, Pröschel PA. Measurement of masticatory forces and implant loads: A methodologic clinical study. *Int J Prosthodont*. 2002;15(1):20–27. PMID:11887595.
50. Latifi F, Tabrizi R, Kordkheili MH, Shahbazi S, Fernandes GVO. The influence of implant number on stress distribution in the anterior region of mandible reconstructed with fibula bone graft: A finite element analysis. *J Maxillofac Oral Surg*. 2024;25(2):467–474. doi:10.1007/s12663-024-02162-z
51. Silveira MPM, Campaner LM, Bottino MA, Nishioka RS, Borges ALS, Tribst JPM. Influence of the dental implant number and load direction on stress distribution in a 3-unit implant-supported fixed dental prosthesis. *Dent Med Probl*. 2021;58(1):69–74. doi:10.17219/dmp/130847
52. Bittencourt ABBC, Rezende EOP, Campaner M, et al. Stress distribution of multiple implant supported prostheses: Photoelastic and strain gauge analyses of external hexagon and morse taper connections. *J Clin Exp Dent*. 2022;14(3):e235–e240. doi:10.4317/jced.59288
53. Ravidà A, Tattan M, Askar H, Barootchi S, Tavelli L, Wang HL. Comparison of three different types of implant-supported fixed dental prostheses: A long-term retrospective study of clinical outcomes and cost-effectiveness. *Clin Oral Implants Res*. 2019;30(4):295–305. doi:10.1111/clr.13415
54. Naves MM, de Menezes HHM, de Magalhães D, de Araújo CA, Júnior PCS. The influence of interproximal contact on implant-supported fixed partial dentures in the posterior jaw: A photoelastic analysis. *J Oral Implantol*. 2015;41(1):3–9. doi:10.1563/AAID-JOI-D-12-00118
55. Baggi L, Cappelloni I, Di Girolamo M, Maceri F, Vairo G. The influence of implant diameter and length on stress distribution of osseointegrated implants related to crestal bone geometry: A three-dimensional finite element analysis. *J Prosthet Dent*. 2008;100(6):422–431. doi:10.1016/S0022-3913(08)60259-0
56. Lee JI, Lee Y, Kim NY, Kim YL, Cho HW. A photoelastic stress analysis of screw- and cement-retained implant prostheses with marginal gaps. *Clin Imp Dent Relat Res*. 2013;15(5):735–749. doi:10.1111/cid.12134
57. Presotto AGC, Bhering CLB, Mesquita MF, Barão VAR. Marginal fit and photoelastic stress analysis of CAD–CAM and overcast 3-unit implant-supported frameworks. *J Prosthet Dent*. 2017;117(3):373–379. doi:10.1016/j.prosdent.2016.06.011
58. Guichet DL, Yoshinobu D, Caputo AA. Effect of splinting and interproximal contact tightness on load transfer by implant restorations. *J Prosthet Dent*. 2002;87(5):528–535. doi:10.1067/mpr.2002.124589
59. Gibbs CH, Mahan PE, Lundeen HC, Brehnan K, Walsh EK, Holbrook WB. Occlusal forces during chewing and swallowing as measured by sound transmission. *J Prosthet Dent*. 1981;46(4):443–449. doi:10.1016/0022-3913(81)90455-8

Marginal bone loss in screw-retained zirconia fixed prostheses supported by Ti-base or multi-unit abutments: A systematic review and meta-analysis

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Abstract

This systematic review aimed to evaluate the marginal bone loss (MBL) associated with screw-retained zirconia fixed dental prostheses (FDP) supported by either titanium-base (Ti-base) or multi-unit abutments.

A comprehensive literature search was conducted in accordance with the PRISMA 2020 guidelines, using PubMed, Scopus, Web of Science, and the Cochrane Library, covering publications up to May 2024. The inclusion criteria focused on clinical trials and cohort studies reporting quantitative MBL outcomes for zirconia screw-retained prostheses supported by either Ti-base or multi-unit abutments, with a minimum follow-up of 6 months. Data extraction and risk-of-bias assessments were performed independently by 2 reviewers. A meta-analysis was conducted using a random-effects model to pool the MBL values.

Of the 752 articles screened, 10 studies met the inclusion criteria, comprising 684 implants in 412 patients. The mean MBL ranged from 0.19 to 1.26 mm over follow-up periods of 12 to 60 months. The pooled mean MBL was 0.68 mm (95% CI: 0.54–0.81 mm; $I^2 = 47\%$). Subgroup analyses revealed no significant differences between the Ti-base- and multi-unit-supported restorations.

Screw-retained zirconia FDP supported by Ti-base or multi-unit abutments are associated with minimal MBL within clinically acceptable thresholds. Long-term prospective studies are warranted to confirm these findings and further assess biological and technical complication rates.

Keywords: marginal bone loss, fixed prosthesis, Ti-base abutment, multi-unit abutment, zirconia prosthesis

Cite as

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Highlights

- This systematic review and meta-analysis assessed the marginal bone loss (MBL) associated with screw-retained zirconia prostheses supported by titanium-base (Ti-base) or multi-unit abutments.
- Both abutment types demonstrated MBL values within clinically acceptable limits (<1.5 mm).
- No statistically significant difference in MBL was observed between Ti-base- and multi-unit-supported restorations.
- Multi-unit abutments (MUAs) may provide biomechanical advantages in full-arch and immediate-loading situations.
- These findings support the clinical viability of both abutment designs for zirconia-based prosthetic rehabilitation.

Introduction

Marginal bone loss (MBL) remains one of the most clinically relevant parameters for assessing the long-term success of dental implants and prosthetic rehabilitation. Progressive peri-implant bone loss can lead not only to esthetic and biological complications, but may ultimately compromise implant stability and result in implant failure. The etiology of MBL is multifactorial and includes mechanical stress, microbial colonization, peri-implant inflammation, prosthetic design, and the type of abutment connection used.^{1,2}

With the increasing use of metal-free prosthetic materials, zirconia has emerged as a favorable alternative to metal-based frameworks due to its excellent mechanical strength, chemical stability and biocompatibility.^{3,4} Screw-retained zirconia fixed dental prostheses (FDP) are now widely used for both single-unit and full-arch restorations. The zirconia superstructure is commonly supported by titanium-base (Ti-base) abutments or multi-unit abutments (MUAs), which serve as intermediaries between the implant and the prosthetic superstructure. Multi-unit abutments are frequently used to compensate for implant angulation and facilitate the fabrication and insertion of screw-retained prostheses. However, this configuration introduces an additional prosthetic screw and, consequently, a secondary mechanical interface that may be susceptible to loosening or fracture under functional loading. Moreover, the coronal surface of MUAs may provide limited retention, particularly in terms of the available bonding area for cement-retained superstructures, potentially affecting the long-term stability of the restoration. These mechanical and prosthodontic considerations have prompted increasing interest in alternative abutment designs, such as Ti-bases, which provide a more streamlined connection and allow direct bonding to the zirconia prosthetic component.

The type of abutment may influence the degree of MBL observed post-restoration. Titanium-base abutments are characterized by a single-piece connection at the implant level and typically rely on the adhesive cementation of the zirconia component onto the Ti base. In contrast, MUAs position the restorative platform above the peri-implant tissue level and provide a more

prosthetically driven, screw-retained solution, particularly useful in full-arch rehabilitation and in achieving a passive fit.^{5,6}

Several clinical studies have reported the MBL outcomes associated with both Ti-base and multi-unit abutments. Vatenas et al., in a randomized clinical trial (RCT) with a 1-year follow-up, observed significantly lower MBL in patients restored with multi-unit-supported zirconia prostheses as compared to those restored with prostheses directly connected at the implant level.¹ Similarly, Lind et al. reported a case series in which switching from direct zirconia-to-implant connections to multi-unit-supported restorations resulted in the reversal of early, non-plaque-induced bone loss, suggesting a potential biomechanical and biological protective effect.²

Despite these findings, the available literature remains heterogeneous, with considerable variability in follow-up periods, loading protocols and MBL assessment methods. Moreover, most studies have employed observational or retrospective design, with only a limited number of RCTs available. These limitations contribute to ongoing uncertainty regarding the actual impact of abutment selection on MBL.

Therefore, the objective of this systematic review and meta-analysis was to comprehensively evaluate the MBL associated with screw-retained zirconia prostheses supported by either Ti-base or multi-unit abutments in single- and multiple-implant restorations. The analysis aimed to determine whether abutment design has a statistically and clinically significant effect on MBL outcomes.

Methods

Protocol and registration

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines, ensuring transparency, reproducibility and methodological rigor. The review protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD42025567891.

Focused question and study objective

To address the clinical relevance of abutment type in zirconia-supported restorations, a PICO strategy was employed. The population included patients rehabilitated with zirconia FDP supported by dental implants (P). The intervention group comprised cases restored with screw-retained zirconia prostheses supported by Ti-base abutments (I), while the comparator group involved restorations supported by MUAs (C). The primary outcome measured was MBL, expressed in millimeters (mm) and assessed radiographically (O). The aim of the review was to determine whether the type of abutment significantly influences peri-implant bone remodeling over time.

Eligibility criteria

Eligible studies were limited to clinical investigations conducted in humans, including RCTs and prospective or retrospective cohort studies, provided that they reported quantitative MBL values associated with screw-retained zirconia prostheses supported by Ti-base or multi-unit abutments. A minimum follow-up period of 6 months was required. Only articles published in English between 2010 and May 2024 were considered. Studies were excluded if they involved in vitro or animal models, failed to report MBL outcomes, focused on cemented restorations, or used materials other than zirconia. Case reports, narrative reviews, letters to the editor, and studies without accessible full text were also excluded.

Search strategy

A comprehensive search strategy was implemented using the databases PubMed, Scopus, Web of Science, and the Cochrane Library. The final search was conducted on May 15, 2024. The search terms combined free-text terms and medical subject headings (MeSH) related to zirconia prostheses, abutment types and MBL. The detailed search syntax was as follows: (“zirconia” OR “ceramic framework”) AND (“Ti-base” OR “titanium base” OR “multi-unit abutment”) AND (“fixed prosthesis” OR “fixed dental prosthesis” OR “FDP”) AND (“marginal bone loss” OR “MBL”) AND (“dental implant”). Additionally, the reference lists of selected full-text articles were manually screened to identify further relevant studies.

Study selection process

Two reviewers (A.R. and M.M.) independently screened the titles and abstracts of all retrieved records. Full-text articles were obtained for studies considered potentially eligible. Any disagreement regarding study inclusion was resolved through consensus, and, where necessary, a third reviewer (M.G.) was consulted.

Data extraction

Data were independently extracted by two reviewers (A.R. and M.M.), using a standardized data extraction form. The following variables were recorded for each study: authors; year of publication; country; study design; number of patients and implants; type of abutment (Ti-base or multiple-unit); implant location (anterior/posterior; maxilla/mandible); follow-up duration; radiographic measurement method; reported MBL values (mean $\pm$ standard deviation ($M \pm SD$)); loading protocols; and reported biological or mechanical complications.

Statistical analysis

Quantitative synthesis was performed using Review Manager, v. RevMan 5.4 (<https://www.cochrane.org>), and R meta package (<https://www.r-project.org>). Given the expected clinical and methodological heterogeneity across studies, a random-effects model was applied to estimate pooled MBL and its 95% confidence intervals (CIs). Heterogeneity was evaluated using the I^2 statistic, τ^2 , and Cochran's Q test. Subgroup analysis was conducted to compare MBL between Ti-base and multi-unit abutments. In addition, potential publication bias was assessed through funnel plot visualization and Egger's regression test.

Results

Study selection

The systematic search yielded a total of 752 articles. After the removal of 132 duplicates, 620 titles and abstracts were screened. Of these, 45 full-text articles were assessed for eligibility, and 10 studies met the inclusion criteria for qualitative and quantitative analysis.^{1–10} The study selection process is illustrated in the PRISMA 2020 flowchart (Fig. 1). As this study was a systematic review and meta-analysis of previously published studies, no power calculation was required, and no new clinical data were collected.

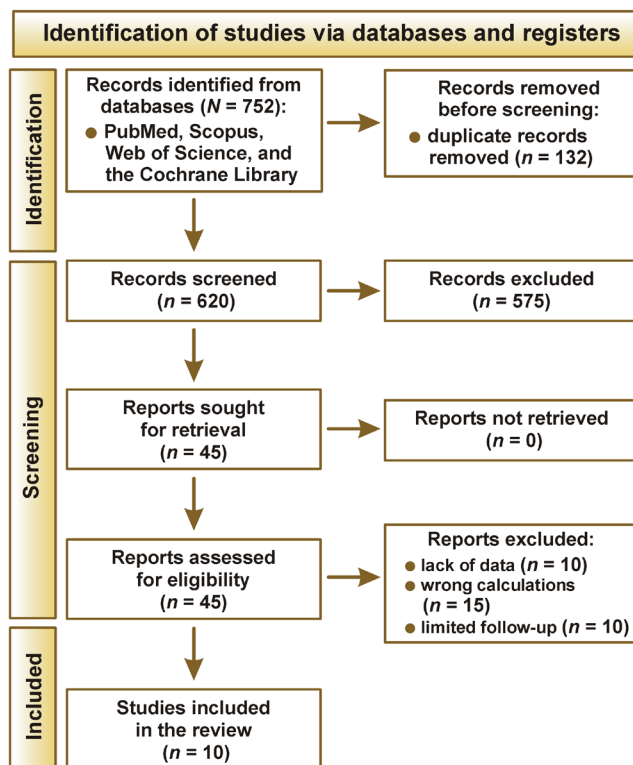
Characteristics of the included studies

The 10 included studies involved a total of 412 patients and 684 implants, with follow-up periods ranging from 12 to 60 months. Eight studies were prospective in design, while 2 were retrospective cohort studies. Marginal bone loss was assessed using standardized periapical radiographs or cone-beam computed tomography CBCT in all cases. The type of abutment used (Ti-base vs. multi-unit) and implant location (anterior vs. posterior; maxilla vs. mandible) varied across studies. The mean patient age ranged from 42 to 68 years. Table 1 summarizes the main characteristics of the included studies, including study design, sample size, abutment type, follow-up duration, and MBL values.

Table 1. Characteristics of the included studies

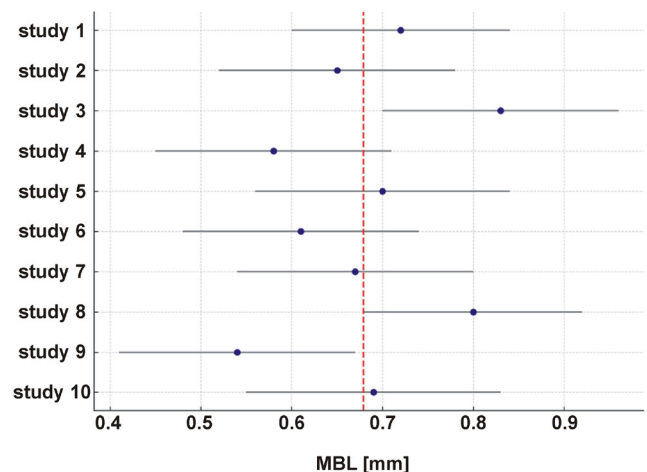
Study	Study design	Sample size (patients)	Sample size (implants)	Abutment type	Follow-up [months]	Mean MBL [mm]
Vatenas et al. ¹ 2023	RCT	45	74	multi-unit	12	0.54
Lind et al. ² 2022	case series	20	32	multi-unit	18	0.60
Bouhy et al. ³ 2020	prospective cohort	52	85	Ti-base	24	0.72
Enkling et al. ⁴ 2021	retrospective	38	60	Ti-base	12	0.83
Al-Rimawi et al. ⁵ 2019	RCT	60	92	multi-unit	36	0.58
Yanagi et al. ⁶ 2018	prospective cohort	48	80	Ti-base	60	0.70
De Freitas et al. ⁷ 2012	retrospective	30	44	Ti-base	18	0.61
Goldstein et al. ⁸ 2017	prospective cohort	41	72	multi-unit	24	0.67
Romanos et al. ⁹ 2016	RCT	50	68	multi-unit	36	0.80
Sennerby et al. ¹⁰ 2015	RCT	28	77	Tt-base	24	0.69

MBL – marginal bone loss; RCT – randomized controlled trial.

**Fig. 1.** PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 flow diagram of the study

Quantitative analysis of marginal bone loss

The pooled mean MBL across all studies was 0.68 mm (95% CI: 0.54–0.81 mm), as calculated using a random-effects model. The level of heterogeneity was moderate ($I^2 = 47\%$; $Q = 16.8$; $p = 0.040$). The forest plot of the meta-analysis is presented in Fig. 2.

**Fig. 2.** Forest plot showing marginal bone loss (MBL) in zirconia screw-retained prostheses supported by Ti-base or multi-unit abutments

Effect sizes represent the mean MBL [mm] with 95% confidence intervals (CIs). The red dashed line indicates the pooled mean value.

Subgroup analysis: Ti-base vs. multi-unit abutments

Subgroup analysis was conducted to compare MBL between the restorations supported by Ti-base abutments and those supported by MUAs. Although both groups demonstrated MBL values within the clinically acceptable range (<1.5 mm), no statistically significant difference was found between the 2 abutment types ($p > 0.05$). These findings suggest that both prosthetic solutions are comparable in terms of peri-implant bone preservation.

Risk-of-bias assessment

The Cochrane Risk of Bias 2.0 tool (RoB 2.0) was used to assess the methodological quality of the included studies across 5 domains: the randomization process; deviations from the intended interventions; missing data; the measurement of the outcome; and selective reporting. Two independent reviewers conducted the assessments, and any disagreement was resolved through discussion or consultation with a third reviewer. A visual summary of the domain-level risk-of-bias assessments is presented in Fig. 3.

Discussion

The present systematic review and meta-analysis aimed to assess the extent of MBL in screw-retained zirconia prostheses supported by either Ti-base or multi-unit abutments. Based on 10 included clinical studies with varying study designs and follow-up periods, the pooled mean MBL was 0.68 mm, which was well below the clinically accepted threshold of 1.5 mm for implant success proposed by Albrektsson et al.¹¹

Comparison of abutment types and outcomes

Across the analyzed studies, both abutment types demonstrated favorable bone stability over time. For instance, Vatenas et al., in an RCT, reported one of the lowest MBL values (0.54 mm) in patients rehabilitated with multi-unit abutments over a 12-month follow-up period.¹ Similarly, Lind et al., in a case series, observed modest MBL (0.60 mm) with MUAs, and suggested that the vertical repositioning of the prosthetic interface may help mitigate early peri-implant bone remodeling.²

Bias	Randomization	Deviations	Missing data	Measurement	Reporting
study 1	low	some concerns	low	low	low
study 2	some concerns	low	low	some concerns	low
study 3	low	low	some concerns	low	low
study 4	high	some concerns	low	low	some concerns
study 5	low	low	low	low	low
study 6	low	some concerns	low	some concerns	low
study 7	some concerns	low	low	low	low
study 8	low	low	low	low	low
study 9	low	low	low	some concerns	low
study 10	some concerns	some concerns	low	low	low

Fig. 3. Risk-of-bias assessment summary

Domain-level judgments across 5 risk categories (randomization, deviations from the intended interventions, missing data, the measurement of the outcome, and selective reporting) for the 10 included studies. green – low risk; yellow – some concerns; red – high risk.

Conversely, Ti-base abutments also yielded acceptable outcomes, although slightly higher MBL values were observed in some cases. Bouhy et al.³ and Enkling et al.⁴ reported mean MBL values of 0.72 mm and 0.83 mm, respectively, in Ti-base-supported prostheses. Both studies hypothesized that the proximity of the restoration–implant interface to the bone crest in direct-to-implant configurations may result in greater bone remodeling, particularly under immediate or early loading protocols.⁵

These findings are consistent with the frameworks proposed by Al-Rimawi et al.⁵ and Yanagi et al.,⁶ which suggest that abutment height and its positioning relative to the mucosal seal may play an important role in preserving the crestal bone.

Design and methodological heterogeneity

A notable finding of this review is the substantial heterogeneity in study design, ranging from RCTs to retrospective case series. This variability may affect the strength and comparability of the evidence, and may partly account for the moderate heterogeneity observed in the meta-analysis ($I^2 = 47\%$).

For example, De Freitas et al., with a relatively long follow-up period of 18 months, reported a mean MBL of 0.61 mm in a Ti-base cohort; however, the study lacked randomization and blinding, increasing the risk of bias.⁷ In contrast, Goldstein et al., in a multicenter RCT, demonstrated lower MBL values with MUAs, and applied strict inclusion criteria and standardized measurement protocols.⁸

Such methodological differences – particularly in radiographic calibration, baseline measurement timing and abutment height standardization – limit the ability to draw definitive conclusions regarding the superiority of one abutment type over the other.⁸ Beyond prosthetic and mechanical factors, peri-implant inflammation is a key contributor to MBL and long-term implant failure. The early detection of inflammatory changes before radiographically detectable bone loss occurs is therefore essential. Recent studies have proposed non-invasive diagnostic approaches, including crevicular fluid analysis and implant sulcus thermography, as effective tools for the early identification of peri-implant mucositis and subclinical inflammation. It has been demonstrated that these techniques can detect inflammatory changes around implants even in asymptomatic patients, suggesting their potential role in preventive implant maintenance protocols.

These findings emphasize the importance of integrating biological monitoring with prosthetic planning to preserve peri-implant bone stability over time

Biomechanical and clinical implications

From a biomechanical standpoint, MUAs may provide stress-distributing benefits due to their supracrestal positioning, particularly in full-arch cases. This is supported by

Romanos et al., who reported stable MBL with multi-unit configurations in edentulous patients undergoing immediate loading.⁹ However, other researchers cautioned that even minor mismatches at the abutment–implant interface may induce micromovements and bacterial infiltration,^{10,12–15} particularly when cementation is involved in Ti-base systems.^{16–24}

Although Ti-base abutments offer a simplified and more direct connection between the implant and prosthesis, their clinical use is not without limitations. In particular, Ti-bases with reduced bonding height have been associated with an increased risk of decementation over time, especially when inadequate surface treatment or minimal bonding protocols are employed. These abutments rely heavily on the integrity of the adhesive interface, and the loss of cement retention may lead to prosthetic instability, complications, or even failure. Several studies have suggested that increased bonding height and optimized surface conditioning may improve the long-term retention and success of Ti-base restorations.^{11,23,24}

Despite these differences, the overall MBL values remained within acceptable clinical limits across all included studies, supporting the use of both approaches according to individual anatomical, functional and esthetic considerations.

Limitations of the current evidence

This review is not without limitations. First, the number of high-quality RCTs remains limited, with only 4 included studies using randomization and blinding. Second, there is a lack of long-term (≥ 5 years) data on multi-unit-supported zirconia restorations. Furthermore, most studies lacked patient-reported outcome measures (PROMs) and did not report implant- or prosthesis-level complications in a standardized manner.

Future directions

Well-designed multicenter randomized trials with standardized radiographic protocols, longer follow-up durations and broader clinical endpoints are needed. Future studies should also investigate the effects of prosthetic screw access location, abutment height and surface characteristics on MBL.

Conclusions

This systematic review and meta-analysis demonstrated that screw-retained zirconia FDP supported by either Ti-base or multi-unit abutments are associated with minimal MBL, well within clinically acceptable limits. The pooled MBL across the 10 included clinical studies was 0.68 mm, with no statistically significant difference observed between the 2 abutment types.

While MUAs appear to offer slight biomechanical advantages, particularly in full-arch or immediate-loading scenarios, Ti-base abutments also demonstrated stable long-term outcomes when used in carefully selected cases. These findings suggest that both prosthetic platforms can be considered reliable in terms of peri-implant bone stability.

However, due to moderate heterogeneity among the available studies and the lack of long-term prospective data, clinical decision making should remain case-specific. Future high-quality RCTs with standardized radiographic protocols and comprehensive outcome reporting are essential to strengthen the evidence base.

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. Vatenas I, Linkevicius T, Rinkeviciute R. The effect of multi-unit or implant-level fixed partial dentures on peri-implant tissues: 1-year follow-up of a randomized clinical trial. *J Oral Med Dent Res.* 2023;4(2):1–10. doi:10.52793/JOMDR.2023.4(2)-48
2. Lind KH, Ulvik IM, Berg E, Leknes KN. Reversible, non-plaque-induced marginal bone loss around an osseointegrated implant: A case report. *Clin Case Rep.* 2022;10(6):e05946. doi:10.1002/ccr3.5946
3. Bouhy A, Rompen E, Lamy M, Legros C, Lecloux G, Lambert F. Maxillary implant overdenture retained by four unsplinted attachments and opposed by a natural or fixed dentition: One-year clinical outcomes. *Clin Oral Implants Research.* 2020;31(8):747–767. doi:10.1111/clr.13623
4. Enkling N, Nauli J, Kraus D, et al. Short strategic implants for mandibular removable partial dentures: One-year results from a pilot randomized crossover abutment type study. *Clin Oral Implants Res.* 2021;32(10):1176–1189. doi:10.1111/clr.13815
5. Al-Rimawi A, Shaheen E, Albdour EA, Shujaat S, Politis C, Jacobs R. Trueness of cone beam computed tomography versus intra-oral scanner derived three-dimensional digital models: An ex vivo study. *Clin Oral Implants Res.* 2019;30(6):498–504. doi:10.1111/clr.13434
6. Yanagi A, Sumita Y, Hattori M, et al. Clinical survey over the past 35 years at the clinic for maxillofacial prosthetics Tokyo Medical and Dental University. *J Prosthodont Res.* 2018;62(3):309–312. doi:10.1016/j.jpor.2017.12.002

7. De Freitas RF, de Carvalho Dias K, da Fonte Porto Carreiro A, Barbosa GA, Ferreira MA. Mandibular implant-supported removable partial denture with distal extension: A systematic review. *J Oral Rehabil.* 2012;39(10):791–798. doi:10.1111/j.1365-2842.2012.02326.x
8. Goldstein G, Andrawis M, Choi M, Wiens J, Janal MN. A survey to determine agreement regarding the definition of centric relation. *J Prosthet Dent.* 2017;117(3):426–429. doi:10.1016/j.prosdent.2016.08.014
9. Romanos GE, Aydin E, Locher K, Nentwig GH. Immediate vs. delayed loading in the posterior mandible: A split-mouth study with up to 15 years of follow-up. *Clin Oral Implants Res.* 2016;27(2):e74–e79. doi:10.1111/clr.12542
10. Sennerby L, Andersson P, Pagliani L, et al. Evaluation of a novel cone beam computed tomography scanner for bone density examinations in preoperative 3D reconstructions and correlation with primary implant stability. *Clin Implant Dent Relat Res.* 2015;17(5):844–853. doi:10.1111/cid.12193
11. Albrektsson T, Tengvall P, Amengual-Peñafiel L, Coli P, Kotsakis G, Cochran DL. Implications of considering peri-implant bone loss a disease, a narrative review. *Clin Implant Dent Relat Res.* 2022;24(4):532–543. doi:10.1111/cid.13102
12. Altınbaş E, Türkaslan SS, Demirekin ZB. Evaluation of complications and marginal bone loss observed in prosthetic restorations applied to different implant abutment connection types: A retrospective study. *Prosthesis.* 2025;7(3):46. doi:10.3390/prosthesis7030046
13. Potdukhe SS, Iyer JM, Nadgere JB. Evaluation of marginal bone level, technical and biological complications between screw-retained and cement-retained all-ceramic implant-supported crowns on zirconia abutment: A systematic review and meta-analysis. *J Indian Prosthodont Soc.* 2024;24(1):25–35. doi:10.4103/jips.jips_524_23
14. Rosa A, Pujia AM, De Angelis R, Arcuri C. Narrow implants and overdentures in the total rehabilitation of atrophic edentulous jaws: Review of clinical aspects with meta-analysis. *Prosthesis.* 2024;6(1):41–52. doi:10.3390/prosthesis6010003
15. Bescós FR, Salgado Peralvo AO, Chamorro Petronacci CM, et al. Marginal bone loss and associated factors in immediate dental implants: A retrospective clinical study. *Int J Implant Dent.* 2025;11(1):25. doi:10.1186/s40729-025-00602-0
16. Minervini G, Franco R, Martelli M, et al. Low-level laser treatment's ability to reduce dry socket pain. *Acta Odontol Scand.* 2024;83:631–641. doi:10.2340/aos.v83.42261
17. Rosa A, Pujia AM, Arcuri C. Complete full arch supported by short implant (<8 mm) in edentulous jaw: A systematic review. *Appl Sci.* 2023;13(12):7162. doi:10.3390/app13127162
18. Franco R, Rosa A, Lupi E, Capogreco M. The influence of dental implant roughness on biofilm formation: A comprehensive strategy. *Dent Hypotheses.* 2023;14(3):90–92. https://ricerca.univaq.it/bitstream/11697/263700/2/the_influence_of_dental_implant_roughness_on.8.pdf. Accessed May 1, 2025.
19. Armentia M, Abasolo M, Coria I, Saintier N. Effect of the geometry of butt-joint implant-supported restorations on the fatigue life of prosthetic screws. *J Prosthet Dent.* 2022;127(3):477.e1–477.e9. doi:10.1016/j.prosdent.2021.12.010
20. El-Mahdy M, Aboelfadl A, Ahmed F, El-Banna A, Wahsh M. Strain-gauge analysis and fracture resistance of implant-supported PEKK hybrid abutments restored with two crown materials: An in vitro study. *Dent Med Probl.* 2023;60(3):497–503. doi:10.17219/dmp/170311
21. Zuffellato N, Massariol M, Gandini A, Dovigo S. Intraorally welded wing abutments supporting full-arch prostheses: A retrospective clinical study. *Dent Med Probl.* 2022;59(3):333–341. doi:10.17219/dmp/149470
22. Krawiec M, Olchowcy C, Kubasiewicz-Ross P, Hadzik J. Role of implant loading time in the prevention of marginal bone loss after implant-supported restorations: A targeted review. *Dent Med Probl.* 2022;59(3):475–481. doi:10.17219/dmp/150111
23. Divakar S, Rathee M, Jain P, Malik S, Tomar SS, Alam M. Finite element analysis of stress distribution in customized root-analogue zirconia implants: A comparative in vitro study. *Dent Med Probl.* 2025;62(1):99–105. doi:10.17219/dmp/152315
24. Kubasiewicz-Ross P, Hadzik J, Dominiak M. Osseointegration of zirconia implants with three varying surface textures and a titanium implant: A histological and micro-CT study. *Adv Clin Exp Med.* 2018;27(9):1173–1179. doi:10.17219/acem/69246

Assessing survival in recurrent or metastatic head and neck cancer patients after radiotherapy with/without targeted molecular therapy: A concise systematic review

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Abstract

This systematic review evaluates the outcomes of different radiotherapeutic strategies in the management of head and neck cancers (HNCs), including curative-intent radiotherapy (RT), palliative stereotactic body radiotherapy (SBRT), multimodal approaches combining surgery and RT, and immunotherapy-based combinations.

A structured literature search was conducted using 4 electronic databases – PubMed, BioMed Central (BMC), Scopus, and Ovid – to identify studies relevant to RT for head and neck squamous cell carcinoma (HNSCC), with a focus on the Quad Shot regimen and other hypofractionated approaches. The search strategy included keywords such as “palliative radiotherapy”, “head and neck cancer”, “Quad Shot”, “hypofractionated RT”, and “recurrent HNSCC”, and was limited to studies published in English up to March 2025.

Fourteen studies were included in this systematic review, comprising a total of 26,734 patients. Curative RT demonstrated favorable outcomes in large cohorts, particularly in terms of overall survival (OS) and local control (LC). Palliative SBRT regimens provided substantial symptomatic relief and disease control with minimal toxicity among elderly and inoperable patients.

Multimodal treatment options yielded the most promising survival outcomes, particularly in patients with early-stage disease. Furthermore, the combination of immunotherapy and RT showed potential synergistic effects, improving both OS and progression-free survival (PFS).

Keywords: prognosis, survival, radiotherapy, HNC, Quad Shot

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Highlights

- Combining surgery, radiation therapy (RT) and immunotherapy yields the most favorable outcomes in terms of overall survival (OS) and disease control, particularly in early-stage cancers with favorable immune responsiveness.
- For elderly patients or those unable to undergo surgery, hypofractionated radiation therapy, such as the Quad Shot regimen and stereotactic body radiation therapy (SBRT), represents an effective treatment option. It is generally well tolerated, provides local disease control and offers substantial symptomatic relief.
- Research examining the combination of RT and immune checkpoint inhibitors (ICIs), such as PD-1/PD-L1 inhibitors, suggests that this approach may significantly improve OS and progression-free survival (PFS).

Introduction

Head and neck cancers (HNCs) represent a heterogeneous group of malignancies arising from the mucosal surfaces of the upper aerodigestive tract. Globally, they account for more than 650,000 new cases and 330,000 deaths annually, placing a substantial burden on health-care systems and patients.^{1,2} Risk factors include tobacco use, alcohol consumption and oncogenic viral infections, including human papillomavirus (HPV), Epstein–Barr virus (EBV) and cytomegalovirus (CMV), with prognosis heavily influenced by tumor site, stage, and patient comorbidities.^{1,3–5}

Researchers emphasize the synergistic role of the gut microbiota in modulating immune responses, potentially enhancing the effectiveness of immunotherapy in cancer management.^{6,7}

Systemic inflammation has also emerged as an important modifier of cancer risk and progression. Banthia et al. reported a significant association between periodontal disease and overall cancer risk, with odds ratios (ORs) of 3.986 and 4.286 for clinical attachment loss (CAL) and the periodontal disease index (PDI), respectively, among cancer patients as compared to controls.⁸ Chrysanthakopoulos investigated a possible correlation between the periodontal disease indices and lung cancer.⁹ These findings highlight the potential role of oral-systemic inflammatory markers as adjunctive prognostic tools in oncology.

Multiple therapeutic modalities are employed in the management of HNCs, including surgery, radiotherapy (RT), systemic therapies (chemotherapy and immunotherapy), and combinations thereof.^{1,10,11} Definitive RT is a cornerstone of organ preservation and is commonly used in both curative and palliative settings. Treatment outcomes vary by modality – curative-intent RT can achieve 2-year local control (LC) rates exceeding 85% in selected populations, while palliative approaches prioritize symptom relief and disease control with acceptable toxicity.^{1,12}

Some authors point to the potential value of integrating artificial intelligence (AI) tools into the early detection of oral cancer, which may ultimately improve treatment selection and RT planning, thereby contributing to better clinical outcomes.^{13,14}

Recent advances have highlighted the potential advantages of combining molecularly targeted therapies with conventional RT in the management of recurrent or metastatic HNCs. When used in combination with RT, agents such as cetuximab have demonstrated modest improvement in survival and LC, particularly in patients who are not suitable candidates for platinum-based chemotherapy.¹⁵ Furthermore, as the overexpression of epidermal growth factor receptor (EGFR) and other biomarkers may influence treatment response and prognosis, studies have emphasized the importance of tailoring therapy according to individual molecular profiles.¹⁶

Immunotherapy, particularly immune checkpoint inhibitors (ICIs), has emerged as a promising adjunct to RT, with combination approaches demonstrating enhanced tumor control and improved survival outcomes in recurrent or metastatic settings.^{17–19}

However, treatment selection must balance efficacy and toxicity while taking into account patient-specific factors, such as age and performance status.

Given that no systematic assessment has been conducted to date, the current evidence remains fragmented, varies in methodology, and frequently lacks standardized outcome reporting, resulting in inconsistent and potentially suboptimal clinical decision-making. Consequently, a comprehensive synthesis of the existing evidence is urgently needed to inform optimal treatment strategies and improve survival outcomes in this high-risk population.

The primary objective of this study was to systematically evaluate the outcomes associated with curative, palliative and multimodal RT strategies, with particular emphasis on overall survival (OS), disease-free survival (DFS), LC, toxicity, the Karnofsky performance status (KPS), and the emerging role of immunotherapy-based regimens.

Methods

The study was registered on the International Prospective Register of Systematic Reviews (PROSPERO) platform (registration ID: CRD42024575386).

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.²⁰

A focused review question was developed using the PICO framework:

- Population: Adult patients with HNC;
- Intervention: Radiotherapy, including curative, palliative and multimodal approaches;
- Comparison: Not applicable;
- Outcome: OS, DFS, LC, toxicity, KPS, and quality of life (QoL).

Using Boolean operators, a comprehensive literature search was conducted in the PubMed, BioMed Central (BMC), Ovid, and Scopus databases up to March 2025. The search strategy included combinations of terms such as: (“head and neck cancer” OR “HNSCC”) AND (“radiotherapy” OR “SBRT” OR “Quad Shot”) AND (“curative” OR “palliative” OR “multimodal” OR “surgery”) AND (“survival” OR “toxicity” OR “quality of life”). Initially, no restrictions were placed on language or publication date; however, only full-text publications in English were included in the final analysis. Before the screening process, duplicate records were removed.

Eligibility criteria

Inclusion criteria:

- studies reporting the clinical outcomes of RT in patients with histologically confirmed HNSCC;
- studies including ≥10 patients;
- the use of curative-intent RT, palliative stereotactic body radiation therapy (SBRT) or multimodal treatment (e.g., surgery and RT);
- outcomes including OS, DFS, LC, KPS, or toxicity.

Exclusion criteria:

- abstract-only studies or conference proceedings;
- non-English articles;
- animal or in vitro studies;
- studies focused exclusively on thyroid or salivary gland tumors;
- imaging-only endpoints without clinical outcome data.

Two independent reviewers screened the titles and abstracts, followed by the full-text assessment of potentially eligible studies. Disagreement was resolved by consensus. Data were extracted using a standardized table, including study design, country, sample size, patient characteristics, type and dose of RT, number of treatment cycles/fractions, reported outcomes, and study limitations.

The study selection process is illustrated in the PRISMA flow diagram (Fig. 1), based on the screening and selection data.²⁰

The Newcastle–Ottawa Scale (NOS) was used to assess the risk of bias in the included observational studies. The scale evaluates 3 domains: the selection of study groups; the comparability of groups; and the ascertainment of outcomes in cohort studies or exposures in case–control studies. Two reviewers independently assessed each study, with discrepancies resolved through consultation or discussion with a third reviewer. Studies were classified as having a low, moderate or high risk of bias based on their total NOS score.²¹

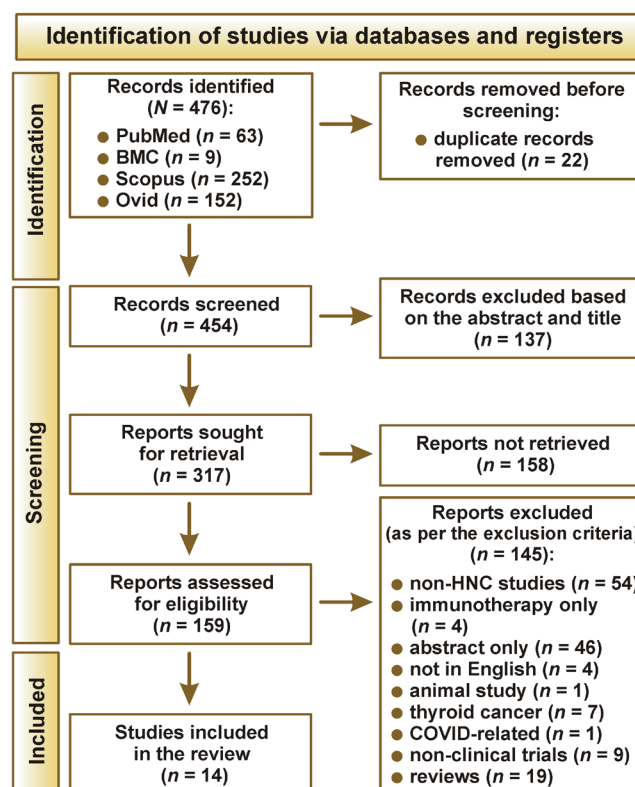


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 flow diagram

BMC – BioMed Central; HNC – head and neck cancer; COVID – coronavirus disease.

A quantitative meta-analysis was not conducted because of substantial heterogeneity among the included studies in terms of study design, patient populations, RT regimens, treatment combinations, outcome measures, and follow-up periods. Statistical pooling of the data was not considered appropriate due to variations in radiation doses and fractionation schedules, the inconsistent reporting of outcomes such as OS, progression-free survival (PFS) and QoL, and the inclusion of both curative- and palliative-intent treatment. Therefore, a narrative synthesis was considered more appropriate for accurately presenting and interpreting the findings of this systematic review.

Results

The risk of bias was assessed across the 14 included studies,^{1,3,10,12,17–19,22–28} with most demonstrating a low risk of bias in terms of selection, comparability and outcome assessment. Nine studies achieved a score of 8, indicating strong methodological quality. Four studies received a score of 6, while 1 study scored 7, suggesting a moderate risk of bias, primarily due to limited adjustment for confounders or smaller sample sizes. The robustness of the findings across most studies was largely attributed to their cohort designs, and the use of large databases or prospective follow-up (Table 1).

Table 1. Risk of bias in the included studies (Newcastle–Ottawa Scale (NOS))

Study	Design	Selection	Comparability	Outcome	Total NOS score	Risk of bias
Rudoltz et al. ¹ 1999	retrospective cohort	3	1	2	6	moderate
Nguyen et al. ³ 2023	prospective cohort	4	2	2	8	low
Glatzel et al. ¹⁰ 2022	retrospective chart review	3	1	2	6	moderate
Luo et al. ¹² 2020	prospective case series	4	2	2	8	low
Luukkaa et al. ¹⁷ 2003	RCT	3	1	2	6	moderate
Crawford et al. ¹⁸ 2024	retrospective	4	2	2	8	low
Bostel et al. ¹⁹ 2020	multicenter retrospective study	4	2	2	8	low
Hall et al. ²² 2009	retrospective chart review	4	1	2	7	moderate
Chen et al. ²³ 2016	observational	4	2	2	8	low
Vargo et al. ²⁴ 2012	multicenter retrospective study	3	1	2	6	moderate
Gogineni et al. ²⁵ 2020	retrospective	4	2	2	8	low
Fan et al. ²⁶ 2020	retrospective	4	2	2	8	low
Schubert et al. ²⁷ 2020	prospective	4	2	2	8	low
Roden et al. ²⁸ 2017	review of NCDB	4	2	2	8	low

RCT – randomized controlled trial; NCDB – National Cancer Database.

A total of 14 studies were included in this review, encompassing diverse geographic regions. The largest number of studies originated from North America, with 6 studies from the USA and 1 from Canada, demonstrating a notable concentration of research in this region. Additionally, 3 studies were conducted in Asia (2 from China and 1 from Taiwan), while 3 studies were conducted in Europe (one each from Finland, the UK and Germany). One study was conducted in Australia (Table 2). This geographic distribution highlights broad international interest in the research area while also reflecting regional differences in study design, patient populations and clinical practices. Such diversity may strengthen the generalizability of the findings, but also underscores the importance of considering contextual factors when interpreting the results.

Curative-intent radiotherapy studies

Studies evaluating curative-intent RT collectively included 12,483 patients from countries including Finland,¹⁷ the UK²² and Taiwan.²³ Radiotherapy doses ranged from 60 to 72 Gy, with fractionation typically consisting of 30–36 fractions. Rudoltz et al.¹ and Vargo et al.²⁴ reported 6-month to 1-year OS rates

exceeding 75%, with high LC rates (>85%) and preserved KPS. Disease-free survival was consistently above 60% in these studies. The multimodal integration of RT with surgery or concurrent chemotherapy further improved outcomes, particularly in early-stage disease. Toxicity was generally acceptable, with rates of grade ≥ 3 toxicity below 25%.

Palliative stereotactic body radiotherapy (Quad Shot)

Quad Shot regimens were investigated in patients with recurrent or metastatic HNC across several countries, including China,¹² Germany¹⁹ and the USA.^{25,26} Typical regimens delivered 14–14.8 Gy in 4 fractions, repeated over 3–4 cycles. Collectively, these studies included more than 800 patients, and demonstrated improvement in symptom relief and short-term LC. Overall survival ranged from 6 to 13 months, with generally favorable tolerability. Fan et al. reported improved QoL and reduced symptom burden with minimal grade ≥ 3 toxicity.²⁶ Gogineni et al. reported improved OS when Quad Shot RT was combined with immunotherapy, particularly anti-programmed death-1 (PD-1) agents, suggesting a potential synergistic benefit.²⁵

Table 2. General characteristics of the included studies

Study	Design	Country	Population	Intervention (RT dose/fraction)	Key findings	Limitations	Risk of bias
Rudoltz et al. ¹ 1999	retrospective cohort	USA	69 patients with recurrent HNSCC	14.8 Gy/4 fx over 2 days	6-month OS 35%, effective palliation	small sample, retrospective data	moderate
Nguyen et al. ³ 2023	prospective cohort	Canada	18 elderly/inoperable HNSCC patients	14.8 Gy/4 fx every 3–4 weeks	symptom relief 75%, minimal toxicity	non-randomized, small cohort	low
Glatzel et al. ¹⁰ 2022	retrospective chart review	USA	40 patients with metastatic HNSCC	14 Gy/4 fx	6-months OS 25%, tolerable toxicity	lack of the control group, retrospective	moderate
Luo et al. ¹² 2020	prospective case series	China	60 patients with locoregionally advanced HNSCC	3 cycles of 14 Gy/4 fx	symptom control 68%, improved PFS	no randomization, potential selection bias	low
Luukkaa et al. ¹⁷ 2003	RCT	Finland	110 patients with advanced HNSCC	60–66 Gy/ 33 fx	2-year OS 72%, significant survival benefit	conventional RT, not palliative-specific	moderate
Crawford et al. ¹⁸ 2024	retrospective	Australia	patients diagnosed with PNS of cSCC via the ophthalmic nerve (V1)	surgical resection with adjuvant RT (59 Gy/29 fx)	5-year OS 61.9%, DSS 74.6% and DFS 62.1%	single-center design, small sample, incomplete data capture	low
Bostel et al. ¹⁹ 2020	multicenter retrospective study	Germany	patients with spinal bone metastases from HNC	palliative RT	palliative RT as an effective treatment option to treat spinal bone metastases of HNC	multicenter analysis of a rare event	low
Hall et al. ²² 2009	retrospective chart review	UK	23 elderly HNSCC patients	3 cycles of 14 Gy/4 fx	6-month OS 20%, good QoL	very small sample, retrospective data	moderate
Chen et al. ²³ 2016	observational	Taiwan	elderly patients with locally advanced HNSCC and a high comorbidity index	RT, concurrent chemotherapy, sequential RT and chemotherapy, or surgery with adjuvant treatment	aggressive treatment improves survival	data from the Taiwan National Health Insurance and cancer registry databases were analyzed	low
Vargo et al. ²⁴ 2012	multicenter retrospective study	USA	130 patients with recurrent HNSCC	SBRT 20–40 Gy 5 fx	1-year OS 40%, improved KPS	high variation in patients' selection	moderate
Gogineni et al. ²⁵ 2020	retrospective	USA	elderly and medically inoperable HNC patients	SBRT	SBRT as a viable treatment option in elderly and medically inoperable HNC patients	small sample	low
Fan et al. ²⁶ 2020	retrospective	China	previously irradiated HNC	Quad Shot regimen (3.7 Gy twice daily over 2 consecutive days at 4-week intervals per cycle, up to 4 cycles)	the Quad Shot regimen is a feasible and effective last-line local treatment option for previously irradiated HNC	heterogenous patient population, potential for observer bias, underestimation of late toxicity, confounding factors in modality comparison	low
Schubert et al. ²⁷ 2020	prospective	USA	locally advanced HNSCC	RT in combination with PD-1/PD-L1	median OS 11.6 months in the al-RT group and 4.2 months in the sl- RT group	small sample	low
Roden et al. ²⁸ 2017	review of NCDB	USA	<70-year-olds with clinical stage I–II SCC of the tonsil	RT, surgery, and surgery + RT	5-year OS 78.8% for surgery + RT, 66.7% for surgery and 64.5% for RT	lack of data available for the HPV status; the HPV status has been shown to be a major prognostic indicator in surgical and nonsurgical patients	low

RT – radiotherapy; HNSCC – head and neck squamous cell carcinoma; PNS – perineural spread; cSCC – cutaneous squamous cell carcinoma; HNC – head and neck carcinoma; SCC – squamous cell carcinoma; fx – fraction; SBRT – stereotactic body radiotherapy; PD-1 – programmed death-1; PD-L1 – programmed death ligand-1; OS – overall survival; PFS – progression-free survival; DSS – disease-specific survival; DFS – disease-free survival; QoL – quality of life; KPS – Karnofsky performance status; al-RT – all-lesion RT; sl-RT – single-lesion RT; HPV – human papillomavirus.

Multimodal strategies including immunotherapy

Combination therapies, particularly those incorporating surgery, RT and systemic agents, such as targeted therapies or ICIs, demonstrated the most favorable survival outcomes. Nguyen et al.³ and Crawford et al.¹⁸ reported 2-year OS rates exceeding 80% with combination regimens. Schubert et al.²⁷ and Roden et al.²⁸ reported improved DFS rates of up to 70% and durable LC. Importantly, immune-based approaches, as reported by Gogineni et al.²⁵ and Fan et al.,²⁶ demonstrated potential benefits in selected patients, including improved OS, reduced recurrence and acceptable toxicity. These approaches may be particularly valuable for patients with HNC who are medically unfit for surgery or have previously untreated disease, offering meaningful disease control and potential survival extension.

Discussion

Researchers have evaluated the efficacy of hypofractionated RT regimens, such as Quad Shot and SBRT, as palliative treatment for head and neck tumors.^{29,30} The Quad Shot regimen, which consists of short courses of low-dose radiation delivered twice daily over 2 days, is generally preferred for frail patients because of its tolerability, convenience, and potential for repeat administration. In contrast, SBRT delivers higher radiation doses in fewer fractions with precise targeting and may provide superior LC in selected patients with limited disease burden and a good functional status. Several studies included in this review suggest that SBRT may offer improved tumor control and more durable disease control in appropriately selected patients. However, the Quad Shot regimen remains a valuable option, particularly for older patients, those with significant comorbidities, or those with a poor performance status. The choice between these regimens should be individualized based on patient characteristics, treatment goals, expected outcomes, and treatment burden.³¹

The Quad Shot regimen comprises a total dose of 44.4 Gy, delivered in 3.7-Gy fractions twice daily over 2 consecutive days per cycle, with a recovery interval of 2–4 weeks between the 3 prescribed cycles. Although this regimen is generally well tolerated, with grade ≥ 3 toxicity rates of approx. 5%, it has demonstrated a median latency to progression of 3–5 months, indicating limited durability of LC.²⁶

The oropharynx emerged as the predominant tumor site investigated using the Quad Shot technique among the included studies.

Although the Quad Shot regimen has demonstrated promising palliative outcomes, the supporting evidence is primarily derived from small, single-institution studies with limited sample sizes and heterogeneous patient

populations. Most studies lacked control groups, making direct comparisons with alternative palliative approaches difficult. Additionally, the subjective nature of symptom relief and QoL assessments, often based on non-standardized or retrospective reporting, may introduce measurement bias. Variations in RT delivery, including the number of cycles and dose per fraction, as well as differences in concurrent therapies, further complicate the interpretation of the findings.

Stereotactic body radiotherapy, on the other hand, is a highly precise form of RT that delivers a high dose of radiation to the tumor in a limited number of treatment sessions, typically 1–5 fractions.³² It uses advanced imaging and motion-management techniques to localize the tumor with submillimeter precision, thereby minimizing radiation exposure to the surrounding healthy tissues. It has become an established treatment modality for tiny, well-defined tumors in the lung, liver and spine, and is increasingly being used for recurrent or unresectable head and neck malignancies.

In the palliative setting, SBRT offers the potential advantages of rapid symptom relief, improved LC and a shorter overall treatment duration. These benefits may be particularly relevant for patients with limited life expectancy or a poor performance status. Studies have indicated that SBRT can achieve durable tumor responses while preserving QoL; however, it may also carry a higher risk of acute toxicity in anatomically complex head and neck regions. Careful patient selection and multidisciplinary treatment planning are therefore essential to maximize therapeutic benefits while minimizing toxicity.³³

Quality-of-life assessment

The included studies highlight symptom relief as a key outcome, which inherently relies on subjective reporting. Although some studies reported improvement in KPS, detailed and standardized QoL assessments were not consistently reported across all studies. This inconsistency makes it challenging to compare the true impact of different RT regimens on patients' overall well-being.

Radiotherapy delivery variability

There was substantial variation in RT delivery across the included studies. Some studies used hypofractionated RT with specific dose and fractionation schemes (e.g., 14.8 Gy/4 fractions, 14 Gy/4 fractions, or the Quad Shot regimen), whereas others employed SBRT with different fractionation schedules (e.g., 20–40 Gy in 5 fractions) or preoperative RT. The number of treatment cycles also varied, with some studies using a single cycle or a limited number of cycles and others employing multiple cycles. This variability makes it difficult to isolate the effects of specific RT approaches on clinical outcomes.

Concurrent therapies

Several studies incorporated multimodal treatment strategies, combining RT with surgery, chemotherapy or immunotherapy. The influence of these concurrent therapies on OS and other clinical outcomes can be difficult to disentangle from the effect of RT itself. For instance, Nguyen et al.,³ Chen et al.²³ and Roden et al.²⁸ evaluated multimodal treatment approaches. Studies investigating the combination of ICHs with RT showed promising results; however, the individual contribution of each treatment modality requires further investigation.²⁷

In summary, variability in QoL measures, RT administration and concurrent treatment modalities complicates the interpretation of the available evidence and limits the ability to draw definitive conclusions regarding the optimal RT strategies for HNC.

Regarding follow-up duration, several studies included in this review had limitations that may have hindered a comprehensive assessment of long-term treatment outcomes. Studies such as Rudoltz et al.,¹ Glatzel et al.¹⁰ and Bostel et al.,¹⁹ focusing on treatment feasibility, specific treatment outcomes or palliative measures, had varying and, in some cases, relatively short follow-up periods that did not capture the full spectrum of late toxicity that can develop months or years after treatment. Although early treatment-related toxicity was generally well documented, late complications, such as fibrosis, xerostomia and osteoradionecrosis, may have been underreported in studies with limited follow-up.^{1,19}

Furthermore, in the context of HNC, long-term disease progression, including local recurrence and distant metastasis, is a critical consideration. Studies with shorter follow-up periods may provide an overly optimistic assessment of disease control and potentially underestimate the recurrence rates over time. This is particularly relevant for studies evaluating novel approaches, such as immunotherapy combined with RT, in which the durability of treatment response and long-term survival benefits require extended observation. For example, Nguyen et al.³ and Schubert et al.²⁷ highlight the importance of long-term follow-up when assessing the efficacy of novel therapeutic approaches. Studies with longer follow-up periods further demonstrate the importance of evaluating long-term outcomes. For example, Luukkaa et al.¹⁷ and Hall et al.²² provide insights into survival outcomes over extended periods, highlighting the potential for late events to influence overall treatment success.

In conclusion, the variability in follow-up duration across the included studies underscores the need for caution when interpreting the long-term effectiveness and safety of HNC treatment. Longer follow-up is essential to fully characterize late toxicity and the true extent of long-term disease control.

Moreover, biomarker data, such as PD-L1 expression and tumor mutational burden, were inconsistently reported, limiting subgroup analyses and the potential for personalized treatment. This is particularly relevant to studies evaluating immunotherapy-based combinations, such as Schubert et al.,²⁷ in which biomarker data are important for understanding treatment response.³ Limited follow-up periods may also fail to capture delayed immune-related toxicity or long-term survival benefits, which are essential for fully evaluating the efficacy and safety of these treatment regimens.

The growing evidence supporting the combination of RT and immunotherapy represents a significant shift in the management of HNSCC. This shift is supported by studies demonstrating that immune-based approaches, particularly those combining ICIs with RT, may improve OS and reduce recurrence, particularly in tumors with favorable immune responsiveness. For example, Schubert et al. reported promising results with the combination of PD-1/programmed death ligand-1 (PD-L1) inhibitors and RT.²⁷

While surgery and chemoradiotherapy remain important treatment modalities, particularly in localized and locally advanced disease, emerging strategies incorporating ICIs offer new opportunities for durable disease control in advanced settings.

Surgery plays a crucial role in achieving LC in early-stage HNSCC. Roden et al. evaluated the role of surgery in the management of early-stage tonsil cancer, highlighting its importance in achieving favorable local disease control.²⁸ Chemoradiotherapy remains a standard treatment approach for locally advanced HNSCC, with the aim of improving locoregional control and OS. Chen et al. evaluated treatment outcomes in elderly patients with locally advanced HNSCC, including those treated with chemoradiotherapy.²³

However, in advanced or recurrent/metastatic HNSCC, where traditional treatment options have limitations, the combination of immunotherapy and RT offers the potential for improved outcomes. Studies reported promising results in terms of OS, DFS and LC with these combination approaches. Schubert et al. demonstrated improved DFS and durable LC with immune-based approaches.²⁷ Gogineni et al.²⁵ and Fan et al.²⁶ also reported promising outcomes with SBRT and the Quad Shot regimen, including improved OS and reduced recurrence.

These findings suggest that, while surgery and chemoradiotherapy remain essential treatment modalities, the integration of immunotherapy, particularly in combination with RT, is reshaping the therapeutic landscape of HNSCC. This approach may offer improved outcomes, particularly for patients with advanced or recurrent/metastatic disease.

The heterogeneity of radiation regimens used across the included studies represents a major limitation of this review. The dose, fractionation, and number of treatment cycles varied considerably among the RT protocols. For example, curative-intent RT protocols delivered doses

of up to 72 Gy in 30–36 fractions, SBRT regimens ranged from 20–40 Gy in 5 fractions, and palliative approaches such as the Quad Shot regimen delivered 14–14.8 Gy in 4 fractions per cycle, often repeated for 3–4 cycles. This variability makes direct comparisons of treatment efficacy and toxicity difficult.

Additionally, follow-up durations varied substantially across studies. Some reports focused primarily on short-term symptom relief or early survival outcomes, such as 6-month OS, whereas others followed patients for several years. These differences limit the ability to comprehensively assess long-term outcomes, including late toxicity, disease recurrence and sustained LC. Rudoltz et al.¹ and Bostel et al.,¹⁹ for example, provided valuable information regarding short-term palliation; however, their relatively short follow-up periods may have resulted in the under-reporting of late complications or recurrence events. In contrast, studies such as Luukkaa et al.¹⁷ and Hall et al.²² included longer follow-up periods, emphasizing the importance of prolonged observation when assessing treatment durability.

The standardization of RT protocols and more consistent follow-up durations in future studies would improve the comparability and clinical applicability of findings in this complex patient population.

Limitations

This review has several additional limitations. First, substantial heterogeneity in study designs, treatment regimens and outcome reporting limited the ability to perform a quantitative meta-analysis. Second, most of the included studies were retrospective, increasing the risk of selection bias. Third, the relatively small sample sizes of studies evaluating immunotherapy-based approaches warrant caution when generalizing their findings. Additionally, the performance status, comorbidities and molecular biomarkers, such as PD-L1 expression, were inconsistently reported across studies, further limiting the ability to perform meaningful subgroup analyses and draw definitive conclusions.

Conclusions

Quad Shot RT remains a safe and effective palliative strategy for symptom relief in patients with advanced HNSCC. However, the integration of ICIs with RT, particularly in combination with the Quad Shot regimen, has demonstrated promising outcomes in terms of OS and PFS, with acceptable toxicity. Multimodal strategies combining surgery, RT and immunotherapy currently represent a promising approach to maximizing survival and disease control. Future prospective studies are needed to confirm these findings and optimize patient selection for individualized treatment approaches.

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. Rudoltz MS, Perkins RS, Luthmann RW, et al. High-dose-rate brachytherapy for primary carcinomas of the oral cavity and oropharynx. *Laryngoscope*. 1999;109(12):1967–1973. doi:10.1097/00005537-199912000-00013
2. McDowell L, Rischin D, Gough K, Henson C. Health-related quality of life, psychosocial distress and unmet needs in older patients with head and neck cancer. *Front Oncol*. 2022;12:834068. doi:10.3389/fonc.2022.834068
3. Nguyen NP, Thariat J, Gorobets O, et al. Immunotherapy and hypofractionated radiotherapy in older patients with locally advanced cutaneous squamous-cell carcinoma of the head and neck: A proposed paradigm by the International Geriatric Radiotherapy Group. *Cancers (Basel)*. 2023;15(20):4981. doi:10.3390/cancers15204981
4. Duš-Ilnicka I. Neglected area of oral cancer: A word about the International Agency for Research on Cancer (IARC) "Handbook of Oral Cancer Prevention". *Dent Med Probl*. 2024;61(4):479–480. doi:10.17219/dmp/190468
5. Payaradka R, Ramesh PS, Vyas R, et al. Oncogenic viruses as etiological risk factors for head and neck cancers: An overview on prevalence, mechanism of infection and clinical relevance. *Arch Oral Biol*. 2022;143:105526. doi:10.1016/j.archoralbio.2022.105526
6. Minervini G, Shivakumar S, Ronsiville V, Franco R, Cicciù M, Marrapodi MM. Microbiological aspects of cancer progression: A systematic review conducted according to the PRISMA 2020 guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. *Dent Med Probl*. 2024;61(5):739–746. doi:10.17219/dmp/183712
7. Yi M, Jiao D, Qin S, Chu Q, Li A, Wu K. Manipulating gut microbiota composition to enhance the therapeutic effect of cancer immunotherapy. *Integr Cancer Ther*. 2019;18:1534735419876351. doi:10.1177/1534735419876351
8. Banthia R, Jain P, Jain AK, Belludi SA, Agarwal N, Patidar M. Evaluation of the association between periodontal disease and total cancer risk: A cross-sectional study. *Dent Med Probl*. 2024;61(6):843–850. doi:10.17219/dmp/175001
9. Chrysanthakopoulos NA. Correlation between periodontal disease indices and lung cancer in Greek adults: A case–control study. *Exp Oncol*. 2016;38(1):49–53. PMID:27031720.

10. Glatzel M, Büntzel J, Schröder D, Küttner K, Fröhlich D. High-dose-rate brachytherapy in the treatment of recurrent and residual head and neck cancer. *Laryngoscope*. 2002;112(8 Pt 1):1366–1371. doi:10.1097/00005537-200208000-00008
11. Gao X. Multimodality treatment for esophageal squamous cell carcinoma [doctoral thesis; Erasmus University Rotterdam, The Netherlands]. 2023. https://pure.eur.nl/ws/portalfiles/portal/97660683/full_thesis_digitalremoved_1_-_64c007be61cf7.pdf. Accessed June 10, 2025.
12. Luo HS, Xu HY, Du ZS, et al. Prognostic significance of baseline neutrophil count and lactate dehydrogenase level in patients with esophageal squamous cell cancer treated with radiotherapy. *Front Oncol*. 2020;10:430. doi:10.3389/fonc.2020.00430
13. Beristain-Colorado MdP, Castro-Gutiérrez MEM, Torres-Rosas R, et al. Application of neural networks for the detection of oral cancer: A systematic review. *Dent Med Probl*. 2024;61(1):121–128. doi:10.17219/dmp/159871
14. İlhan B, Lin K, Guner P, Wilder-Smith P. Improving oral cancer outcomes with imaging and artificial intelligence. *J Dent Res*. 2020;99(3):241–248. doi:10.1177/0022034520902128
15. Petrelli F, Coinu A, Riboldi V, et al. Concomitant platinum-based chemotherapy or cetuximab with radiotherapy for locally advanced head and neck cancer: A systematic review and meta-analysis of published studies. *Oral Oncol*. 2014;50(11):1041–1048. doi:10.1016/j.oraloncology.2014.08.005
16. He X, Lei X, Cheng Y, Zhu H. Neoadjuvant chemotherapy vs upfront surgery for resectable locally advanced oral squamous cell carcinoma: A retrospective single center study. *Adv Clin Exp Med*. 2025;34(8):1307–1319. doi:10.17219/acem/192623
17. Luukkaa M, Minn H, Aitasalo K, et al. Treatment of squamous cell carcinoma of the oral cavity, oropharynx and hypopharynx – an analysis of 174 patients in south western Finland. *Acta Oncol*. 2003;42(7):756–762. doi:10.1080/02841860310010907
18. Crawford L, Bowman J, Gandhi M, Porceddu SV, Panizza B. Clinical outcomes in perineural spread of cutaneous squamous cell carcinoma via the ophthalmic nerve. *Head Neck*. 2024;46(9):2214–2222. doi:10.1002/hed.27836
19. Bostel T, Rühle A, Rackwitz T, et al. The role of palliative radiotherapy in the treatment of spinal bone metastases from head and neck tumors – a multicenter analysis of a rare event. *Cancers (Basel)*. 2020;12(7):1950. doi:10.3390/cancers12071950
20. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst Rev*. 2021;10(1):89. doi:10.1186/s13643-021-01626-4
21. Wells GA, Shea B, O'Connell D, et al. The Newcastle–Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa: Ottawa Hospital Research Institute. 2011.
22. Hall SF, Groome PA, Irish J, O'Sullivan B. Radiotherapy or surgery for head and neck squamous cell cancer: Establishing the baseline for hypopharyngeal carcinoma? *Cancer*. 2009;115(24):5711–5722. doi:10.1002/cncr.24635
23. Chen JH, Yen YC, Yang HC, et al. Curative-intent aggressive treatment improves survival in elderly patients with locally advanced head and neck squamous cell carcinoma and high comorbidity index. *Medicine (Baltimore)*. 2016;95(14):e3268. doi:10.1097/MD.00000000000003268
24. Vargo JA, Wegner RE, Heron DE, et al. Stereotactic body radiation therapy for locally recurrent, previously irradiated nonsquamous cell cancers of the head and neck. *Head Neck*. 2012;34(8):1153–1161. doi:10.1002/hed.21889
25. Gogineni E, Rana Z, Vempati P, et al. Stereotactic body radiotherapy as primary treatment for elderly and medically inoperable patients with head and neck cancer. *Head Neck*. 2020;42(10):2880–2886. doi:10.1002/hed.26342
26. Fan D, Kang JJ, Fan M, et al. Last-line local treatment with the Quad Shot regimen for previously irradiated head and neck cancers. *Oral Oncol*. 2020;104:104641. doi:10.1016/j.oraloncology.2020.104641
27. Schubert P, Rutzner S, Eckstein M, et al. Prospective evaluation of all-lesion versus single-lesion radiotherapy in combination with PD-1/PD-L1 immune checkpoint inhibitors. *Front Oncol*. 2020;10:576643. doi:10.3389/fonc.2020.576643
28. Roden DF, Schreiber D, Givi B. Multimodality treatment of early-stage tonsil cancer. *Otolaryngol Head Neck Surg*. 2017;157(1):62–68. doi:10.1177/0194599817696290
29. Upadhyay R, Gogineni E, Tocaj G, et al. Palliative Quad Shot radiation therapy with or without concurrent immune checkpoint inhibition for head and neck cancer. *Cancers (Basel)*. 2024;16(5):1049. doi:10.3390/cancers16051049
30. Puraia S, Kumar J, Yadav AK, Zaidi AK, Kumar R, Singh G. Comparison of conventional palliative radiotherapy fractionation schedule with Quad Shot regimen in locally advanced head and neck cancer patients: A randomised clinical trial. *J Clin Diagn Res*. 2024;18(12):XC01–XC07. doi:10.7860/JCDR/2024/74806.20397
31. Corry J, Peters LJ, D'Costa I, et al. The 'QUAD SHOT' – a phase II study of palliative radiotherapy for incurable head and neck cancer. *Radiother Oncol*. 2005;77(2):137–142. doi:10.1016/j.radonc.2005.10.008
32. Folkert MR, Timmerman RD. Stereotactic ablative body radiosurgery (SABR) or stereotactic body radiation therapy (SBRT). *Adv Drug Deliv Rev*. 2017;109:3–14. doi:10.1016/j.addr.2016.11.005
33. Mohamad I, Karam I, El-Sehemy A, et al. The evolving role of stereotactic body radiation therapy for head and neck cancer: Where do we stand? *Cancers (Basel)*. 2023;15(20):5010. doi:10.3390/cancers15205010

Exploring the association between diabetes and oral lichen planus: A systematic review

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Abstract

Persistent inflammatory oral mucosal lesions characteristic of oral lichen planus (OLP) have attracted considerable attention due to their potential association with diabetes mellitus (DM). This systematic review aimed to synthesize evidence from different study designs, including cross-sectional and case–control studies, to clarify the complex relationship between OLP and DM. The study was conducted according to predefined objectives and eligibility criteria. Using the PECOS framework, the review evaluated the prevalence, risk factors and comorbidities associated with DM among individuals with clinically diagnosed OLP. A search strategy combining Medical Subject Heading (MeSH) terms and relevant keywords was applied across multiple databases. The findings demonstrated variability in the reported prevalence and clinical associations between type 2 DM (T2DM) and OLP. Some studies reported a strong correlation, whereas others yielded conflicting findings, potentially reflecting methodological differences. Cross-sectional studies generally demonstrated high methodological quality, supporting the reliability of findings. Case–control studies showed greater variability in methodological quality, with some areas requiring improvement. Quality assessment using the Newcastle–Ottawa Scale (NOS) indicated generally good methodological quality, although differences in selection and comparability were observed across studies. The risk-of-bias assessment identified potential sources of bias, emphasizing the need for cautious interpretation of the available evidence. This study underlines the importance of standardized management procedures, emphasizes individualized patient care and supports further research into the mechanisms underlying the association between OLP and DM.

Keywords: HbA1c, diabetes mellitus type 2, oral lichen planus, metabolic control, clinical association

Highlights

- The association between oral lichen planus (OLP) and diabetes mellitus (DM) varied across studies.
- Type 2 DM was associated with a higher prevalence of OLP in some studies, whereas others found no significant association between the 2 conditions.
- Diagnostic and methodological differences may explain these inconsistent findings.

Introduction

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disorder characterized by painful erosions and reticulated white striae affecting the oral mucosa. Despite extensive research on this topic, the etiology of OLP remains complex due to immune dysfunction, hereditary factors and systemic associations. One such systemic association is diabetes mellitus (DM), a metabolic disorder.^{1,2} Type 2 DM (T2DM) is characterized by insulin resistance, hyperglycemia and systemic inflammation, and is associated with various oral manifestations, including periodontal disease, candidiasis and delayed wound healing. Several studies have reported a higher prevalence of OLP in patients with DM, suggesting a bidirectional relationship.^{3,4} A meta-analysis by Mozaffari et al. demonstrated a significant correlation between DM and OLP, highlighting an increased risk of OLP in individuals with DM.⁵ Similarly, Dave et al. confirmed this association and emphasized the role of systemic conditions and medications on the clinical presentation of OLP.⁶ Further supporting evidence comes from a systematic review by Otero Rey et al., which demonstrated a higher prevalence of OLP among individuals with DM.⁷

The mechanisms underlying the association between OLP and DM remain incompletely understood, but several plausible explanations have been proposed. Hyperglycemia-induced oxidative stress may be an important contributing factor, as it can promote immune dysregulation and chronic inflammation, exacerbating OLP lesions.^{2,8} Genetic susceptibility may also play a role, with studies identifying shared genetic markers between OLP and DM, suggesting that immune system alterations in predisposed individuals may increase susceptibility to both conditions.⁹ Beyond genetic factors, insulin resistance and altered cytokine profiles in diabetic patients contribute to a persistent pro-inflammatory state that may increase susceptibility to OLP.¹⁰ Additionally, hypertension, which frequently coexists with DM, may contribute to systemic inflammation and potentially exacerbate oral mucosal damage.¹¹ The association between OLP and DM is further supported by evidence suggesting that individuals with OLP may exhibit early metabolic disturbances, highlighting the potential value of metabolic screening for the detection

of prediabetes before it progresses to DM.¹² Epidemiological studies have also highlighted demographic variations in the coexistence of OLP and DM, indicating that population-specific factors should be considered when evaluating this association.^{2,8} Clinical observations further suggest that DM may influence not only susceptibility to OLP but also its clinical severity, with a higher prevalence of erosive forms reported among patients with DM. This association may be related to delayed wound healing and chronic inflammatory processes commonly observed in DM.¹³ Given the association of OLP with several systemic conditions, a multidisciplinary approach involving oral medicine specialists, dermatologists and endocrinologists is essential for comprehensive patient management.^{14,15} From a broader perspective, systematic reviews and meta-analyses have revealed substantial geographic variation in the prevalence and incidence of OLP, suggesting that environmental, genetic and systemic health factors may all contribute to its development.¹⁶ Collectively, these findings underscore the importance of early metabolic screening in patients with OLP and support an integrated, patient-specific approach to management.

The relationship between OLP and DM has been widely investigated due to the potential metabolic and inflammatory effects of DM on the development of OLP.^{5,17} Although several studies have reported a higher prevalence of OLP among patients with DM, the exact nature of this association remains unclear. Some studies, including those by Nosratzahi et al., found no significant correlation, indicating that genetic predisposition, environmental factors and variations in diagnostic criteria may contribute to the inconsistent findings.¹⁸ Additionally, OLP has been associated with other systemic disorders, including hypertension, further complicating its pathogenesis.^{11,19} Geographic variations in the prevalence of OLP, as highlighted by Dammak et al., emphasize the importance of considering population-specific and regional factors.¹³ Given the chronic nature of OLP and its potential systemic associations, this review aimed to evaluate the existing evidence, assess prevalence and risk factors, and explore diagnostic and therapeutic advancements to improve disease management and patient outcomes. The objective was also to enhance diagnostic accuracy, refine therapeutic strategies and promote interdisciplinary collaboration for improved disease management and patient outcomes.

Material and methods

Study design

This systematic study investigated the relationship between DM and OLP using a comprehensive methodological approach.

Protocol

Before the systematic review was initiated, a detailed protocol outlining the objectives, inclusion criteria, search strategy, and methodology was developed. The protocol was designed to enhance transparency and provide a structured framework for the review process.

PECOS framework

The study followed the PECOS framework, as outlined below:

- Population (P): Individuals clinically diagnosed with OLP from diverse backgrounds and settings;
- Exposure (E): DM as the primary exposure, with its association with OLP being evaluated;
- Comparison (C): Patients with OLP with and without DM, compared in terms of prevalence, risk factors and comorbidities;
- Outcome (O): The association between DM and OLP, including prevalence, co-occurrence, risk factors, and related comorbidities;
- Study design (S): Cross-sectional, case–control and observational studies.

Search strategy

A comprehensive literature search was conducted in PubMed®/MEDLINE, Embase, the Cochrane Library, Scopus, and Web of Science to identify relevant studies published between 2013 and 2023. The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The search strategy combined Medical Subject Headings (MeSH) terms and keywords related to OLP and DM. Boolean operators (AND, OR) were used to refine specificity and inclusivity. Keywords related to OLP included “Oral LP”, “Mucosal Lichen Planus”, “Oral Lesions”, and “Oral Inflammatory Disorder”. Terms related to DM included “DM”, “Diabetic”, “Metabolic Disorder”, and “Hyperglycemia”. To capture the association between the 2 conditions, terms such as “Link”, “Correlation” and “Interplay” were used. A total of 138 records were retrieved, screened and assessed by 3 independent reviewers (VPV, GM, HU). Any disagreements were resolved through discussion. After applying the predefined PRISMA-based inclusion and exclusion criteria, 7 studies were selected for the final analysis.

Eligibility criteria

The included studies were required to have been published in peer-reviewed journals between 2013 and 2023 and to investigate the potential association between DM and OLP using clinical or observational research designs. Only studies published in English were considered, and articles reporting relevant information on comorbidities, risk factors and the prevalence of DM and OLP were included. Studies published in languages other than English, those that did not examine the association between DM and OLP, and studies that did not use a clinical or observational research design were excluded. Editorials, reviews and case reports without original research were also excluded. This selection process ensured the inclusion of studies relevant to the comprehensive analysis of the relationship between DM and OLP.

Data extraction, collection and synthesis

Data extraction was performed using a systematic approach. Extracted information was organized according to study design, participant characteristics and outcome measures, facilitating consistent synthesis and comparison across the included studies. Of the 138 records screened, only 7 studies were included in the present review (Fig. 1).

Quality assessment

The methodological quality of the included studies was assessed using established instruments appropriate for observational and clinical research. The assessment considered variables such as sample size, statistical analysis, methodology, and study design.

Risk assessment

A risk-of-bias assessment was conducted to identify potential sources of bias and limitations in the included studies. During this process, the internal validity of the studies was assessed, and the potential influence of confounding variables on the reported associations was considered.

Results

Study characteristics and findings

This systematic review included 7 studies conducted between 2013 and 2023, exploring the association between T2DM and OLP (Table 1). The studies utilized cross-sectional, case–control and comparative designs, with sample sizes ranging from 50 to 525 participants. The mean age of participants varied across studies, although most

Table 1. Studies investigating the association between oral lichen planus (OLP) and diabetes mellitus (DM)

Study	Study type/methodology	Country	Patients with DM, <i>n</i>	OLP patients, <i>n</i>
Al-Maweri et al. 2013 ³	• cross-sectional study • 391 patients with T2DM and 391 controls • demographic, medical and dental data evaluated • oral examination performed according to the WHO criteria, with biopsy when necessary	Malaysia	391	2 (0.5%)
Mohsin et al. 2014 ²⁰	• cross-sectional study • 395 patients with T2DM and 405 controls • oral examination performed according to the WHO criteria, with biopsy when necessary	Pakistan	395	7 (1.8%)
Nosratzehi et al. 2015 ¹⁸	• case-control study • 50 patients with OLP and 50 healthy controls • HbA1c and FBS evaluated for DM diagnosis	Iran	–	50
Rashmi et al. 2018 ⁸	• observational case-control study	India	75	3 (2.67%)
Adhikari and Karki 2022 ¹	• comparative cross-sectional study	Nepal	16% of OLP cases (8 patients)	50
Sun et al. 2024 ²¹	• cross-sectional study • 525 patients with DM and 525 controls • data on glucose levels, DM duration, complications, treatment, systemic diseases, and medications	China	525	–
Rodríguez-Fonseca et al. 2023 ²²	• case-control study	Spain	24 (8.73%) in OLP group 13 (4.73%) in the control group	275

WHO – World Health Organization; OLP – oral lichen planus; T2DM – type 2 diabetes mellitus; HbA1c – glycated hemoglobin; FBS – fasting blood glucose; OMLs – oral mucosal lesions.

Age (patients/controls) [years] <i>M</i> ± <i>SD</i>	Main findings	Conclusions
54.71 ± 8.48/53.04 ± 12.06	OMLs were present in 45.5% of patients with T2DM ($p = 0.042$). Significant findings included denture stomatitis ($p = 0.018$), angular cheilitis ($p = 0.006$), and geographic tongue ($p = 0.017$). Poor metabolic control increased the risk of OMLs. The prevalence of OLP was 0.5% in patients with T2DM and 0.0% in controls ($p = 0.499$), indicating no significant association between T2DM and OLP.	T2DM was associated with a higher prevalence of OMLs, particularly fungal infections. Poor glycemic control was correlated with worse oral conditions, but no clear association between T2DM and OLP was identified.
53 ± 9.8/48 ± 7.2	OMLs were significantly more prevalent in patients with T2DM (60.8%) than in controls (39.2%) ($p < 0.0001$), with common benign lesions including coated tongue (26.8%) and fissured tongue (15.9%). OLP was present in 1.8% of patients with T2DM and 1.0% of controls ($p = 0.348$).	Patients with T2DM had a significantly higher prevalence of benign oral mucosal lesions. However, no clear association was found between T2DM and OLP or potentially malignant disorders.
44.5 ± 13.24/44.3 ± 11.44	OLP involved the buccal mucosa in 76% of cases, and reticular/papular forms accounted for 70% of cases. No significant differences in HbA1c or FBS were observed.	No significant association was found between OLP and DM.
53.43 ± 9.98/52.37 ± 10.05	Lichen planus was present in 3 individuals, including 2 in the diabetic group and 1 in the control group. A significant association was observed between DM and deterioration of periodontal health ($p < 0.001$). Multiple oral lesions were more prevalent in patients with diabetes (42.67%) than in controls (9.33%). Leukoplakia was found in 10 patients, with a prevalence of 6.67% in both groups. Malignant tumors were found only in patients with diabetes (4%).	The study suggests a potential association between DM and oral mucosal changes, including periodontal disease, abscess formation and potentially malignant disorders. However, OLP was not significantly associated with DM in this study.
42.3 (OLP)	The prevalence of DM was significantly higher in patients with OLP (16%) than in controls (4%) ($p = 0.046$). DM was more common in males with OLP (75%) than in females (25%). Hypertrophic OLP (30%) and eruptive OLP (27.5%) were the most common variants. DM was more prevalent in patients with OLP with a disease duration of >2 years.	The prevalence of DM was significantly higher in patients with OLP.
not specified	The prevalence of OLP was higher in patients with DM (2.3%/2.2%) than in controls (0.6%) ($p = 0.019/0.022$). No significant association was found between OLP and age, sex, glucose level, DM duration, complications, treatment, systemic diseases, or medication history.	DM was associated with a higher prevalence of OLP, but no correlation was found with other patient-related factors.
59.60 ± 12.18 (OLP)	The prevalence of prediabetes was higher in patients with OLP (21.45%) than in controls (14.55%) ($p = 0.035$). DM (≥ 126 mg/dL) was found in 8.73% of patients with OLP and 4.73% of controls ($p = 0.061$). Atrophic-erosive OLP was more frequent in older individuals (>60 years) and was associated with a higher likelihood of prediabetes. Among diabetic participants, 46.87% of patients with OLP and 50% of controls had uncontrolled glycemia despite treatment. A significant association was observed between oral antidiabetic treatment and atrophic-erosive OLP ($p = 0.005$).	The study suggests a potential association between OLP and prediabetes/DM, particularly in atrophic-erosive forms. Screening of glycemic status in patients with OLP is recommended for the early detection of DM.

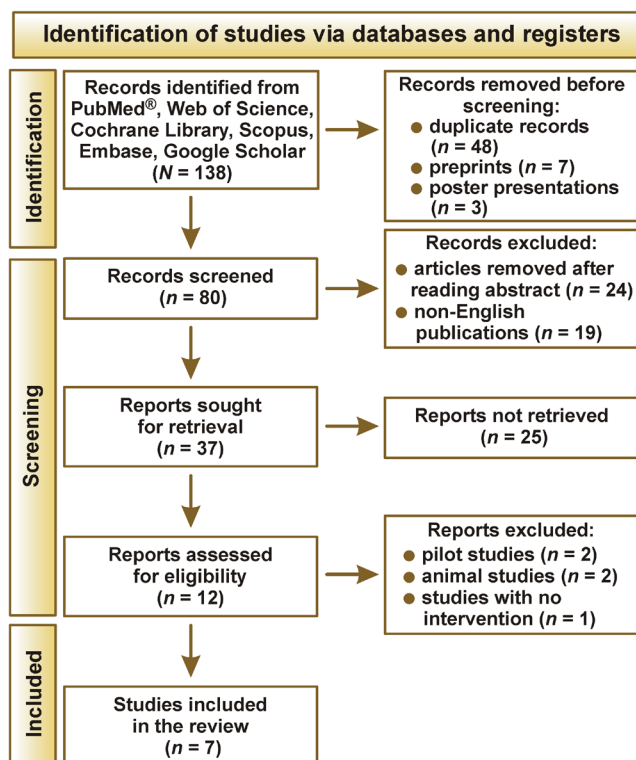


Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram of the study selection process

publications focused on middle-aged and older populations. Some studies have suggested a higher prevalence of OLP in individuals with DM. Al-Maweri et al. found oral mucosal lesions (OMLs) in 45.5% of patients with T2DM, a prevalence that was significantly higher than that in controls ($p = 0.042$). However, the prevalence of OLP (0.5%) did not differ significantly between the groups ($p = 0.499$).³ Similarly, Sun et al. reported a higher prevalence of OLP in patients with DM (2.3% vs. 0.6% in controls, $p = 0.019$), indicating a potential link.²¹ However, other studies did not establish a significant association. Mohsin et al. observed a higher prevalence of benign OMLs in patients with T2DM (60.8% vs. 39.2%, $p < 0.0001$) but found no significant association with OLP (1.8% in T2DM patients vs. 1.0% in controls, $p = 0.348$).²⁰ Nosratzahi et al. reported no significant differences in glycated hemoglobin (HbA1c) levels or fasting blood sugar between patients with OLP

and controls, suggesting that DM may not be a major factor in the development of OLP.¹⁸

Risk factors for diabetes and oral lichen planus

Several studies highlighted systemic factors potentially associated with the development of OLP in patients with DM. Rodríguez-Fonseca et al. found a higher prevalence of prediabetes in OLP patients (21.45%) compared to controls (14.55%, $p = 0.035$), with DM (≥ 126 mg/dL) detected in 8.73% of patients with OLP vs. 4.73% of controls ($p = 0.061$).²² Atrophic-erosive OLP was more frequent in older individuals (>60 years) and showed a significant association with oral antidiabetic treatment ($p = 0.005$).²² Adhikari and Karki reported a significantly higher prevalence of DM in patients with LP (16%) compared to controls (4%, $p = 0.046$), with DM being more common in males (75%) and in patients with a longer duration of LP.¹ Rashmi et al. found a significant correlation between DM and poorer periodontal health ($p < 0.001$) but no significant association with OLP (prevalence of 2.67%).⁸ Overall, while some studies suggest a potential link between T2DM and OLP, others indicate that confounding factors, such as genetic predisposition and diagnostic variations, may affect findings. These results highlight the need for standardized methodologies to clarify the nature of the relationship between DM and OLP.

Quality assessment

The quality of the included cross-sectional studies was assessed using the Newcastle–Ottawa Scale (NOS) and is presented in Table 2, with scores ranging from 8 to 10. Mohsin et al.²⁰ and Sun et al.²¹ received the highest scores (10/10), reflecting high methodological quality, including well-defined selection criteria, comparability and robust outcome assessment. Al-Maweri et al.³ scored 9/10, with 1 criterion not fulfilled within the selection domain, while Adhikari and Karki¹ had the lowest score (8/10) due to limitations in the selection domain. Despite these differences, all included cross-sectional studies demonstrated sufficient methodological rigor, reinforcing their reliability in evaluating the association between DM and OLP.

Table 2. Quality assessment of the cross-sectional studies using the Newcastle–Ottawa Scale (NOS)

Study	Selection 1	Selection 2	Selection 3	Selection 4	Comparability	Outcome 1	Outcome 2	Total
Al-Maweri et al. 2013 ³	*	*	—	**	**	**	*	9
Mohsin et al. 2014 ²⁰	*	*	*	**	**	**	*	10
Adhikari and Karki 2022 ¹	*	—	—	**	**	**	*	8
Sun et al. 2024 ²¹	*	*	*	**	**	**	*	10

Table 3. Quality assessment of the case–control studies using the Newcastle–Ottawa Scale (NOS)

Nosratzahi et al. 2015 ¹⁸	*	*	—	*	*	*	*	*	7
Rodríguez-Fonseca et al. 2023 ²²	*	*	—	*	*	*	*	*	7

The case–control studies, assessed using the NOS and presented in Table 3, had scores ranging from 6 to 7. Nosratzahi et al.¹⁸ and Rodríguez-Fonseca et al.²² both scored 7/9, indicating good methodological quality according to the applied assessment criteria. Rashmi et al.⁸ had the lowest score (6/9) due to limitations in selection and exposure assessment, which could introduce potential bias. While all studies contributed valuable insights, some methodological limitations were noted. Overall, the included studies maintained moderate to high quality, supporting the reliability of their findings.

Figures 2 and 3 illustrate the risk-of-bias assessment for the included studies. Figure 2 presents a tabular summary, showing the evaluation of 7 domains across 4 cross-sectional studies. Green circles indicate a low risk of bias, while red circles denote a high risk of bias. Notably, Adhikari and Karki¹ exhibit a high risk of bias in the Selection 2 and 3 domains, whereas Al-Maweri et al.³ demonstrate a high risk of bias only in the Selection 3 domain. Figure 3 provides a graphical representation, indicating that most studies have a low risk of bias. However, the Selection 2 and Selection 3 domains demonstrate some concerns, with Selection 3 having the highest proportion of high-risk assessments. These figures highlight potential methodological inconsistencies across studies. Figure 4 presents a tabular summary of the risk-of-bias assessment for 3 case–control studies across 7 domains. Nosratzahi et al.¹⁸ show a high risk of bias only in the Selection 3 domain, whereas the study by Rashmi et al.⁸ demonstrates a high risk of bias in the Selection 3 and Exposure 3 domains. Rodríguez-Fonseca et al. exhibit a low risk of bias across most domains, with little concern in Selection 3.²² Figure 5 represents these findings graphically, illustrating that most studies have a low risk of bias, but the Selection 3 and Exposure 3 domains show some concerns.

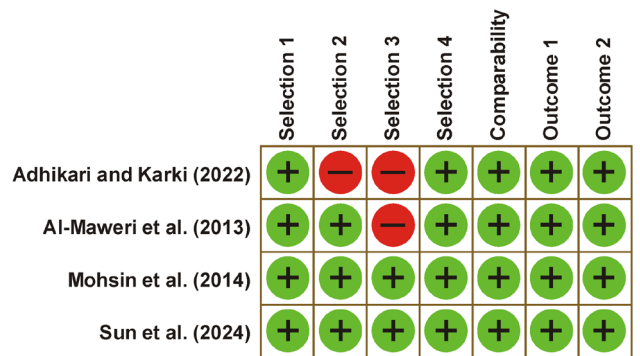


Fig. 2. Risk of bias in individual cross-sectional studies included in the review

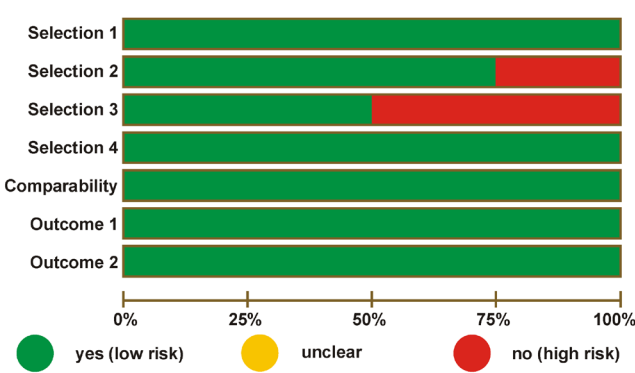


Fig. 3. Risk of bias across the included cross-sectional studies

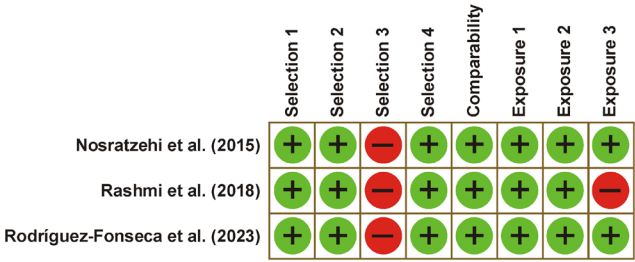


Fig. 4. Risk of bias in individual case–control studies included in the review

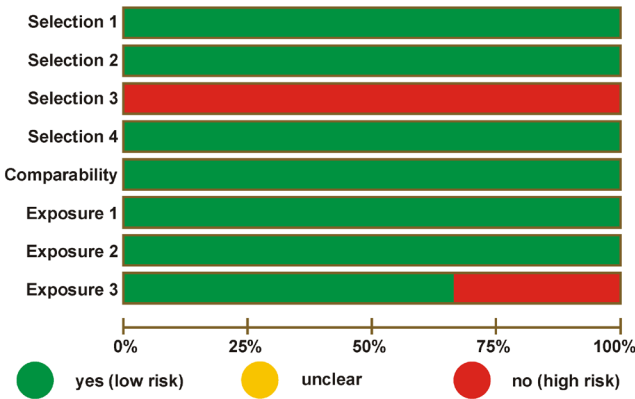


Fig. 5. Risk of bias across the included case–control studies

Discussion

This systematic review examined the complex relationship between DM, a chronic metabolic disorder with systemic inflammatory consequences, and OLP, a potentially premalignant inflammatory oral condition. By synthesizing evidence from cross-sectional and case–control

studies, this review evaluated the prevalence of OLP in individuals with DM, its clinical implications, the risk of prediabetes and DM in OLP patients, and the potential role of OLP as a marker of systemic health. The findings highlight the need for individualized patient management and standardized diagnostic and therapeutic approaches. Several studies have reported an association between OLP and DM, with some indicating a higher prevalence of OLP among patients with DM. A systematic review and meta-analysis by Mallah et al.¹⁷ confirmed this link, highlighting the inflammatory nature of both conditions and suggesting that chronic hyperglycemia-induced oxidative stress, immune dysregulation and microvascular changes may contribute to the development of OLP in diabetic individuals. Similarly, Varma et al. emphasized that insulin resistance and metabolic disturbances in DM could predispose patients to inflammatory oral conditions, including OLP.²³

Endocrine disorders, particularly thyroid disorders, have been linked to the pathogenesis of OLP. Arduino et al. identified early thyroid dysfunction in newly diagnosed patients with OLP, suggesting a complex interplay between endocrine and immune mechanisms.²⁴ These findings emphasize the importance of comprehensive systemic evaluation, especially in patients with OLP and coexisting metabolic or hormonal abnormalities. The systemic nature of OLP extends beyond its oral manifestations, warranting an interdisciplinary approach to patient care. Hasan et al. underscored the comorbidities associated with OLP, including cardiovascular diseases, metabolic syndrome and hypertension.¹⁰ This aligns with the findings of Grabowska-Szelag et al., who described cases of Grinspan's syndrome, a triad of OLP, DM and hypertension, suggesting that OLP may coexist with a broader spectrum of systemic disorders.¹⁹ Similarly, Dammak et al. reported a higher prevalence of OLP in patients with DM, supporting the hypothesis that metabolic dysfunction contributes to the development of OLP.¹³

Epidemiological studies suggest that patients with OLP show an increased risk of developing prediabetes or DM. Părlătescu et al. observed borderline glycemic levels (100–125 mg/dL) in many patients with OLP, highlighting the importance of early metabolic screening.²⁵ A systematic review and meta-analysis by Mallah et al. further reinforced this association, demonstrating a significant relationship between the prevalence of DM and OLP.¹⁷ Additionally, González-Serrano et al. compared the prevalence of oral mucosal diseases in diabetic and non-diabetic populations, confirming a higher occurrence of OLP in diabetic individuals, independent of other risk factors.²⁶ Other studies, such as those by Atefi et al.⁴ and Mohsin et al.,²⁰ further reinforce this association, suggesting that DM may not only increase the risk of OLP but also influence its severity and progression. Inflammatory markers have also been studied in the context of OLP and DM. Gadiock et al. evaluated systemic inflammation in patients with OLP using neutrophil CD64 expression, revealing

elevated inflammatory responses.²⁷ Drummond et al. further explored the role of insulin-induced hypoglycemia in triggering inflammatory markers, suggesting a bidirectional relationship between metabolic disturbances and inflammatory conditions such as OLP.²⁸ Additionally, studies by Rohani et al. and Rodríguez-Fonseca et al. suggest that patients with OLP may benefit from screening for metabolic disorders, given the potential for undiagnosed DM.^{12,22} Diagnostic accuracy is crucial in OLP, particularly due to its potential premalignant nature. Cheng et al. emphasized the importance of standardized histopathological and clinical criteria to ensure precise diagnosis and effective management.²⁹ Given the association between OLP and metabolic disorders, appropriate clinical and metabolic monitoring may therefore be warranted. Moreover, Yuwanati et al. highlighted the significant impact of OLP on oral health-related quality of life, stressing the psychosocial burden faced by affected individuals, especially those with systemic comorbidities such as DM.³⁰

The findings of this review highlight the clinical value of metabolic screening in patients with OLP. Given the bidirectional relationship between OLP and DM, healthcare professionals should remain aware of the possibility of coexisting metabolic abnormalities in patients with OLP. Routine fasting plasma glucose assessment may aid in the early diagnosis of DM, enabling timely intervention. Furthermore, effective management of OLP in patients with DM may require a multidisciplinary approach involving oral medicine specialists, dermatologists and endocrinologists. Overall, this systematic review highlights the intricate relationship between OLP and DM and reinforces the importance of comprehensive patient assessment. The available evidence suggests that OLP may be associated with metabolic disturbances, supporting metabolic screening and interdisciplinary management. Future research should focus on elucidating the precise pathophysiological mechanisms linking these conditions in order to refine diagnostic and therapeutic strategies.

Strengths and limitations

The present systematic review provides useful insights into the relationship between OLP and DM, focusing particularly on the possible pathophysiological connection and highlighting the need for a multidisciplinary approach to the issue. One of the significant advantages of the study under consideration is the inclusion of a large number of populations and study designs in the literature review. In addition, the review emphasizes the necessity of taking metabolic disorders into account when diagnosing and treating OLP. However, there are several limitations that should be pointed out. Variations in diagnostic criteria used to diagnose both conditions may have affected the prevalence rates. Variations in the clinical and histopathological definitions of OLP and inconsistencies in the assessment of glycemic status make it difficult to establish

a direct causal relationship. The majority of the included studies were cross-sectional, preventing determination of whether DM precedes OLP or vice versa. Additionally, heterogeneity in sample sizes and geographic distribution limits the generalizability of the findings. Despite these limitations, this review synthesizes the available evidence and emphasizes the need for future longitudinal studies with standardized diagnostic criteria to better understand the relationship between OLP and DM and its clinical implications.

Conclusions

Within the limitations of this study, the available evidence suggests that OLP may be more prevalent among individuals with DM, emphasizing the need for a comprehensive approach to evaluation of oral and systemic health. Although previous studies have shed light on several issues, the interaction between OLP and DM is still not fully understood. Further research is needed to understand the mechanisms involved and their causal relationship, as well as to determine the effect of DM control on OLP. Such studies may inform the development of integrated health-care strategies and reinforce the importance of interdisciplinary collaboration in the management of patients with these coexisting conditions.

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

During the preparation of the manuscript, the authors used DeepL Translate (DeepL SE, Cologne, Germany) for language editing and grammar correction.

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References

- Adhikari S, Karki A. Lichen planus and its association with diabetes mellitus. *Asian J Med Sci*. 2022;13(4):73–78. doi:10.3126/ajms.v13i4.41111
- Ara SA, Mamatha GP, Rao BB. Incidence of diabetes mellitus in patients with lichen planus. *Int J Dent Clinics*. 2011;3(1):29–33.
- Al-Maweri SAA, Ismail NM, Ismail ARI, Al-Ghashm A. Prevalence of oral mucosal lesions in patients with type 2 diabetes attending hospital universiti sains malaysia. *Malays J Med Sci*. 2013;20(4):39–46. PMID:24043995.
- Atefi N, Majedi M, Peyghamban S, Ghourchian S. Prevalence of diabetes mellitus and impaired fasting blood glucose in patients with lichen planus. *Med J Islam Repub Iran*. 2012;26(1):22–26. PMID:23482842.
- Mozaffari HR, Sharifi R, Sadeghi M. Prevalence of oral lichen planus in diabetes mellitus: A meta-analysis study. *Acta Inform Med*. 2016;24(6):390–393. doi:10.5455/aim.2016.24.390-393
- Dave A, Shariff J, Philipone E. Association between oral lichen planus and systemic conditions and medications: Case–control study. *Oral Dis*. 2021;27(3):515–524. doi:10.1111/odi.13572
- Otero Rey EM, Yáñez-Busto A, Rosa Henriques IF, López-López J, Blanco-Carrión A. Lichen planus and diabetes mellitus: Systematic review and meta-analysis. *Oral Dis*. 2019;25(5):1253–1264. doi:10.1111/odi.12977
- Rashmi B, Chinna SK, Rodrigues C, Anjaly D, Bankur PK. Oral mucosal changes in type II diabetes mellitus patients: A case–control study. *Int J Oral Care Res*. 2018;6(2):32–35.
- Yao M, Lu Y, Liu T, et al. Genetic and therapeutic for oral lichen planus and diabetes mellitus: A comprehensive study. *BMC Oral Health*. 2024;24(1):1226. doi:10.1186/s12903-024-04962-8
- Hasan S, Ahmed S, Kiran R, Panigrahi R, Thachil JM, Saeed S. Oral lichen planus and associated comorbidities: An approach to holistic health. *J Family Med Prim Care*. 2019;8(11):3504–3517. doi:10.4103/jfmpc.jfmpc_749_19
- De Porras-Carrique T, Ramos-García P, González-Moles MÁ. Hypertension in oral lichen planus: A systematic review and meta-analysis. *Oral Dis*. 2024;30(4):1793–1805. doi:10.1111/odi.14727
- Rohani B. Oral manifestations in patients with diabetes mellitus. *World J Diabetes*. 2019;10(9):485–489. doi:10.4239/wjd.v10.i9.485
- Dammak N, Chokri A, Sioud S, Hentati H, Selmi J. Oral lichen planus and diabetes mellitus: Prevalence and clinical features in a Tunisian population. *Int J Frontline Res*. 2022;1(1):33–37. doi:10.56355/ijfrls.2022.1.1.0004
- González-Moles MÁ, Warnakulasuriya S, González-Ruiz I, et al. Worldwide prevalence of oral lichen planus: A systematic review and meta-analysis. *Oral Dis*. 2021;27(4):813–828. doi:10.1111/odi.13323
- Cassol-Spanemberg J, Rodríguez-de Rivera-Campillo ME, Otero-Rey EM, Estrugo-Devesa A, Jané-Salas E, López-López J. Oral lichen planus and its relationship with systemic diseases. A review of evidence. *J Clin Exp Dent*. 2018;10(9):e938–e944. doi:10.4317/jced.55145
- 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
- Mallah N, Ignacio Varela-Centelles P, Seoane-Romero J, Takkouche B. Diabetes mellitus and oral lichen planus: A systematic review and meta-analysis. *Oral Dis*. 2022;28(8):2100–2109. doi:10.1111/odi.13927
- Nosratzahi T, Arbabi-Kalati F, Arefpoor Z. Lack of association between diabetes mellitus and oral lichen planus in Zahedan (South-East of Iran). *Caspian J Dent Res*. 2015;4:8–12. doi:10.22088/cjdr.4.2.8
- Grabowska-Szelag K, Tomera-Niekowal I, Kęsek B. Lichen planus coexisting with diabetes mellitus and hypertension (Grisnpan's syndrome) – description of two cases. *J Stoma*. 2018;71(5):449–456. doi:10.5114/jos.2018.84648
- Mohsin SF, Ahmed SA, Fawwad A, Basit A. Prevalence of oral mucosal alterations in type 2 diabetes mellitus patients attending a diabetic center. *Pak J Med Sci*. 2014;30(4):716–719. PMID:25097503.
- Sun Y, Chen D, Deng X, et al. Prevalence of oral lichen planus in patients with diabetes mellitus: A cross-sectional study. *Oral Dis*. 2024;30(2):528–536. doi:10.1111/odi.14323

22. Rodríguez-Fonseca L, Llorente-Pendás S, García-Pola M. Risk of prediabetes and diabetes in oral lichen planus: A case-control study according to current diagnostic criteria. *Diagnostics (Basel)*. 2023;13(9):1586. doi:10.3390/diagnostics13091586
23. Varma K, Shukla P. Association of diabetes mellitus in patients with lichen planus. *Indian J Clin Exp Dermatol*. 2017;3(1):14–16. doi:10.18231/2455-6769.2017.0004
24. Arduino PG, Karimi D, Tirone F, et al. Evidence of earlier thyroid dysfunction in newly diagnosed oral lichen planus patients: A hint for endocrinologists. *Endocr Connect*. 2017;6(8):726–730. doi:10.1530/EC-17-0262
25. Părlătescu I, Nicolae C, Oancea R, Funieru C. Oral lichen planus and diabetes: A clinical study. *Rom J Diabetes Nutr Metab Dis*. 2020;27(4):303–308. doi:10.46389/rjd-2020-1045
26. González-Serrano J, Serrano J, López-Pintor RM, Paredes VM, Casañas E, Hernández G. Prevalence of oral mucosal disorders in diabetes mellitus patients compared with a control group. *J Diabetes Res*. 2016;2016:5048967. doi:10.1155/2016/5048967
27. Gadiok KS, Veeraraghavan VP, Mony U, Arvind M, Gayathri R, Kavitha S. Evaluation of systemic inflammation in lichen planus using neutrophil CD64. *Texila Int J Public Health*. 2024. https://www.texilajournal.com/v2/adminlogin/download.php?category=article&file=6_TJ2601.pdf. Accessed December 9, 2024.
28. Drummond JB, Barbosa IG, Dantzer R, Teixeira AL. The effect of insulin-induced hypoglycemia on inflammatory markers: A systematic review. *Brain Behav Immun*. 2018;73:41–50. doi:10.1016/j.bbi.2018.05.003
29. Cheng YSL, Gould A, Kurago Z, Fantasia J, Muller S. Diagnosis of oral lichen planus: A position paper of the American Academy of Oral and Maxillofacial Pathology. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;122(3):332–354. doi:10.1016/j.oooo.2016.05.004
30. Yuwanati M, Gondivkar S, Sarode SC, et al. Impact of oral lichen planus on oral health-related quality of life: A systematic review and meta-analysis. *Clin Pract*. 2021;11(2):272–286. doi:10.3390/clinpract11020040

Status of knowledge on emergency dental trauma management among medical students: 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

Dental injuries frequently occur in children and adolescents. It is common for multiple types of injuries to affect the teeth and the surrounding tissues. Medical doctors often provide initial treatment; therefore, it is crucial for them to have the knowledge and skills necessary to provide first aid for traumatic dental injuries (TDIs).

The present study aimed to analyze, synthesize, and evaluate the quality of the existing research on medical students' knowledge, skills and attitudes regarding the provision of first aid for dental injuries.

The electronic medical databases Web of Science, PubMed, Wiley Online Library, Dentistry & Oral Sciences Source, and Scopus were searched. To identify additional publications, the reference lists of relevant articles were manually reviewed. The analysis included publications in English and Polish, including original research studies, such as randomized trials, cohort studies and cross-sectional surveys, while excluding case reports, reviews, commentaries, letters, and conference abstracts. The credibility of the studies was assessed using the Cochrane Collaboration's risk-of-bias tool. Due to substantial methodological and statistical heterogeneity, a meta-analysis was not possible; instead, a narrative synthesis was conducted to summarize the findings.

The search identified 55 studies, of which 10 were included in the systematic review, involving a total of 2,424 medical students. All studies focused on tooth avulsion; 3 also addressed tooth fractures, and 1 discussed tooth luxation.

The systematic review indicates that medical students often lack the knowledge and skills required to manage dental trauma in emergency situations, highlighting the need to include dental traumatology courses in medical education. Early training in the management of dental injuries should be implemented and integrated into first-aid training to improve preparedness for such situations.

Keywords: medical students, dental trauma, tooth fracture, tooth avulsion, emergency management

Cite as

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Highlights

- The study assessed medical students' knowledge, skills and attitudes regarding dental injury management.
- The study identified limited competence among medical students in managing dental trauma during emergencies.
- The study emphasizes the importance of incorporating dental traumatology courses into medical curricula.

Introduction

Traumatic dental injuries (TDIs) involve damage to the teeth, periodontal tissues and the soft tissues of the oral cavity.^{1,2} These injuries represent a significant global health concern and are often associated with sports activities, falls, impacts, or accidents. Children and adolescents are affected more frequently than adults.^{1–3} Data published by the International Association of Dental Traumatology (IADT) in 2020 indicate that dental injuries account for 5% of all injuries in children aged up to 6 years.^{1,2} Additionally, 25% of school-aged children have experienced a dental injury, and 33% of adults have reported an injury to their permanent teeth, with the majority of these injuries occurring before the age of 19.^{1,2}

Dental trauma can occur as an isolated injury affecting only the teeth and the surrounding supporting tissues, or it can accompany other maxillofacial injuries, referred to as dentofacial injuries.³ As a result, patients with TDIs may initially seek care from emergency medical doctors.^{4,5} The range of symptoms resulting from post-traumatic damage to dental tissues is diverse. These symptoms can vary from transient conditions requiring only periodic follow-up to complex complications that may ultimately lead to tooth loss. Addressing dental injuries presents significant challenges in both diagnosis and treatment. The prognosis largely depends on how quickly appropriate care is provided after the trauma and on the type of first aid administered.^{1,3,5} To improve the likelihood of a successful outcome and increase the potential for maintaining a healthy, complete dentition, it is essential for emergency department (ED) medical personnel to be familiar with the basic procedures for providing first aid in dental emergencies.

Emergency care plays a crucial role in determining the prognosis and the effectiveness of specific specialist treatment that may be required later. The time interval between an injury and the provision of appropriate care can significantly influence the risk of complications, such as pulp necrosis, root resorption, bone loss, ankylosis, or even tooth loss.^{2,3} Furthermore, complications resulting from a lack of treatment or delayed intervention can interfere with the proper development of the stomatognathic system, negatively affecting the self-esteem, psychological well-being and the overall quality of life (QoL) of young patients. Understanding basic first-aid protocols for traumatic injuries to oral tissues – such as the immobilization

of a luxated tooth, the replantation of an avulsed tooth, or the proper storage of fragments from a fractured tooth crown – can help prevent complications and spare patients from complex and costly dental procedures.^{1,2} Therefore, it is essential for medical professionals to possess the knowledge and skills necessary to provide first aid in cases of TDIs.^{5,6}

Education on dental trauma is very important for medical students, as they will inevitably need to use this knowledge to provide emergency dental care for stabilizing acute TDIs before referring patients to a dentist for further treatment. Understanding how to assess and manage TDIs is crucial for medical professionals, as their actions significantly impact patient outcomes. This educational focus aims to prevent inadequate emergency treatment in these circumstances. However, studies indicate that in many countries, dental curriculum and training, including topics such as dental traumatology, are lacking in medical education programs.^{7–9} To address the gap in medical training, it is essential for medical students to receive additional education on dental trauma. This training will help them understand the importance of accurately assessing and managing TDIs, including the retention and replantation of damaged teeth.^{6,7}

Therefore, the objective of this systematic review was to selectively collect and evaluate the available scientific evidence on medical students' knowledge, skills and attitudes regarding the provision of first aid for dental injuries.

Methods

Information sources and search strategy

The present systematic review of the available literature on medical students' knowledge, skills and attitudes regarding TDIs was performed according to the Cochrane Collaboration guidelines.¹⁰ The literature search was conducted according to a previously prepared protocol that specified the eligibility criteria, the search strategy, the study selection method, and the planned methodology for data analysis and synthesis.¹⁰

The search strategy adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (Fig. 1).¹¹ The electronic medical databases Web of Science, PubMed, Wiley Online Library, Dentistry & Oral Sciences Source, and Scopus

were searched. Additionally, to identify publications not indexed in these databases, the electronic search was complemented by a manual search of the reference lists of all relevant articles. The analysis included publications in English and Polish. Original research studies, including randomized trials, cohort studies and cross-sectional surveys, were eligible for inclusion. Case reports, reviews, editors' commentaries, letters to the editor, and conference abstracts were excluded. To optimize sensitivity and minimize potential bias, no date restrictions were applied to the inclusion of studies. Duplicate studies were identified and removed. The search strategy used Medical Subject Headings (MeSH) terms and Boolean operators. The specific search terms included "knowledge" OR "skills" OR "attitudes" AND "dental trauma" AND "medical students". The final search was conducted on January 24, 2025.

Eligibility criteria

The initial selection of articles was conducted by analyzing the titles and abstracts identified through the database search, using the PICO framework (Population, Intervention, Comparison, and Outcome).¹¹ The research question was specified as follows: Population (P): medical students; Intervention (I): questionnaires assessing the knowledge, skills and attitudes regarding TDIs; Comparison (C): not applicable; Outcome (O): knowledge results.

Study selection

The study selection process was carried out independently by 2 researchers (P.K. and B.J.), who had previously calibrated their approach based on 20% of the publications. The agreement between the reviewers was good, with a kappa value of 0.83. The 2 researchers initially screened the titles and abstracts to remove duplicates, and to assess articles for inclusion in the review according to the pre-defined screening criteria. The full texts of potentially eligible articles were then obtained and evaluated to determine their eligibility for inclusion in the final analysis. A third reviewer (T.P.-K.) resolved any disagreement between the 2 reviewers.

Data collection process

Data were extracted from the included studies, focusing on demographic information, the purpose of each study, the methods employed, the results, and the conclusions. Survey questions with similar structures and assessment criteria were grouped into what we referred to as question groups (QGs). These groups were then analyzed and visually represented as the Knowledge-Skills-Attitudes Heat Map (KSA Heat Map).¹² Each color indicated the level of knowledge, skills or attitudes among the surveyed medical students, categorized as follows: <25%; 25–50%; 51–75%; and >75%. A heat map visually represents data

using color coding to show varying values, making it easier to understand and analyze complex datasets. Two reviewers (P.K. and B.J.) independently extracted the data from the included studies, and subsequently checked and verified the extracted data. Any discrepancies between the 2 reviewers were resolved through discussion and consensus with a third reviewer (T.P.-K.).

Risk-of-bias assessment

The credibility of the studies was evaluated using the Cochrane Collaboration's risk-of-bias 2 tool (RoB 2). Each positive response was assigned 1 point. The following criteria were established: ≥ 7 points indicated a low risk of bias (high study credibility); 4–6 points indicated a moderate risk of bias; and ≤ 3 points indicated a high risk of bias (low study credibility).¹⁰ The credibility of each publication was assessed independently by 2 researchers (P.K. and B.J.), with any discrepancies resolved by a third researcher (T.P.-K.).

Synthesis

Due to substantial methodological and statistical heterogeneity among the included studies, the available data did not allow for a meaningful meta-analysis to be conducted. Instead, an extensive narrative synthesis of the data was performed. The findings of all included studies are summarized and presented through textual descriptions and tables.

Results

Study selection

Through a systematic search of medical information databases, a total of 55 publications were identified. After removing duplicates and applying the established search criteria, the scientific reports were initially screened based on their titles and abstracts. A total of 32 publications were deemed suitable for further analysis based on their full texts. Finally, 10 publications were selected for inclusion in the analysis (Fig. 1).

Study characteristics

The studies included in this review were conducted between 2015 and 2024 across various continents, including Asia,^{13–17} South America,¹⁸ Europe,^{19–21} and Africa.²² All studies were cross-sectional (Tables 1 and 2).

In total, the publications surveyed 2,424 medical students, with the number of participants in individual studies ranging from 84¹⁷ to 761.¹⁶ The studies by Coşgun and Coşgun,¹⁷ Oleszkiewicz and Emerich¹⁹ and Enabulele and Omo²² focused exclusively on final-year students. In contrast,

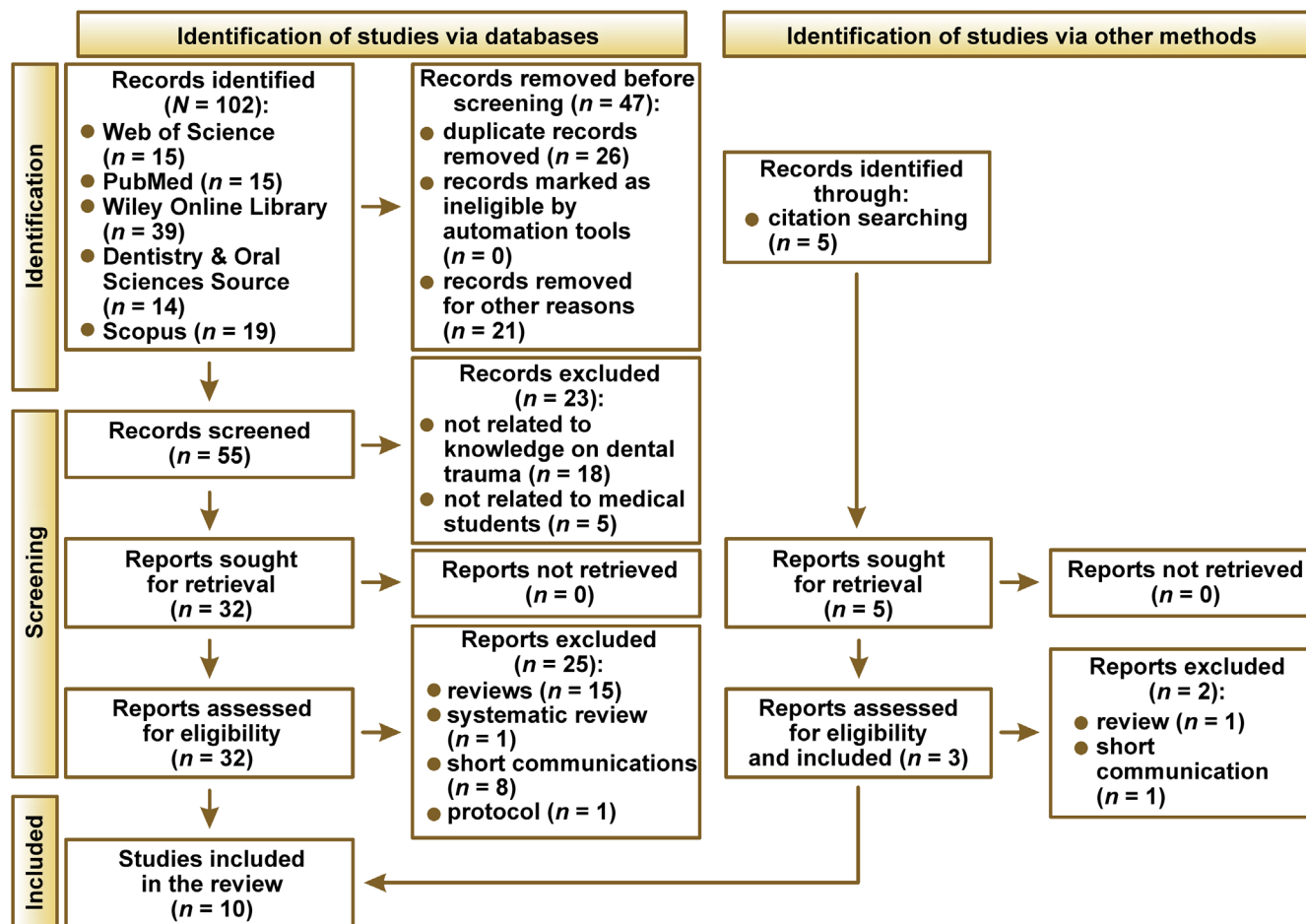


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the study selection process

the other studies included participants from various years of study: some included third-year students only,¹⁴ while others involved students in the latter half of their studies (4th, 5th and 6th years),^{13,16} third- to fifth-year students,²⁰ or students from all years of study.¹⁵ Notably, 2 publications did not specify the year of study of the surveyed participants (Table 1).^{18,21}

The systematic review analyzed publications that assessed medical students' knowledge, skills or attitudes related to TDIs. All of the studies focused on tooth avulsion. Additionally, 3 studies included questions about tooth fracture^{16,17,21} and 1 study also addressed tooth luxation (Table 2).²¹ The surveys used in these studies collected the sociodemographic data of the participants, and included questions regarding dental trauma training, previous experience, attitudes toward dental trauma, the self-assessed knowledge of dental trauma, and scenario-based questions on the emergency management of dental trauma.

Results of individual studies

The KSA Heat Maps reflecting the studies included in the systematic review are presented in Tables 3 and 4. Eight studies examined whether participants had received

information or training regarding TDIs.^{13–15,17,18,20–22} Of these, 7 studies reported that less than 25% of respondents had received such information,^{13–15,17,18,20,22} while 1 study reported a proportion of 37.80%.²¹ The predominant sources of knowledge were the Internet, family, friends, dentists, and medical journals. Furthermore, less than 20% of respondents reported having attended a first-aid course related to the management of dental trauma during the study period.^{13,15,18,20–22}

Two publications evaluated medical students' knowledge of dental trauma management,^{13,21} while 7 studies assessed students' self-perceived level of knowledge.^{14–18,21,22} In the study conducted by Varghese et al., 98.40% of students demonstrated a low level of knowledge (less than 60% correct answers), while the remaining students scored within the medium range (60–80%).¹³ Moreover, the authors found no statistically significant difference in the knowledge levels between fourth- and fifth-year students. The mean number of correct answers to the 10 questions was 3.4 (3.2 for fourth-year students and 3.5 for fifth-year students).¹³ In the study by Ivkošić et al., students answered an average of 4.32 ± 1.67 questions correctly out of 10.²¹ Most respondents self-assessed their knowledge as limited or insufficient.^{14–18,21,22} A significant proportion of students considered dental education related

Table 1. Demographic characteristics of the studies included in the systematic review

Study	Country	Sample size <i>n</i>	Year of study of the participants <i>n</i> (%)	Sex <i>n</i> (%)	Age [years] <i>M</i> ± <i>SD</i> or <i>M</i>
Varghese et al. ¹³ 2017	Malaysia	190	4 th – 82 (43.2) 5 th – 108 (56.8)	M – 77 (40.5) F – 113 (59.5)	NA
Duruk and Daşkıran ¹⁴ 2022	Turkey	430	3 rd	M – 316 (73.5) F – 114 (26.5)	22.12 ± 2.46
Soğukpınar Önsüren and Ipek ¹⁵ 2021	Turkey	224	1 st – 39 (17.4) 2 nd – 34 (15.2) 3 rd – 44 (19.6) 4 th – 34 (15.2) 5 th – 34 (15.2) 6 th – 39 (17.4)	M – 99 (44.2) F – 125 (55.8)	NA
Al-Haj et al. ¹⁶ 2022	Saudi Arabia	761	4 th – 215 (28.3) 5 th – 333 (43.8) 6 th – 213 (28.0)	NA	NA
Coşgun and Coşgun ¹⁷ 2024	Turkey	84	final year	M – 33 (39.3) F – 51 (60.7)	NA
Rodrigues et al. ¹⁸ 2021	Brazil	305	NA	M – 93 (30.5) F – 212 (69.5)	22.1 ± 3.3
Oleszkiewicz and Emerich ¹⁹ 2015	Poland	115	final year	NA	24.7
Ivancic Jokic et al. ²⁰ 2017	Croatia	86	3 rd – 34 (39.5) 4 th – 22 (25.6) 5 th – 30 (34.9)	M – 30 (34.9) F – 56 (65.1)	22.7
Ivkošić et al. ²¹ 2020	Croatia	135	NA	M – 31 (23.0) F – 104 (77.0)	NA
Enabulele and Omo ²² 2015	Nigeria	94	final year	M – 61 (64.9) F – 33 (35.1)	26.3 ± 3.4

M – mean; *SD* – standard deviation; M – male; F – female; NA – data not available.

to the management of dental trauma to be essential or very important.^{14,16,17,20,22}

Questions regarding the appropriate procedures for managing tooth fracture were addressed in 3 publications.^{16,17,21} In the studies conducted by Coşgun and Coşgun¹⁷ and Ivkošić et al.,²¹ the proportion of students demonstrating the knowledge of the appropriate management of crown fractures was in the 25–50% range. In contrast, the study by Al-Haj et al. reported a higher proportion of participants with the knowledge of the appropriate management procedure, ranging from 50% to 75%.¹⁶ Additionally, 1 publication addressed the management of luxated teeth, revealing that only 2.20% of respondents were aware of the correct approach.²¹

The knowledge of the appropriate management of avulsed primary teeth was evaluated in 3 publications.^{13,14,21} The percentage of students who demonstrated the knowledge of the appropriate procedure varied considerably, ranging from 21%¹³ to 75%.²¹ Regarding the recognition of the importance of replanting an avulsed permanent tooth, the percentage of surveyed students ranged from 7.10% to 77.60%.^{13–22} Despite this, fewer than 25% of respondents in 4 publications demonstrated the ability and competence to provide injured individuals, patients or caregivers with the accurate information

regarding the appropriate procedure,^{13,14,16,17} while 34.00% demonstrated this capability in 1 study.²² The proportion of students who knew how to manage the replantation of an avulsed tooth into its socket ranged from 2.95% to 34.00%.^{13,17,18,22} Fewer than 52% of respondents were aware of the critical extra-alveolar time.^{13–15,18,21,22} The percentage of respondents who knew how to properly handle a replanted tooth was below 50% in 3 publications,^{13,21,22} while 2 studies reported figures ranging from 50% to 75%,^{14,16} and 1 study reported an awareness rate of 88.30%.²⁰ The knowledge of the appropriate cleaning technique for an avulsed tooth prior to replantation, as well as the appropriate storage medium, was demonstrated by fewer than 75% of respondents across the publications.^{13–22} In the study conducted by Varghese et al., when asked about the appropriate procedure in the absence of an avulsed tooth to prevent aspiration, 41.60% of medical students responded that a chest X-ray should be performed.¹³

Quality and risk-of bias assessment

Five publications demonstrated a low risk of bias, with scores ranging from 7 to 9 points, and were therefore considered highly credible.^{13,14,16,17,21} In contrast, 4 publications

Table 2. Characteristics of the studies included in the systematic review

Study	Study design	Purpose of the study	Research conduct	Source of questionnaire	Type of questions	Number of questions	Medical problem (kind of dental trauma)	Reliability	Validity	Conclusions
Varghese et al. ¹³ 2017	cross-sectional	to assess the knowledge, skills and attitudes regarding the emergency management of avulsed teeth among medical students	stationary	Abu-Dawoud et al.*	objective	23	tooth avulsion	pilot-tested	yes	low level of knowledge on the first-aid management of dental trauma
Duruk and Daşkıran ¹⁴ 2022	cross-sectional	to assess the knowledge of medical students about emergency procedures to be taken in the case of tooth avulsion	online	structured, self-applied questionnaire	objective	18	tooth avulsion	pilot-tested	yes	lack of knowledge about the emergency management of avulsed teeth
Soğukpınar Önsüren and Ipek ¹⁵ 2021	cross-sectional	to assess the level of knowledge and attitudes of medical students regarding TDIs	stationary	structured, self-applied questionnaire	objective	17	tooth avulsion	unknown	unknown	low level of knowledge about dental injuries
Al-Haj et al. ¹⁶ 2022	cross-sectional	to assess the basic knowledge and attitudes of medical students about the emergency management of TDIs	online	structured self-applied questionnaire	objective	17	tooth fracture, tooth avulsion	pilot-tested	yes	insufficient level of knowledge about dental injuries
Coşgun and Coşgun ¹⁷ 2024	cross-sectional	to determine the approaches of senior-year students at the faculty of medicine toward dental trauma cases	online	previous articles	objective	demographic data and 10 questions	tooth fracture, tooth avulsion	pilot-tested	yes	insufficient level of knowledge about dental injuries
Rodrigues et al. ¹⁸ 2021	cross-sectional	to assess the knowledge of medical students about emergency procedures to be taken in the case of avulsion of permanent teeth	stationary	structured, self-applied questionnaire	objective	18	tooth avulsion	non-validated questionnaire	yes	no theoretical and practical experience regarding dental trauma management
Oleszkiewicz and Emerich ¹⁹ 2015	cross-sectional	to investigate the state of knowledge and attitudes regarding first-aid procedures after dental injury among medical students	stationary	structured, self-applied questionnaire	objective	8	tooth avulsion	pilot-tested	yes	poor knowledge about dental injuries
Ivancic Jokic et al. ²⁰ 2017	cross-sectional	to assess the knowledge of medical students about treatment procedures in the clinical cases involving dental injury	stationary	structured, self-applied questionnaire	objective	23	tooth avulsion	non-validated questionnaire	unknown	low level of knowledge among medical students
Ikrošić et al. ²¹ 2020	cross-sectional	to assess the level of knowledge of medical students regarding dental trauma management	stationary	previous article	objective	24	tooth fracture, tooth luxation, tooth avulsion	pilot-tested	yes	limited knowledge regarding dental trauma, dental injuries, and emergency management procedures
Enabulele and Omo ²² 2015	cross-sectional	to assess the knowledge of medical students about the emergency management of traumatic tooth avulsion	stationary	structured, self-applied questionnaire	objective	NA	tooth avulsion	pilot-tested	yes	little or no awareness of emergency management procedures for avulsed teeth

TDI – traumatic dental injury; NA – data not available.

* Abu-Dawoud M, Al-Enezi B, Andersson L. Knowledge of emergency management of avulsed teeth among young physicians and dentists. *Dent Traumatol*. 2007;23(6):348–355. doi:10.1111/j.1600-9657.2006.00477.x

Table 3. Knowledge-Skills-Attitudes Heat Map (KSA Heat Map) of responses to the questions regarding receiving dental trauma information and the knowledge level of the participants

Study	Received previous dental trauma management information/training (QG1)		Source of the information (if QG1 yes)					Any first-aid course on dental trauma management during the study period (QG2)			Level of knowledge among medical students (QG3)			Self-assessment of knowledge (QG4)				Importance of dental education on dental trauma management (QG5)				
	no	yes	Internet/ TV/radio	family/friends	dentists	medical literature	undergraduate course	yes	low	some	high	I don't know/ very poor	I know a little	sufficient but incomplete	I know enough	not	slightly	fairly	important	very important		
Varghese et al. ¹³ 2017		12.60	4.70	3.10	2.10	1.60	1.10	5.20	98.40	3.4*						10.00	9.00	63.70	17.34			
Duruk and Daşkıran ¹⁴ 2022		16.00	2.40		3.10	1.60	4.30					57.60	30.20	6.20	6.00	26.40			73.60			
Soğukpınar Önsüren and İpek ¹⁵ 2021		7.60	1.30		3.57	0.45	1.78	5.90				70.60			29.40							
Al-Haj et al. ¹⁶ 2022												69.10	22.60	9.30	9.30	11.00	18.00		71.00			
Coşgun and Coşgun ¹⁷ 2024		4.80										10.70	85.70	3.50					77.30			
Rodrigues et al. ¹⁸ 2021		23.00	0.30	8.60	1.00	7.20	1.30	0.30				61.50	40.00		0.30							
Oleszkiewicz and Emerich ¹⁹ 2015																						
Ivancic Jokic et al. ²⁰ 2017		15.10	1.09	1.36	2.99	1.36		15.10								1.80		35.70	62.50			
Ivkošić et al. ²¹ 2020		37.80						9.60	4.32 ±1.67*			31.90	45.90	22.20	0.00	3.00	5.20	63.70	21.50	6.70		
Enabulele and Omo ²² 2015		2.10						7.40				98.90			1.10	10.60			89.40			

Data presented as percentages [%].

Color indications: beige – <25% of participants; yellow – 25–50% of participants; orange – 51–75% of participants; red – >75% of participants; blue – no representation; green – other data.

QG – question group; * average number of correct answers per 10 questions.

Table 4. Knowledge-Skills-Attitudes Heat Map (KSA Heat Map) of responses to the questions regarding dental trauma management

Study	Correct responses regarding										
	tooth fracture procedure	luxated tooth procedure	management of avulsed primary teeth	importance of the replanting of avulsed permanent teeth	ruling out the aspiration of avulsed teeth	readiness to give instructions on the emergency management of avulsed teeth	management of an avulsed tooth with replantation into the socket	critical extra-alveolar time	proper handling of an avulsed tooth	proper cleaning of an avulsed tooth before replantation	proper storage medium
Varghese et al. ¹³ 2017			21.10	35.80	41.60	4.20	6.80	41.60	36.30	28.40	13.10
Duruk and Daşkıran ¹⁴ 2022			35.70	58.50		5.60		51.17	56.30	39.00	3.90
Soğukpınar Önsüren and Ipek ¹⁵ 2021				17.40				47.10			7.60
Al-Haj et al. ¹⁶ 2022	71.60			28.40		6.40			55.30	34.80	42.00
Coşgun and Coşgun ¹⁷ 2024	34.50			7.10		4.70	7.10			64.20	7.19
Rodrigues et al. ¹⁸ 2021				63.93			2.95	51.80			56.39
Oleszkiewicz and Emerich ¹⁹ 2015				8.10						62.60	61.00
Ivancic Jokic et al. ²⁰ 2017				77.60					88.30		43.20
Ivkošić et al. ²¹ 2020	46.70	2.20	74.80	46.70					40.00	17.80	8.90
Enabulele and Omo ²² 2015				7.50		34.00	34.00	25.50	46.80		1.10

Data presented as percentages [%].

Color indications: beige – <25% of participants; yellow – 25–50% of participants; orange – 51–75% of participants; red – >75% of participants; blue – no representation.

demonstrated a moderate level of credibility,^{15,18,19,22} while 1 publication was classified as having low credibility, with a score of 3 points (Table 5).²⁰

Discussion

Dental injuries are prevalent during the developmental years.^{1,3} The most frequent causes are falls and sports-related injuries, with the upper incisors being the most commonly affected teeth. It is not uncommon for various types of injuries to occur simultaneously to the tooth and the surrounding tissues.^{1,3,23} Each post-traumatic dental injury necessitates prompt treatment.^{2,3} The specific type of injury determines the appropriate dental specialist care required. The effectiveness of treatment is largely influenced by the time elapsed between the injury and the initiation of care, as well as by the adequacy of the treatment provided. Emergency interventions may include repositioning a displaced tooth, replanting an avulsed tooth, and stabilizing the tooth or its fragments. Although such

injuries primarily affect children and adolescents, their esthetic and psychological effects may extend well into adulthood. The potential complications arising from these injuries include impaired root development, tooth resorption, pulp necrosis, crown discoloration, and, in severe cases, tooth loss.

Tooth avulsion, which occurs when a tooth is completely displaced from its socket, accounts for 0.5–3% of all dental injuries.²⁴ The prognosis of this type of injury, which involves damage to the neurovascular bundle and the surrounding periodontal tissues, depends on the prompt administration of appropriate treatment. It should always be regarded as a dental emergency. According to the IADT guidelines, the preferred course of action is the immediate replantation of the tooth, ideally within 1 h, and preferably at the site of the accident.^{1,2} At the site of the injury, after assessing the condition of the socket, the avulsed tooth should be carefully reinserted into the socket by holding it by the crown and aligning it with the position of the adjacent teeth.³ Following this initial intervention, the patient should be referred to a dentist for further treatment.

Table 5. Summary measures used to assess the risk of bias in the included studies

Study	Were the criteria for including participants in the sample clearly defined?	Was the sample effectively selected and representative of the target student population?	Were the study materials and methods described clearly?	Anonymity	Were objective standard criteria used for the measurement of the condition?	Was the questionnaire validated?	Was the survey tested with a pilot sample?	Were the questions clear and easy for respondents to understand?	Was statistical analysis performed?	Risk of bias
Varghese et al. ¹³ 2017	yes	yes	yes	unknown	yes	yes	yes	yes	yes	low
Duruk and Daşkıran ¹⁴ 2022	yes	yes	yes	yes	yes	yes	yes	yes	yes	low
Soğukpınar Önsüren and İpek ¹⁵ 2021	no	no	no	unknown	yes	yes	unknown	yes	yes	moderate
Al-Haj et al. ¹⁶ 2022	yes	yes	yes	yes	yes	yes	yes	unknown	yes	low
Coşgun and Coşgun ¹⁷ 2024	no	no	yes	yes	yes	yes	yes	yes	yes	low
Rodrigues et al. ¹⁸ 2021	yes	yes	yes	unknown	yes	no	no	yes	yes	moderate
Oleszkiewicz and Emerich ¹⁹ 2015	no	yes	no	unknown	yes	yes	yes	unknown	yes	moderate
Ivancic Jokic et al. ²⁰ 2017	no	no	no	yes	yes	no	unknown	yes	unknown	high
Ivkošić et al. ²¹ 2020	yes	no	yes	yes	yes	yes	yes	yes	yes	low
Enabulele and Omo ²² 2015	no	no	yes	unknown	yes	yes	yes	unknown	yes	moderate

The success of tooth replantation largely depends on the length of time the tooth remains outside its socket. When a tooth is replanted more than 1 h after the injury, this is referred to as delayed replantation. The prognosis in such cases is less favorable due to an increased risk of complications, particularly the high likelihood of periodontal ligament necrosis.² In cases of delayed replantation, the tooth should be stored and transported in an appropriate storage medium, such as Hanks' balanced salt solution (HBSS), physiological saline, milk, or, when no other suitable medium is available, saliva.¹ Although tooth avulsion carries a significant risk of complications, replantation should always be attempted. Unfortunately, delayed replantation often has a poor long-term prognosis, as the periodontal ligament (PDL) may no longer be viable, potentially resulting in bone resorption and the loss of the alveolar process.³ The primary aim of replantation is to preserve the tooth for esthetic, functional and psychological reasons, while also maintaining the integrity of the alveolar bone.

Delayed replantation can result in ankylosis, which is characterized by the fusion of the mineralized structures of the tooth root with the surrounding alveolar bone. This condition results in the obliteration of the periodontal crevice. Post-traumatic tooth ankylosis poses a considerable challenge, particularly in young patients. The development of the alveolar process is closely associated with tooth eruption, which facilitates the vertical growth of the alveolar bone. In cases of ankylosis, the affected tooth remains positioned below the level of the adjacent teeth, as it becomes progressively embedded in the bone while the neighboring teeth continue to erupt. This condition can impede the vertical development of the maxillary alveolar process, resulting in the ankylosed tooth being positioned below the normal occlusal level, the potential tipping of the adjacent teeth, midline deviation, and the supra-eruption of the opposing teeth. Consequently, an open bite may develop, significantly affecting both smile esthetics and the overall function.^{2,3}

Out of the 10 analyzed publications included in the review, 7 focused solely on students' knowledge regarding tooth avulsion.^{13–15,18–20,22} Three publications also included questions concerning procedures for tooth fracture.^{16,17,21} Although future medical professionals may not be directly involved in the reattachment of tooth fragments, it is crucial for them to understand the potential value of these fragments for reconstructive purposes. The fragments should be preserved securely, and whenever possible, the young patient should be referred to a dentist at the earliest opportunity.

A medical graduate should attain specific learning outcomes throughout their studies, focusing on knowledge, skills and attitudes. Knowledge involves the acquisition of information through learning, enabling students to comprehend a variety of issues. Skills refer to the ability to apply this knowledge, encompassing the practical "know-how" necessary to perform tasks and solve problems. This

ensures that students can effectively implement what they have learned. Attitudes involve the ability to integrate knowledge, skills, and personal and social competencies within the medical profession. This preparation equips students to interact appropriately with patients and their caregivers.²⁵ These learning outcomes define what students should know, understand, and be able to accomplish upon completing their education. Beyond imparting theoretical knowledge, medical education aims to foster appropriate professional attitudes and develop students' skills in diagnosis, treatment planning, and patient care.

The systematic review of the analyzed publications reveals that medical students demonstrate insufficient knowledge, skills and competencies regarding first-aid procedures for TDIs. While many students were aware that an avulsed permanent tooth can be replanted,^{13,14,16,18,20,21} only a small proportion were able to perform this important procedure independently.^{13,17,18,22} Additionally, few students were familiar with the appropriate methods for protecting and storing an avulsed tooth.^{13–15,17,21,22} The findings indicate that, although students recognize their deficiencies in providing first aid for dental injuries,^{14–18,21,22} not all consider such education essential to their training as medical professionals.^{13,14,16,20–22}

Insufficient knowledge and skills in providing appropriate care for patients with dental injuries can be attributed to the lack of relevant courses in medical curricula.^{7,8,24,26–28} It is crucial for aspiring medical doctors to be adequately prepared to implement effective pre-dental treatment strategies. The evident lack of awareness among physicians regarding dental injuries highlights the need to integrate dental traumatology into medical education.^{6,29,30} Such integration would help ensure a high standard of care for patients with dental injuries. Moreover, incorporating dentistry, particularly with an emphasis on dentofacial injuries, into medical curricula would facilitate the development of the essential knowledge, skills and attitudes required for the basic diagnosis and management of post-traumatic dental injuries.^{9,29–33} Given the importance of achieving favorable treatment outcomes while minimizing complications, it is vital for future physicians to be well versed in this area.

While it may be unrealistic to expect doctors to be fully trained in all aspects of dental trauma care, they remain responsible for appropriately managing dental emergencies. Therefore, they should possess a fundamental understanding of how to manage dental trauma. The aim of teaching dental trauma to medical students is not to prepare them to perform these procedures routinely, but rather to equip them with the knowledge necessary to respond effectively to emergencies when they arise. Dental injuries often occur unexpectedly and at inconvenient times, meaning that patients frequently present to EDs as their first point of medical contact. Final-year medical students, in particular, may benefit more from education in dental trauma, as they have already established a solid foundation

in pre-clinical and clinical medical sciences, unlike first-year students. E-learning modules could enable medical students to learn at their own pace without disrupting their regular academic schedules.^{6,30}

In a study by Yeng et al., medical students evaluated an online course focused on dental trauma.²⁶ The findings indicated that the students considered the course understandable and beneficial to their future medical careers.²⁶ However, there is a recognized need to organize practical courses to help students develop essential clinical skills, as an online format alone may not provide sufficient hands-on experience. The combination of theoretical knowledge regarding patient management and clinical training has demonstrated positive outcomes, highlighting the importance of problem-based education in preparing medical students to interact with patients and effectively manage dental trauma cases.³⁴

Limitations and strengths

The data obtained from the reviewed studies present difficulties in terms of comparison, as the student cohorts in the individual studies were not homogeneous. Notably, 3 publications specifically focused on students who were approaching the completion of their academic programs, thereby involving individuals with the highest levels of knowledge and qualifications.^{17,19,22} In contrast, other studies included respondents from various academic years. Of particular note, the study conducted by Soğukpınar Önsüren and İpek included first- and second-year students as well.¹⁵

Another potential source of bias is the size of the study groups, which in survey research is influenced by the number of individuals in the target population. In 5 publications, the study group sizes were representative of the overall student population.^{13,14,16,18,19} However, in the remaining studies, the sample sizes were insufficient to accurately represent the target population.^{15,17,20–22}

The objective of this study was to analyze, synthesize, and evaluate the findings of the published scientific research concerning the knowledge, skills and attitudes of medical students regarding the provision of initial first aid for post-traumatic dental injuries. To our knowledge, this is the first report to assess medical students' knowledge of dental trauma. A study conducted by Tewari et al. investigated the global awareness of the prevention and emergency management of TDIs among non-dental healthcare professionals.³⁵ The results indicated that the knowledge of the emergency management of these injuries was generally low or insufficient in many cases. Additionally, there was a significant lack of research evaluating the prevention of TDIs. The authors concluded that this gap in the knowledge and understanding of first-aid procedures may stem from inadequate training in dental trauma during educational programs,³⁴ as well as the absence of effective continuing education courses designed to enhance professionals' expertise and confidence.³⁶

This systematic review has a limitation related to language bias, as it includes exclusively studies published in English or Polish. Consequently, the review may have overlooked relevant findings and insights from research published in other languages, which could have provided valuable data and further strengthened the overall analysis.

Conclusions

This systematic review of the literature indicates that medical students have limited knowledge, skills and inadequate attitudes regarding the management of dental trauma in emergency situations. These findings highlight the urgent need to incorporate dental traumatology into the educational curriculum for future physicians. It is crucial to begin educating medical students about dental injuries early in their training and to progressively expand their knowledge as they advance through their studies and professional careers. Furthermore, incorporating awareness of dental injuries into first-aid training courses could significantly improve individuals' preparedness to respond effectively in such situations.

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. Bourguignon C, Cohenca N, Lauridsen E, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and luxations. *Dent Traumatol.* 2020;36(4):314–330. doi:10.1111/edt.12578
2. Levin L, Day PF, Hicks L, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: General introduction. *Dent Traumatol.* 2020;36(4):309–313. doi:10.1111/edt.12574
3. Andreasen JO, Andreasen FM, Andersson L, eds. *Textbook and Color Atlas of Traumatic Injuries to the Teeth*. 5th ed. Oxford, UK: Wiley–Blackwell; 2018.

4. Mallya PL, Kundabala M, Srikant N, Midday AR. Awareness about the management of avulsed tooth among medical interns in Mangalore, India. *Indian J Public Health Res Dev*. 2018;9(10):212–216. doi:10.5958/0976-5506.2018.01343.8
5. Steeves E, Doyle T, Mello I. Perceptions of emergency department physicians regarding the management of traumatic dental injuries. *Dent Traumatol*. 2023;39(5):462–468. doi:10.1111/edt.12844
6. Yeng T, O'Sullivan AJ, Shulruf B. Learning about dental trauma for medical students. *Dent Traumatol*. 2020;36(3):237–240. doi:10.1111/edt.12530
7. Skapetis T, Gerzina T, Hu W. Management of dental emergencies by medical practitioners: Recommendations for Australian education and training. *Emerg Med Australas*. 2011;23(2):142–152. doi:10.1111/j.1742-6723.2011.01384.x
8. Trivedy C, Kodate N, Ross A, et al. The attitudes and awareness of emergency department (ED) physicians towards the management of common dentofacial emergencies. *Dent Traumatol*. 2012;28(2):121–126. doi:10.1111/j.1600-9657.2011.01050.x
9. Yeng T, O'Sullivan AJ, Shulruf B. Appropriate management of traumatic dental injuries at the hospital emergency department provides a positive impact on patient outcomes: Exemplar case study. *AMJ*. 2019;12(9):255–262. doi:10.35841/1836-1935.12.9.255-262
10. Higgins JP, 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>.
11. 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
12. Tewari N, Goel S, Haldar P, Mathur VP, Srivastav S. An effective method to summarise the outcomes of questionnaire-based studies in systematic reviews. *Acta Odontol Scand*. 2022;80(8):569–572. doi:10.1080/00016357.2022.2052957
13. Varghese E, Zhen-Ying JN, Law LL, Samson RS, Soe HH. Knowledge, attitude, and practice of emergency management of tooth avulsion among medical students in Melaka, Malaysia: A cross-sectional study. *World J Dent*. 2017;8(4):273–280. doi:10.5005/jp-journals-10015-1450
14. Duruk G, Daşkiran IC. Evaluation of knowledge on emergency management of avulsed teeth among Turkish medical and dental students. *Pesqui Bras Odontopediatria Clin Integr*. 2022;22:e210092. <https://revista.uepb.edu.br/PBOCI/article/view/1488>.
15. Soğukpınar Önsüren A, İpek S. Are student knowledge levels and attitudes about avulsion dental trauma adequate in the faculty of medicine? *Int Dent Res*. 2021;11(Suppl 1):143–151. doi:10.5577/intdentres.2021.vol11.suppl1.22
16. Al-Haj Ali SN, Farah RI, Alhariri S. Knowledge and attitudes of Saudi medical students about emergency management of traumatic dental injuries. *Int J Environ Res Public Health*. 2022;19(21):14249. doi:10.3390/ijerph192114249
17. Coşgun A, Coşgun MF. Management of dental trauma of last-year medical students attending medical school in Tokat. *Neu Dent J*. 2024;6(1):101–107. doi:10.51122/neudentj.2024.93
18. Rodrigues OF, Gomes Landim KL, Alves RT, Machado FC, Carrada CF. Knowledge of avulsion of permanent teeth emergency management among undergraduate in Brazilian health care students. *Pesqui Bras Odontopediatria Clin Integr*. 2021;21:e0085. <https://revista.uepb.edu.br/PBOCI/article/view/625>.
19. Oleszkiewicz I, Emerich K. How to proceed in case of tooth avulsion: State of student knowledge. *Eur J Paediatr Dent*. 2015;16(2):103–106. PMID:26147814.
20. Ivancic Jokic N, Bakarcic D, Grzic R, Majstorovic M, Sostarek M. What general medicine students of University of Rijeka know about dental avulsion? *Eur J Dent Educ*. 2017;21(4):e131–e134. doi:10.1111/eje.12235
21. Ivkošić I, Gavić L, Jerković D, et al. Knowledge and attitudes about dental trauma among the students of the University of Split. *Acta Stomatol Croat*. 2020;54(3):302–313. doi:10.15644/asc54/3/8
22. Enabulele JE, Omo JO. Knowledge and attitudes of final year medical students to first aid management of traumatic tooth avulsion: A cross-sectional survey. *J Med Biomed Res*. 2015;14(1):54–63. doi:10.4314/jmbr.v14i1
23. Wang G, Wang C, Qin M. A retrospective study of survival of 196 replanted permanent teeth in children. *Dent Traumatol*. 2019;35(4–5):251–258. doi:10.1111/edt.12475
24. Andersson L, Andreasen JO, Day P, Heithersay G, et al. Guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Pediatr Dent*. 2017;39(6):412–419. doi:10.1111/j.1600-9657.2012.01125.x
25. Regulation of the Minister of Science and Higher Education of 26 July 2019 regarding the education standards for preparing individuals to practice the professions of physician, dentist, pharmacist, nurse, midwife, medical laboratory scientist, physical therapist, and paramedic (consolidated text) Dz.U. (Journal of Laws of the Republic of Poland) of 2021, item 755 [in Polish]. <https://isap.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=WDU20210000755>.
26. Yeng T, O'Sullivan AJ, Shulruf B. Medical students' perception of an online dental trauma course in medical education. *Aust Endod J*. 2022;48(1):51–57. doi:10.1111/aej.12601
27. Ulusoy AT, Onder H, Cetin B, Kaya S. Knowledge of medical hospital emergency physicians about the first-aid management of traumatic tooth avulsion. *Int J Paediatr Dent*. 2012;22(3):211–216. doi:10.1111/j.1365-263X.2011.01178.x
28. Raoof M, Vakilian A, Kakoei S, Manochehrifar H, Mohammadalizadeh S. Should medical students be educated about dental trauma emergency management? A study of physicians and dentists in Kerman Province, Iran. *J Dent Educ*. 2013;77(4):494–501. PMID:23576595.
29. Puranik CP, Pickett K, de Peralta T. Evaluation of problem-based learning in dental trauma education: An observational cohort study. *Dent Traumatol*. 2023;39(6):625–636. doi:10.1111/edt.12870
30. Yeng T, O'Sullivan AJ, Shulruf B. A proposal to introduce dental trauma into medical education: An insight. *Dent Traumatol*. 2020;36(4):390–392. doi:10.1111/edt.12542
31. Díaz J, Bustos L, Herrera S, Sepulveda J. Knowledge of the management of paediatric dental traumas by non-dental professionals in emergency rooms in South Araucanía, Temuco, Chile. *Dent Traumatol*. 2009;25(6):611–619. doi:10.1111/j.1600-9657.2009.00835.x
32. Glendor U. Has education of professional caregivers and lay people in dental trauma care failed? *Dent Traumatol*. 2009;25(1):12–18. doi:10.1111/j.1600-9657.2008.00707.x
33. İpek B. Is it necessary to incorporate traumatic dental injury course into the medical education curriculum: A systematic review. *Essent Dent*. 2024;3(3):106–113. doi:10.5152/EssentDent.2024.24017
34. Smolana A, Loster Z, Loster J. Assessment of stress burden among dental students: A systematic literature review and meta-analysis of data. *Dent Med Probl*. 2022;59(2):301–307. doi:10.17219/dmp/133955
35. Tewari N, Jonna I, Mathur VP, et al. Global status of knowledge for the prevention and emergency management of traumatic dental injuries among non-dental healthcare professionals: A systematic review and meta-analysis. *Injury*. 2021;52(8):2025–2037. doi:10.1016/j.injury.2021.06.006
36. Sedky RAF, Ben Dor B, Mustafa DS, et al. Self-assessment skills of undergraduate students in operative dentistry: Preclinical performance and gender. *Dent Med Probl*. 2025;62(6):1035–1042. doi:10.17219/dmp/175276

Current approaches to comprehensive oral care after cancer therapy

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Abstract

Cancer therapies, including radiotherapy, chemotherapy and surgical interventions, are essential components of modern oncology, but are frequently associated with long-term, multifactorial oral complications that can impair patients' oral function and quality of life (QoL). This narrative review integrates current evidence, clinical guidelines and expert consensus to provide a comprehensive overview of contemporary approaches to oral management following cancer treatment.

The aim of this review was to synthesize and critically evaluate current dental strategies for the comprehensive care of individuals after oncologic therapy, identify the most common oral complications, including oral mucositis (OM), xerostomia, dysgeusia, radiation-induced caries, oral candidiasis, osteoradionecrosis (ORN), and medication-related osteonecrosis of the jaw (MRONJ), and highlight the areas requiring further research.

A literature search was conducted across PubMed, Wiley Online Library, the Web of Science, Google Scholar, and ScienceDirect from March to October 2025, focusing on clinical trials, reviews and international guidelines. Preventive measures, including remineralization protocols, rigorous oral hygiene practices and salivary stimulants, were emphasized as the crucial components of supportive care. The emerging therapeutic modalities, such as low-level laser therapy (LLLT) and novel pharmacological agents, were critically assessed with regard to their clinical effectiveness.

Overall, the evidence emphasizes the need for an interdisciplinary, patient-centered approach involving oncologists, dental specialists and allied healthcare professionals to minimize morbidity and optimize clinical outcomes. Multidisciplinary dental care remains indispensable for preventing and managing oral complications, and improving the long-term QoL of cancer survivors.

Keywords: oral mucositis, xerostomia, osteoradionecrosis, post-cancer dental care, interdisciplinary oncology

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Highlights

- The study emphasizes the critical role of oral health in comprehensive and long-term survivorship care for patients treated with cancer therapies.
- The findings demonstrate that early dental intervention significantly reduces the incidence and severity of post-radiotherapy oral complications.
- The study highlights how personalized oral care strategies enhance oral function, reduce morbidity, and improve the quality of life of cancer survivors.
- The findings show that multidisciplinary collaboration among oncologists, dentists, and allied healthcare professionals optimizes oral health outcomes in oncology patients.
- The study supports the implementation of evidence-based protocols to standardize dental oncology practices and improve patient-centered outcomes.

Introduction

Cancers are among the leading causes of death worldwide, particularly among middle-aged and older adults, contributing to chronic pain, reduced quality of life (QoL), and substantial costs associated with the treatment of advanced disease. Although traditionally classified as non-communicable diseases, a growing body of evidence has shown that some cancers are closely linked to viral infections, particularly human papillomavirus (HPV). Human papillomavirus plays a significant role in the development of cervical, laryngeal and oral cancers, with HPV-positive cases accounting for 20–40% of oral cancers.¹

Cancers of the lip, oral cavity and pharynx are among the most common malignant tumors of the head and neck region, accounting for more than 400,000 new cases worldwide each year. Recent studies have indicated that their incidence is unevenly distributed geographically and shows a global upward trend. As medical and social costs continue to rise, oral and pharyngeal cancers in younger populations are becoming an increasingly significant public health concern.² Dental complications in individuals who have survived childhood cancer are often irreversible, as they most commonly affect the permanent dentition.³

Post-treatment dental care represents a vital component of comprehensive oncologic management, particularly in patients undergoing therapy for head and neck malignancies. Current evidence supports the integration of dental professionals into multidisciplinary oncology teams, demonstrating improved oral health outcomes among head and neck cancer survivors. Patients managed within such coordinated frameworks exhibit significantly better periodontal health and oral hygiene as compared to those referred solely to general dental services.⁴ Researchers conducted a dose–response meta-analysis including 25 studies to further clarify and assess the association between tooth loss and cancer risk.⁵ They demonstrated that each additional loss of 10 teeth was associated with a 9% increase in cancer risk. They also estimated that tooth loss was associated with a 3–31% increased risk of various

malignancies, including cancers of the head and neck, esophagus, stomach, colon, pancreas, lung, hematopoietic system, and bladder, in a clear dose-dependent manner.⁵

Oncologic therapies – including radiotherapy, chemotherapy and surgery – are associated with both acute and chronic oral complications, highlighting the need for long-term dental monitoring and intervention.⁶ Acute radiation-induced toxicity often develops early during treatment and may persist for several weeks after therapy, whereas late complications may arise weeks or even years later.⁷

Oral cavity and oropharyngeal cancers are recognized worldwide as major health concerns, given their substantial impact on patients' QoL.⁸ The primary risk factors for oropharyngeal cancer (OPC) include tobacco smoking, alcohol consumption, betel chewing, and HPV infection, which may occur individually or in combination. Unlike tobacco-related cancers of the middle pharynx, whose incidence has declined in many high-income countries, the incidence of HPV-associated OPC has risen sharply over recent decades.⁹

The incidence of OPC in the United States has risen steadily among men since the 1970s. Notably, both the incidence and annual number of OPC cases now exceed those of cervical cancer, making OPC the most common HPV-related malignancy in the United States. Unlike cervical cancer, OPC cannot currently be screened for, as precancerous lesions cannot be reliably identified, and the existing diagnostic tools are insufficient for detecting early-stage, localized tumors.¹⁰

In the context of post-treatment care, it is important to note that patients with HPV-related oral cancer generally have better prognoses, and greater sensitivity to radiotherapy and chemotherapy. The projected increase in HPV-associated cancers also suggests continued improvement in patient survival, reflecting advances in treatment and the overall QoL.¹

Oropharyngeal squamous cell carcinoma (OPSCC), which includes malignancies of the tonsils, base of the tongue, soft palate, and uvula, is now one of the most rapidly increasing cancers in incidence across high-income

countries.¹¹ Over the past several decades, the epidemiology of OPSCC in the United States has undergone notable changes, with important clinical and public health implications for patients after cancer treatment.¹² The standard therapeutic approach for OPSCC generally includes the surgical resection of the tumor, definitive radiotherapy, or combined chemoradiotherapy. Among the treatment-related side effects that adversely affect patients' perceived QoL and functional performance – and therefore require multidisciplinary care, including dental support – are xerostomia, odynophagia and oral thrush.¹¹

Interdisciplinary collaboration improves functional outcomes and helps preserve oral health.^{4,13,14} As cancer survival increases, greater emphasis is being placed on maintaining oral function and comfort. Although many cancer patients exhibit oral health profiles similar to those of the general population, a substantial proportion present with deteriorating restorations, periodontal disease, and other complications exacerbated by cancer therapy, thereby affecting QoL.⁷ Emerging evidence suggests that early radiographic and biochemical changes around implants, such as subtle alterations in crestal bone and elevated levels of pro-inflammatory cytokines, may precede overt clinical signs. This highlights the need for more sensitive monitoring in post-cancer patients, whose reduced regenerative capacity and increased susceptibility to oral complications make early detection essential for maintaining long-term function and comfort.¹⁵ Furthermore, the potential application of stem cell-based therapies with tissue-specific differentiation capacity offers a promising adjunct to peri-implant and post-oncologic oral rehabilitation. Systematic reviews indicate that mesenchymal stem cells (MSCs) can enhance osteoblastic differentiation, promote new bone formation, and improve implant osseointegration through molecular signaling and interactions with biofunctionalized implant surfaces. Although the evidence remains heterogeneous and largely preclinical, these emerging approaches highlight a potential future direction for managing complex tissue defects in cancer survivors.¹⁶

Dentists play an essential role in early diagnosis and prevention through oral hygiene promotion, fluoride use and salivary care.⁶ Patients experiencing oral mucositis (OM), dry mouth, gingival sensitivity, and gingival pain exhibit markedly greater taste alterations and reduced QoL, while functional impairment, such as trismus and dysphagia, further contributes to malnutrition and diminished well-being. These findings underscore the need for early rehabilitation, dietary modification, and coordinated multidisciplinary care.^{17,18} Artificial intelligence (AI), including neural networks (NNs), shows growing promise as a surveillance tool. Many studies report that NNs can identify early oral cancer with accuracies exceeding 85%, offering valuable support to clinicians, particularly in settings with limited access to oral pathology expertise. Risk factor and behavioral assessments should complement AI-based evaluations to enhance diagnostic precision.¹⁹

Although guidelines are available for healthcare professionals caring for patients undergoing oncologic treatment, minimizing common side effects, such as mucositis, xerostomia and radiation-induced caries, depends largely on patients' active involvement in oral self-care, supported by appropriate education. Although these self-care protocols are often supported by a low level of evidence, their implementation remains essential for maintaining oral health.²⁰

Methods

This narrative review synthesizes current evidence and clinical practice guidelines to provide an integrated overview of contemporary approaches to oral management in oncology patients. Its aim was to summarize and critically evaluate modern dental strategies for the comprehensive care of individuals after cancer treatment, highlight major oral complications, and identify the areas requiring further investigation.

Studies were included based on their relevance to post-cancer oral complications, current dental management protocols, peri-implant tissue health, early diagnostic approaches, and the emerging regenerative or stem cell-based therapies. A total of 74 publications were included. The literature comprised clinical practice guidelines, systematic reviews and meta-analyses, narrative or scoping reviews, randomized controlled trials (RCTs), observational studies, epidemiological studies, preclinical/in vitro studies, and case reports or case series. Although no formal risk-of-bias assessment was performed, study selection followed the principles of transparency and relevance consistent with the Scale for the Assessment of Narrative Review Articles (SANRA) recommendations, with a focus on clinical applicability and contribution to contemporary dental oncology practice.

Literature search was conducted in PubMed/MEDLINE, Wiley Online Library, the Web of Science, Google Scholar, and ScienceDirect between March and October 2025. Given the narrative design of this review, study selection was guided by thematic relevance and the contribution of each source to the understanding of contemporary concepts and clinical practice, rather than by rigid, predefined systematic criteria.

Oral mucositis

The oral mucosa, periodontal tissues and dentition are among the regions most commonly affected by radiation-induced toxicity due to their high cellular turnover and essential roles in oral health and function.⁷ These structures are particularly susceptible to ionizing radiation, which can compromise tissue integrity and lead to substantial clinical complications.

Oral mucositis is a frequent and debilitating adverse effect of oncologic therapies. It initially presents as mucosal erythema and atrophy, and may progress to ulceration. One of the earliest signs is leukoedema – an ill-defined, diffuse, milky-white opalescence, most often observed on the buccal mucosa, which typically disappears upon stretching.⁷ Erythema may appear within the first week of fractionated radiotherapy (2 Gy/day)⁷ and within 4–5 days after the initiation of chemotherapy.²¹ The onset of OM reflects epithelial aplasia resulting from cytotoxic injury to rapidly proliferating basal cells, generally occurring 7–14 days after chemotherapy. Early symptoms include burning or tingling sensations, and increased sensitivity to mechanical and chemical stimuli.⁷ Pain, dysgeusia, dysphagia, and odynophagia commonly interfere with nutrition and hydration.²² Lesions frequently involve the lips, buccal mucosa, ventrolateral tongue, and floor of the mouth, typically resolving within 2–4 weeks after the completion of therapy, although complete healing may require up to 3 months.⁷

Chlorhexidine and other biologically active rinses provide antimicrobial and anti-inflammatory benefits, but have limited effects on the molecular pathways underlying OM.¹³ Basic oral care remains fundamental to reducing secondary infections and minimizing mechanical trauma. Symptomatic management is central, particularly in chemotherapy-induced OM, with compounded analgesic rinses (“Magic Mouthwash”) containing diphenhydramine, viscous lidocaine, bismuth subsalicylate, or corticosteroids commonly used for palliation.⁷

Preventive oral hygiene reduces the risk of OM, although its direct effect on the underlying mechanisms of mucosal injury remains unclear.¹³ Chlorhexidine and benzydamine may be used for antiseptic and anti-inflammatory purposes; however, their use may be associated with adverse effects, including mucosal burning, taste alterations, and staining.²³

Chlorhexidine is not recommended for the prevention of OM in patients receiving radiotherapy for head and neck cancer, although it remains useful for gingivitis and plaque control.¹³ Diagnosis relies primarily on clinical history, symptoms and lesion distribution.²⁴ Chemotherapeutic agents most commonly associated with OM include doxorubicin, bleomycin, fluorouracil, and methotrexate.²⁵

For the World Health Organization (WHO) grade I–IV OM, weekly low-level laser therapy (LLLT) is recommended until complete recovery.²¹ Prophylactic photobiomodulation (PBM) initiated 2–3 weeks before cancer treatment, combined with education on oral hygiene, nutritional guidance and risk-factor modification (e.g., tobacco and alcohol use), is strongly advised.²⁶

Analgesic rinses, such as doxepin and methylene blue, may be effective in managing oral mucositis-related symptoms. Methylene blue oral rinse has demonstrated rapid and clinically meaningful analgesic effects in cancer-related oral mucositis, with improvement in oral function when used alongside standard therapy. Its safety profile appears favorable, with no serious adverse events reported,

and only mild, transient effects, such as brief oral burning or temporary discoloration of the lips and teeth, which resolve spontaneously. Owing to its demonstrated efficacy, safety, and limited adverse effects, methylene blue represents a promising supportive option for refractory oral mucositis-associated pain.²⁷ Topical doxepin rinse also appears to be an effective analgesic option for managing oral mucositis-related pain, providing clinically meaningful reductions in symptom intensity. Although generally well tolerated, its use may be accompanied by mild, transient adverse effects, such as oral burning, unpleasant taste, or drowsiness, the latter likely related to partial systemic absorption. Patient acceptability is relatively high, with many individuals expressing willingness to continue therapy when adequate pain relief is achieved. Overall, doxepin represents a useful adjunct to supportive care, offering a symptomatic benefit with an acceptable safety profile.²⁸

Benzydamine is indicated for the prevention of OM in patients receiving moderate-dose radiotherapy (<50 Gy), while a 0.2% morphine oral solution may provide both analgesic and anti-inflammatory effects.²⁹ Topical morphine has been shown to reduce pain intensity, shorten the duration of severe symptoms, and improve functional outcomes, such as swallowing and oral opening. Its safety profile appears favorable, with predominantly mild and transient local adverse effects, most commonly brief burning, dryness or itching, and minimal systemic toxicity. Reported adverse reactions are infrequent and generally do not limit tolerability. Palifermin significantly reduces the incidence, duration and severity of OM, but may cause transient taste disturbances. However, according to the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) guidelines, the available evidence is insufficient or inconsistent to support formal clinical recommendations for these agents. Oral cryotherapy using ice chips is widely employed in chemotherapy-induced OM to reduce mucosal exposure to chemotherapeutic agents through vasoconstriction.^{7,30,31}

Key recommendations are summarized in Table 1.

Xerostomia

Despite efforts to limit radiation exposure to healthy tissues, the salivary glands, the oral mucosa and maxillofacial bones are often unintentionally affected, resulting in functional and structural changes with long-term effects on QoL.⁷ Radiotherapy and certain cytotoxic agents commonly impair salivary gland function, causing hyposalivation and xerostomia.^{26,32} This predisposes patients to dental caries, fungal infections and bacterial overgrowth due to the disruption of the oral microbiota and reduced salivary defense mechanisms.³³ It has been noted that oncologists frequently underestimate the importance of salivary function, whereas they should incorporate its assessment into routine patient evaluation.¹⁷

Table 1. Key recommendations for the management of oral mucositis (OM)

No.	Recommendation	Description
1.	Monitor high-risk areas	Regularly assess the oral mucosa, periodontium and dentition
2.	Recognize early signs	Look for leukoedema, erythema, burning sensations, and ulcers
3.	Maintain oral hygiene	It helps reduce the risk and severity of infections
4.	Use benzydamine cautiously	Effective at <50 Gy doses; may cause side effects
5.	Manage pain effectively ^{7,21–31}	Use “Magic Mouthwash”, doxepin, methylene blue, or 0.2% morphine
6.	Apply PBM ⁷³	Begin before therapy and continue weekly as needed
7.	Consider palifermin ⁷²	For severe OM to reduce its duration and severity
8.	Use oral cryotherapy	Ice chips are used during chemotherapy to protect the oral mucosa
9.	Promote prevention	Educate on oral hygiene, diet, and the avoidance of smoking and alcohol

PBM – photobiomodulation.

Hyposalivation may develop early, sometimes after only a few 2-Gy fractions. Radiation doses >30 Gy are associated with irreversible glandular injury, with severity correlating with the cumulative dose.²⁶ Although the compensatory hypertrophy of the spared glands may occur months later, the total salivary output may decrease by 50–60% within the first week when all major glands are irradiated.⁷

Management involves supportive and pharmacological strategies. Ice chips, adequate hydration (≥ 1.5 L/day), mucosal lubricants, and salivary stimulation with xylitol-containing products are considered first-line measures. Saliva substitutes are generally more effective than water for managing oral dryness.³⁴ In severe cases, muscarinic receptor agonists, such as pilocarpine and cevimeline, may be prescribed.^{26,35–37} Corticosteroids may benefit patients with immune-mediated xerostomia, although they should be used with caution. Anethole trithione may be considered as an adjunctive therapy. Patients should avoid alcohol, caffeine, and acidic or spicy foods,^{26,29,36} and may also benefit from saline/bicarbonate rinses, humidifiers, and moisturizing gels or sprays.^{34,38}

Complementary therapies under investigation include acupuncture, omega-3 fatty acid supplementation, vitamins C and E, green tea polyphenols, and aloe vera, although the evidence supporting their use remains limited. Photobiomodulation and transcutaneous electrical nerve stimulation (TENS) show emerging potential, whereas hyperbaric oxygen therapy (HBOT) has limited supporting evidence and homeopathy lacks the evidence of efficacy.³⁵

Topical pilocarpine (1–2%) effectively stimulates salivary secretion, with lower concentrations (e.g., 0.1%) also demonstrating benefit. A 2% topical formulation may provide salivary stimulation comparable to a 5-mg oral dose, with fewer systemic effects. Novel delivery systems, including

pilocarpine lozenges, aerosols, hydrogels, and nanofiber carriers, are under development. Localized mucosal tablet formulations may optimize therapeutic outcomes while minimizing systemic exposure.³⁹ However, pilocarpine is effective only when functional salivary tissue is preserved. Evidence from controlled clinical studies indicates that its therapeutic benefit is modest, with only minimal increases in the salivary flow reported across trials. Local administration (e.g., ophthalmic drops applied intraorally) is associated with fewer adverse effects than systemic tablet formulations, which frequently cause cholinergic side effects, such as sweating, rhinorrhea, nausea, and excessive salivation. Bethanechol demonstrates comparable efficacy with a more favorable side-effect profile, suggesting that it may be a better-tolerated alternative in some patients.⁴⁰

Lipids act as coating and lubricating agents in the oral cavity by forming a protective layer that reduces friction. Unlike other macromolecules that rely on water retention, lipids interact with oral surfaces primarily through weak dispersion forces, while longer lipid chains provide greater viscosity and longer-lasting adhesion to the mucosa. Studies indicate that plant-based oils, such as sunflower, canola, coconut, and olive oils, may serve as saliva substitutes by improving oral comfort, reducing xerostomia and enhancing the salivary flow. Olive oil may additionally exhibit anti-inflammatory and antibacterial properties.^{41,42}

Sodium hyaluronate (hyaluronic acid – HA), a naturally occurring polysaccharide with mucoadhesive and water-binding properties, is considered a safe and effective therapeutic option, providing viscosity and lubrication comparable to that of natural saliva. It is biocompatible and can be degraded by hyaluronidase. However, attention should be given to its molecular weight: high-molecular-weight HA may inhibit the antimicrobial activity of lysozyme and peroxidase, potentially increasing the risk of infection. Therefore, low-molecular-weight HA (1,000–4,000 kDa) is recommended for use in artificial saliva formulations.^{41,43}

Since the salivary film forms a protective coating over the oral mucosa, its reduction in hyposalivation leaves the epithelium more exposed and susceptible to chemical irritants. A diminished mucosal/salivary film compromises lubrication and barrier function, increasing the risk of irritation in response to topical agents. Therefore, when high-concentration fluoride products are considered, the condition of the oral mucosa should be carefully assessed in patients with hyposalivation, as the absence of an adequate salivary coating may increase their sensitivity to these agents.^{44–46}

Key recommendations are summarized in Table 2.

Taste alterations

Chemotherapy is known to disrupt gustatory function and reduce appetite. Although taste alteration is a common treatment-related toxicity, it is frequently underreported by patients and may be underestimated by clinicians,

Table 2. Key recommendations for the management of xerostomia

No.	Recommendation	Description
1.	Ensure hydration	At least 1.5 L/day; the use of ice chips
2.	Use mucosal lubricants	Such as vegetable oils or moisturizing oral gels
3.	Stimulate saliva ^{32–36}	Use xylitol-containing gum or lozenges
4.	Prescribe pilocarpine or cevimeline	Recommended in moderate to severe cases
5.	Consider topical pilocarpine	Explore alternative delivery systems for local effects
6.	Avoid irritants	Including alcohol, caffeine, acidic and spicy foods
7.	Use rinses	Saline or baking soda solutions to soothe oral tissues
8.	Use corticosteroids cautiously	Only in immune-mediated xerostomia
9.	Explore complementary therapies ^{41–46}	Examples: acupuncture, omega-3 fatty acids, vitamins, aloe vera
10.	Consider PBM or TENS	For salivary stimulation

TENS – transcutaneous electrical nerve stimulation.

as it does not pose an immediate threat to survival. Greater severity of taste alteration has been observed in women than in men, and among individuals with breast cancer as compared to those with gastrointestinal, pulmonary, genitourinary, or hematologic neoplasms. Patients who reported a good or very good appetite exhibited substantially lower levels of taste alteration than those with poor or moderate appetite. No significant associations were identified between taste alteration and either patient age or the number of chemotherapy cycles when analyses were stratified by the appetite level.¹⁷

Dysgeusia

Dysgeusia, a common complication in patients undergoing head and neck radiotherapy (HNRT), often coexists with xerostomia due to the integral role of saliva in taste perception. The condition is dose-dependent, with disturbances in taste sensation typically occurring at radiation doses of approx. 30 Gy. At this threshold, patients may experience a near-complete loss of fundamental taste modalities – namely salty, sweet, sour, and bitter – secondary to damage to gustatory epithelial cells.^{21,47} While mild dysgeusia is generally manageable, more severe forms can significantly impair appetite, reduce oral intake, and negatively affect the nutritional status and QoL.⁷

Partial recovery of taste function usually begins within 20–60 days following the cessation of treatment, with full restoration frequently occurring over a period of 2–4 months.⁷ Chemotherapy can also contribute to transient dysgeusia, often presenting as a metallic taste due to the diffusion of cytotoxic agents into saliva. Drugs

such as cyclophosphamide, methotrexate and 5-fluorouracil may alter taste either by affecting cranial nerves (VII, IX and X), damaging the oral mucosa, or directly impacting taste bud cells.^{22,26}

Although clinical data regarding the efficacy of zinc supplementation are variable, zinc has been proposed to improve taste perception by stabilizing the regulatory proteins involved in taste bud maintenance.⁷ Zinc supplementation also appears to offer therapeutic benefits in reducing the incidence, duration and severity of OM, likely due to its roles in immune function, epithelial repair and antioxidant defense. Its safety profile is generally favorable, with minimal adverse effects reported when used at appropriate doses. However, variability in zinc formulations, dosing strategies, and routes of administration limits the comparability of the existing studies and underscores the need for standardized, long-term clinical trials.⁴⁸ Vitamin D supplementation has also been explored as a potential therapeutic option for mitigating taste alterations. In chronic or persistent cases, dietary counseling and simple nutritional intervention remain important for improving patient outcomes.⁷

Supportive strategies, such as slow, mindful mastication, can enhance flavor perception and stimulate the salivary flow, which may be particularly beneficial in patients with concurrent xerostomia. Additionally, rotating food types during meals may help prevent sensory adaptation and promote dietary diversity, thereby supporting adequate nutrition and overall oral intake.⁷

According to some researchers, future cancer treatment strategies and post-therapy supportive care should prioritize the development of microbiota-modulating interventions and personalized therapeutic approaches to enhance oncologic treatment efficacy. Given that gut microbiota dysbiosis has been associated with chronic inflammation, the production of carcinogenic metabolites and altered immune responses, and considering that inflammatory conditions, including those affecting the oral cavity, are frequently observed in patients undergoing chemotherapy and radiotherapy, dental practitioners should also consider systemic microbiological interactions when aiming to improve patient outcomes.⁴⁹

The principal recommendations are summarized in the Table 3.

Radiation-induced caries

It refers to an aggressive and rapidly progressing form of dental decay that commonly develops following HNRT. Although ionizing radiation can alter the structural integrity of dental hard tissues, the underlying pathogenesis of radiation-induced caries is largely analogous to that of caries associated with other hyposalivation-related conditions. Clinically, it resembles caries observed in patients with xerostomia, but more frequently involves atypical

Table 3. Key recommendations for the management of dysgeusia

No.	Recommendation	Description
1.	Monitor for dysgeusia onset	Especially when radiation doses reach or exceed 30 Gy
2.	Educate patients	Explain the expected recovery timelines (partial: 20–60 days; full: 2–4 months)
3.	Identify chemotherapy-related taste changes ^{17,21}	Note a metallic taste from agents like cyclophosphamide, methotrexate and 5-fluorouracil
4.	Consider zinc supplementation ^{22,47}	It may support taste bud function; clinical results are variable
5.	Assess vitamin D status	Correct deficiency to support the overall oral and taste health
6.	Refer for dietary counseling	It helps ensure adequate nutrition and address altered taste perceptions
7.	Encourage slow, mindful chewing	It enhances flavor perception and stimulates the production of saliva
8.	Recommend food rotation during meals	It prevents taste fatigue and promotes a more balanced nutritional intake

tooth surfaces, such as cusp tips and cervical margins. The disease also exhibits a higher recurrence rate and more rapid progression than conventional caries.²⁶

The elevated risk is primarily attributable to radiation-induced enamel demineralization, shifts toward a cariogenic oral microbiota, poor oral hygiene, and high-carbohydrate dietary patterns.⁵⁰ Xerostomia, commonly induced by radiotherapy, often precedes the onset of radiation-induced caries, which may manifest as early as 3 weeks after treatment and rapidly compromise dental integrity. Radiation further weakens tooth structure, rendering enamel more vulnerable to acid-induced breakdown. Contributing factors include frequent consumption of sugar-laden beverages, which facilitates bacterial adhesion and enhances enamel demineralization.²² Under conditions of reduced salivary flow, *Streptococcus mutans* may proliferate, further increasing the risk of caries in irradiated patients.

Effective prevention requires rigorous oral care protocols, including routine dental evaluations and continuous fluoride therapy. Although custom fluoride trays remain the most efficacious delivery method, alternatives such as fluoride varnishes, rinses and high-fluoride toothpaste are also recommended. Fluoride application should be maintained for as long as hyposalivation persists. When trays are not used, topical fluoride gels or brush-on formulations, combined with dietary modifications, are essential.⁵⁰ Additional strategies include daily sodium fluoride rinses,²⁶ quarterly dental visits and regular professional cleanings.

Nutritional counseling should emphasize reduced consumption of fermentable carbohydrates, decreased snacking frequency and substitution with non-cariogenic sweeteners. Xylitol-containing products may be beneficial due to their ability to inhibit cariogenic bacteria and stimulate the salivary flow. Advanced preventive care may include fluoride varnishes for prolonged enamel protection and

calcium phosphate- or hydroxyapatite-based agents to promote remineralization. Probiotic therapy may also help support a balanced oral microbiome and reduce pathogenic bacterial colonization.³³

In advanced cases, restorative interventions, such as composite restorations, crowns or dental implants, may be required. To preserve enamel integrity, patients should use non-abrasive toothpaste and avoid acidic foods and beverages.^{29,51} Long-term oral health maintenance depends on patient education focused on oral hygiene practices, caries prevention and healthy dietary habits.⁵²

The principal recommendations are summarized in the Table 4.

Oral candidiasis

It is a frequent opportunistic infection in oncology patients, particularly following chemotherapy and radiotherapy, due to treatment-induced immunosuppression and hyposalivation.⁵³ The most common form, pseudo-membranous candidiasis, presents clinically as white, curd-like plaques that can be gently scraped off, typically revealing an erythematous, eroded or bleeding mucosal surface underneath. Another notable subtype, hyperplastic candidiasis, manifests itself as thickened, non-removable white plaques. This variant closely resembles leukoplakia, necessitating careful differential diagnosis because of its potential for malignant transformation.⁷

Oral candidiasis is associated with burning, pain, dysphagia, dysgeusia, and mucosal discomfort. A minimum 2-week course of antifungal therapy is generally required

Table 4. Key recommendations for the management of radiation-induced caries

No.	Recommendation	Description
1.	Maintain daily oral hygiene ^{29,33}	Brush and floss regularly; use high-fluoride toothpaste
2.	Use fluoride regularly	Prefer custom trays; rinses or varnishes as alternatives
3.	Continue fluoride during hyposalivation	Maintain use as long as dry mouth persists
4.	Attend regular dental check-ups	Schedule visits at least every 3 months
5.	Reduce sugar intake	Limit fermentable carbohydrates and between-meal snacking
6.	Avoid sugary medications	Rinse mouth after use if alternatives are not available
7.	Use xylitol products ^{50–52}	It helps reduce cariogenic bacteria and stimulate the salivary flow
8.	Apply remineralizing agents	Use products like calcium phosphate or hydroxyapatite
9.	Protect enamel	Use low-abrasive toothpaste and avoid acidic foods and drinks
10.	Educate patients	Provide guidance on prevention, diet and proper oral hygiene practices

for resolution.⁵⁴ The condition is predominantly caused by *Candida albicans*, and commonly affects the tongue, hard palate and buccal mucosa, often coexisting with angular cheilitis, erythema and mucosal bleeding. Predisposing factors include local and systemic immunosuppression, xerostomia, prolonged use of antibiotics or corticosteroids, immunosuppressive agents, poor denture hygiene, high-sugar diets, and tobacco use.^{29,36}

Diagnostic confirmation is achieved through clinical evaluation supported by microbiological testing, including fungal cultures.^{26,33,36,55} Although a Cochrane meta-analysis reported insufficient evidence to definitively support or oppose the use of antifungal agents in cancer-associated candidiasis,⁷ treatment typically begins with topical antifungal agents, such as nystatin (suspension or lozenges), clotrimazole or miconazole. In refractory or systemic cases, systemic antifungal agents, such as fluconazole or itraconazole, may be necessary. Adjunctive management includes meticulous oral hygiene, mechanical tongue cleaning and antiseptic rinses, such as chlorhexidine or sodium bicarbonate solutions.

The principal recommendations are summarized in the Table 5.

Denture stomatitis

In cases of denture stomatitis resistant to conventional therapies, tea tree oil (*Melaleuca alternifolia*), due to its antifungal properties, may be used alongside the COE-COMFORT™ tissue conditioner. The microwave disinfection of complete dentures is another effective adjunctive method. If these measures fail, temporary discontinuation or replacement of the denture is recommended. Chronic

or recurrent oral candidiasis should prompt medical evaluation to reassess diagnosis and modify treatment accordingly.^{26,33,36,54,55}

Osteoradionecrosis

Osteoradionecrosis (ORN) is a serious complication of HNRT, characterized by radiation-induced hypoxia, hypocellularity, and reduced vascularity within osseous tissues.³⁶ These alterations impair tissue regeneration and compromise mucosal integrity, thereby predisposing patients to bone necrosis and sequestration.⁷ The mandible is the most frequently affected site, owing to its dense bone structure and relatively limited blood supply.

Clinically, ORN presents with exposed necrotic bone, chronic pain, mucosal ulceration, fistula formation, and, in severe cases, pathological fractures, often in the absence of overt infection. Radiographic findings typically include diffuse radiolucencies with ill-defined borders and, in advanced stages, radiopaque sequestra.^{36,56} Osteoradionecrosis is commonly classified according to its clinical course, anatomical extent, severity of symptoms, and response to HBOT. It may significantly impair QoL, particularly in advanced cases.

Multiple risk factors contribute to the development of ORN, including poor oral hygiene, pre-radiotherapy dental or surgical procedures, high radiation doses or fraction sizes, tumor proximity to bone, and direct bone invasion. The condition is more prevalent in males over 55 years of age, particularly those with a history of tobacco and alcohol use. Although most cases occur within 3 years after radiotherapy, delayed onset is also possible. Prevention relies on comprehensive oral evaluation and the stabilization of oral health before and after radiotherapy. Reducing the need for invasive dental procedures and minimizing oral infections and inflammation are critical components of ORN prevention.⁵⁰ Management involves a combination of conservative and surgical strategies, including debridement, wound care, antibiotic therapy, and, when indicated, HBOT.⁸

The principal recommendations are summarized in the Table 6.

Medication-related osteonecrosis of the jaw

Medication-related osteonecrosis of the jaw (MRONJ) is associated with the antiresorptive agents (e.g., bisphosphonates and denosumab) and antiangiogenic therapies used in cancer care. These drugs impair osteoclast function, reduce bone turnover and increase the risk of osteonecrosis.⁵⁷ Medication-related osteonecrosis of the jaw can lead to progressive jawbone destruction and considerable morbidity, particularly with intravenous formulations used in cancer treatment as compared to oral formulations used for osteoporosis.

Table 5. Key recommendations for the management of oral candidiasis

No.	Recommendation	Description
1.	Identify forms	Distinguish between pseudomembranous (removable) and hyperplastic (non-removable) candidiasis
2.	Watch for symptoms	Monitor for burning, pain, dysphagia, dysgeusia, and mucosal bleeding
3.	Assess risk factors	They include immunosuppression, xerostomia, antibiotic use, poor hygiene, sugar intake, and smoking
4.	Diagnose accurately ^{53–55}	Use clinical examination and microbiological cultures if needed
5.	Treat promptly	Initiate topical antifungals; escalate to systemic treatment if necessary
6.	Support oral hygiene	Clean the tongue, use antiseptic rinses, maintain denture hygiene
7.	Prevent recurrence	Encourage denture care, sugar reduction, probiotic use, and smoking cessation
8.	Ensure follow-up	Schedule regular dental check-ups for early identification and management

Table 6. Key recommendations for the management of osteoradionecrosis (ORN)

No.	Recommendation	Description
1.	Conduct pre-radiotherapy dental assessment	Eliminate sources of infection and reduce the need for post-radiotherapy surgical procedures
2.	Stabilize oral health before and after radiotherapy	It helps prevent complications related to oral tissues and bone
3.	Identify high-risk patients	They include smokers, older males, and those receiving high radiation doses
4.	Monitor for symptoms	Look for exposed bone, persistent pain or non-healing oral ulcers
5.	Use radiographs ^{7,36,50}	Detect early bone changes and the presence of sequestra
6.	Start conservative treatment ^{36,50,56}	Begin with local wound care, antibiotics, and minimal surgical debridement
7.	Consider HBOT ^{36,50,56}	For cases that do not respond to conservative measures
8.	Educate patients	Emphasize oral hygiene, smoking cessation and other lifestyle modifications
9.	Coordinate multidisciplinary care	Involve dental, surgical and oncology teams for optimal management

HBOT – hyperbaric oxygen therapy.

Diagnostic criteria include a history of antiresorptive or antiangiogenic therapy, non-healing exposed bone in the maxillofacial region persisting for ≥ 8 weeks, the absence of previous radiotherapy to the jaws, and the absence of jaw metastases.⁵⁷ Evidence suggests that quarterly dental evaluations and preventive protocols, such as prophylactic antibiotics and primary soft-tissue closure following tooth extraction, may reduce the incidence of MRONJ more effectively than reactive care. The benefit of platelet-rich growth factor (PRGF) application following tooth extraction remains inconclusive.⁵⁸

Studies have shown no significant advantage of adding HBOT to standard MRONJ treatment, including antiseptics, antibiotics and surgery, highlighting the need for further research.⁵⁷ A comparative study by Mozzati et al. reported no cases of MRONJ with either minimally invasive or more invasive tooth extraction protocols in patients receiving oral bisphosphonates, suggesting that there is insufficient evidence to favor one surgical approach over the other.⁵⁹

Neurotoxicity

Chemotherapy-related neurotoxicity can result in persistent, diffuse pain localized to the head and neck region. This pain may mimic odontogenic pain, complicating diagnosis. Despite the presence of symptoms, clinical and radiographic examinations typically reveal no dental or mucosal abnormalities, although the thickening of the periodontal ligament (PDL) may be observed in vital teeth.²¹ Symptom

fluctuation and the absence of clear clinical findings present diagnostic challenges in dental settings.

Trismus

Trismus, defined as a restricted ability to open the mouth, is a common complication following oral cancer surgery and radiotherapy. It typically results from postoperative fibrosis and scar contracture, leading to a decreased interincisal distance, often measuring less than 35 mm between the maxillary and mandibular incisors.⁷

Trismus may develop early during radiotherapy, particularly in patients with tumors involving the nasopharynx, palate or maxillary sinuses. If left unaddressed, the condition can lead to a significant reduction in mandibular mobility, impairing essential functions, such as mastication, speech and oral hygiene.²² Reduced jaw opening is frequently observed approx. 9 weeks after the completion of radiotherapy. Denture-wearing patients are particularly affected, as trismus may prevent the proper insertion of prostheses and complicate the fabrication of new appliances because of restricted intraoral access.⁷

The management of trismus emphasizes the early initiation of therapeutic exercises, including jaw stretching and the use of assistive devices, such as bite dilators or tongue depressors, aimed at preserving the range of motion.⁵⁴ While short-term improvement can be achieved, long-term benefits require consistent adherence to these exercises. In chronic cases, the development of dense fibrotic tissue renders passive stretching less effective, underscoring the importance of early detection and intervention to prevent irreversible functional impairment.²²

The principal recommendations are summarized in the Table 7.

Table 7. Key recommendations for the management of trismus

No.	Recommendation	Description
1.	Monitor mouth opening ³	Assess mandibular mobility; trismus is defined as mouth opening <35 mm
2.	Recognize early functional impairment ^{3,9}	Reduced jaw opening may impair oral function and prosthetic use
3.	Initiate therapeutic exercises early ^{9,27}	Begin jaw exercises early to preserve the mandibular range of motion
4.	Use assistive stretching devices ²⁷	Bite dilators or tongue depressors may support stretching and the maintenance of mouth opening

The effective prevention and management of oral complications related to oncologic treatment require an interdisciplinary approach involving dental and medical professionals. Dental care is critical for reducing the risk of complications and enhancing therapeutic outcomes and QoL.

Pre-radiotherapy dental management is particularly important for patients with head and neck cancer. According

to the Canadian Dental Oncology Network (CDON) consensus guidelines, developed using a modified Delphi method, prophylactic tooth extraction may be considered at radiation dose thresholds of 70 Gy for the maxilla and 60 Gy for the mandible.⁶⁰ Management protocols are tailored to anatomical location and the radiation dose, with all patients – regardless of dentition – recommended for referral to a dental oncology clinic for pre-treatment evaluation. The recommended healing interval between tooth extraction and radiotherapy is 7–14 days.⁶⁰ Risk assessment should consider factors such as infectious foci, the oral health status, the type of tumor, and the overall prognosis. Fluoride prophylaxis, oral hygiene education and routine dental follow-up are essential components of comprehensive care aimed at reducing post-radiotherapy complications.^{19,60}

Following anticancer treatment, including chemotherapy, long-term dental care remains essential for controlling infection, restoring function and improving esthetics. Treatment planning should be conducted collaboratively with oncologists.^{21,61} Key elements include consistent daily oral hygiene under the supervision of a dentist or dental hygienist, as well as routine follow-up visits – monthly for the first 3 months, every 3 months during the first year, and every 6 months thereafter for at least 3 years.³⁴ Dental prophylaxis involves the regular removal of plaque and calculus,^{33,36,51} and caries prevention through the use of high-fluoride toothpaste, chlorhexidine rinses (for at least 3 weeks every 3 months) and routine fluoride applications.²⁹

Fluoride prophylaxis is especially critical for patients after radiotherapy to prevent radiation-induced caries. Recommended strategies include:

- high-fluoride toothpaste (5,000 ppm F⁻): daily use to promote remineralization;
- sodium fluoride rinses: 0.05% daily or 0.2% weekly;
- 5% sodium fluoride varnish: every 3 months, particularly in patients with xerostomia;
- 1.23% fluoride gel: applied using trays by a dentist or the patient.³⁶

Xylitol-containing products are especially recommended, as xylitol has been shown to inhibit the growth of *S. mutans*. Patients should be advised to choose water over sugary or acidic beverages, as acidic drinks can contribute to irreversible dental erosion. Additionally, frequent vomiting, commonly associated with cytostatic therapy, may further contribute to enamel degradation through repeated exposure to gastric acid.³³

Post-chemotherapy procedures and prosthodontics

After chemotherapy, treatment-related side effects should be appropriately managed, and regular dental follow-up is particularly important during the first few months after treatment. Invasive procedures, such as tooth extractions or oral surgery, should be delayed for at least

1 year when clinically feasible. If urgent intervention is required, antibiotic prophylaxis should be initiated 48 h before the procedure and continued for 7–15 days afterward. Hyperbaric oxygen therapy may also be considered to reduce the risk of complications.²⁹

The use of dentures should generally be avoided for 1 year after treatment. If necessary, prosthetic rehabilitation should be postponed for 4–6 months following chemotherapy. Special caution is advised in patients treated with bisphosphonates, particularly intravenous formulations, as implant placement carries a risk of MRONJ.⁶¹ Multidisciplinary care is crucial for managing oral complications and improving patients' QoL.

Orthodontic considerations in oncology patients

Orthodontic treatment decisions should account for the patient's general health, caries risk and adherence to oral hygiene practices.⁶² Treatment planning should consider the applied forces, the anticipated treatment duration and the pre-existing root damage, which may be exacerbated during therapy.⁶³ In patients receiving antiresorptive medications, tooth extractions should be approached with caution because of the risk of MRONJ, which has been reported to range from 0.2% to 6.7%.⁶⁴ Bisphosphonates may impair tooth movement and prolong treatment duration. Orthodontic treatment is generally contraindicated during and shortly after intravenous bisphosphonate therapy.

Given the adverse effects of oncologic therapies, such as mandibular retrognathia, reduced craniofacial dimensions and increased tooth mobility, orthodontic treatment should be simplified. Lower-force mechanics are recommended to minimize the risk of root resorption, and mandibular treatment should be avoided where possible.⁶⁵

Orthodontic treatment may be safely resumed 2 years after the completion of chemotherapy or radiotherapy, owing to prolonged alterations in bone metabolism and potential treatment-related discomfort, including nausea, xerostomia, taste alterations, and mucosal sensitivity. However, when treatment has been limited to surgery alone without adjunctive therapies, there is no requirement to delay orthodontic intervention.⁶⁶

Teeth whitening is generally safe when performed correctly, but may cause transient side effects, such as dental hypersensitivity and soft-tissue irritation. Therefore, whitening procedures should be approached cautiously, particularly in post-oncological patients. Patient eligibility should be based on a thorough clinical evaluation of the caries status, non-caries lesions, periodontal health, xerostomia, soft-tissue condition, and tooth sensitivity. To minimize potential risks, lower concentrations of carbamide peroxide applied over longer periods are preferred over high-concentration hydrogen peroxide products.⁶⁷

Immunotherapy-related oral complications and their management

Immune-related adverse events (irAEs) primarily affect the oral mucosa and the salivary glands, often leading to severe symptoms. Mucosal changes may present as lichenoid lesions, erosions and ulcers, accompanied by intense pain and hypersensitivity that can impair oral intake and contribute to malnutrition. Salivary gland dysfunction is also common, presenting as xerostomia, dysphagia, and difficulties with speaking, often accompanied by thick or sticky saliva. Among these side effects, xerostomia is the most frequently reported and typically develops early during treatment. Mucosal disturbances, including inflammation, ulceration and pain – such as those associated with lichenoid and vesiculobullous reactions – generally develop over the following months. Dysgeusia occurs less frequently, but may also develop early and can significantly affect daily functioning. Most oral irAEs arise within the first months of immunotherapy. Due to their chronic nature, symptoms such as dry mouth, taste disturbances and mucosal disease can substantially impair QoL, limiting food intake, speech and sleep.^{68–70}

The early initiation of treatment is crucial to avoid the interruption of anticancer therapy. Standard management includes systemic corticosteroids (e.g., prednisone 40–60 mg/day) and topical steroids, such as dexamethasone 0.05% rinse or clobetasol 0.05% gel, often used at higher-than-standard concentrations. Adjunctive approaches include PBM, which can effectively alleviate mucosal pain, and, in cases of salivary gland dysfunction, sialogogues (e.g., pilocarpine), moisturizing agents, and intraductal salivary gland irrigation, which can significantly improve the salivary flow.⁶⁸

The rapid identification and comprehensive management of these complications are essential for maintaining the continuity of immunotherapy and improving patients' QoL.

The key recommendations addressing the complications discussed above throughout the manuscript are presented in Table 8.

Limitations

This review is subject to several limitations. As a narrative review, it does not follow a systematic methodology for comprehensive evidence synthesis, which may introduce selection bias. The available evidence is characterized by substantial heterogeneity, including differences in study design, patient populations, cancer types, treatment modalities, radiation doses, outcome definitions, and follow-up duration. This heterogeneity limits the direct comparability of findings and may contribute to inconsistent or conflicting results.

Despite advances in supportive oral care, several aspects of post-cancer dental management remain unresolved,

reflecting broader limitations in the current evidence base. In particular, the routine use of chlorhexidine for OM prevention remains controversial; although effective for plaque control and infection prevention, international guidelines do not support its prophylactic use for mucositis, and some studies report increased mucosal irritation. Similarly, the long-term use of high-concentration fluoride in patients with severe xerostomia remains debated. While fluoride prophylaxis is essential for preventing radiation-induced caries, a reduced salivary flow and the impairment of the salivary film may increase mucosal sensitivity, and current guidelines provide limited guidance regarding optimal formulations and dosing in this setting.

Moreover, many of the included studies focus on specific cancer types or treatment settings, which may limit the generalizability of their findings to the broader population of cancer survivors. The relative scarcity of long-term follow-up data – particularly regarding chronic oral complications, implant outcomes and the emerging therapies – further limits conclusions about sustained effectiveness and late adverse effects.

Finally, this review was limited to English-language publications. Given the rapidly evolving nature of oncologic therapies and dental management strategies, new evidence may emerge after the completion of this review, potentially influencing future recommendations.

Conclusions

The long-term management of oral health in cancer survivors is a critical component of multidisciplinary oncology care. As cancer therapies advance and survival rates increase, addressing the persistent and often debilitating oral complications of treatment becomes increasingly important. These complications include OM, xerostomia, dysgeusia, radiation-induced caries, ORN, candidiasis, and functional impairment, such as trismus and dysphagia.

Comprehensive dental care, integrated into cancer management, plays a key role in mitigating these complications. Early dental intervention – particularly before radiotherapy – is essential for risk assessment, preventive care, and reducing post-treatment morbidity. Continued surveillance and personalized strategies, such as high-fluoride therapy, salivary stimulation, nutritional guidance, and patient education, are vital for preserving oral function and QoL.

When interpreting the available clinical guidelines, it becomes clear that recommendations for post-treatment oral care in cancer survivors remain heterogeneous and address different yet complementary aspects of management. The CDON guidelines, although primarily focused on pre-radiotherapy decision-making, acknowledge that patients who have undergone radiotherapy remain at increased long-term risk of dental complications, including radiation-induced caries and ORN.^{60,71}

The MASCC/ISOO and European Society for Medical Oncology (ESMO) guidelines focus predominantly

Table 8. Comparative summary of the key recommendations for oncology-related oral complications

Condition	Main preventive measures	Primary management strategies	Key pharmacological/therapeutic options	Critical clinical notes	References
OM	pre-treatment oral assessment; PBM/LLLT prophylaxis; avoid trauma; risk-factor control	basic oral care; pain and nutritional support; weekly LLLT	doxepin; methylene blue; viscous lidocaine; benzydamine (RT <50 Gy); cryotherapy; Magic Mouthwash	chlorhexidine not recommended; onset 7–14 days post-chemotherapy	7,13,21–31
Xerostomia/hyposalivation	minimize the RT dose; hydration ≥ 1.5 L/day; avoid alcohol/caffeine/acids; xylitol	saliva stimulation and lubrication; substitutes; humidifiers	pilocarpine; cevimeline; sodium hyaluronate (low-molecular-weight HA); plant oils; bethanechol	irreversible glandular injury at RT >30 Gy; early onset after a few 2-Gy fractions	7,26,32–46
Taste alterations/dysgeusia	maintain the salivary flow; diet counseling; hygiene	supportive care; slow chewing; alternate foods	zinc; vitamin D	dose-dependent (occurs at ~ 30 Gy); recovery at 2–4 months	7,17,21,22,26,47,48
Radiation-induced caries	daily hygiene; diet counseling; lifelong fluoride in xerostomia	remineralization; quarterly visits	5,000 ppm F ⁻ ; trays; sodium fluoride rinses, varnish; CPP-ACP; hydroxyapatite; xylitol	may start within weeks; atypical surfaces; fast recurrence	22,26,29,33,50–52
Oral candidiasis	reduce xerostomia; denture hygiene; low sugar intake	2-week course of antifungals; antiseptic rinses; tongue scraping	nystatin; clotrimazole; miconazole; fluconazole; itraconazole	hyperplastic form mimics leukoplakia	7,26,29,33,36,53–55
Denture stomatitis	denture hygiene; avoid nighttime wear	tissue conditioners; disinfection	tea tree oil and COE-COMFORT; microwave disinfection	replace dentures if persistent	26,33,36,54,55
ORN	pre-RT dental clearance; avoid post-RT invasive procedures	conservative care; infection control; debridement	antibiotics; selective HBOT	the mandible most affected; exposed bone; fistulae	7,36,50,56
MRONJ	quarterly visits; prophylactic antibiotics; soft-tissue closure	antiseptics; antibiotics; surgery	analgesics; antimicrobials; PRGF inconclusive	caused by antiresorptive agents; no benefit from HBOT	57–59
Neurotoxicity	awareness during treatment	supportive care	analgesics	mimics odontogenic pain; PDL thickening possible	21
Trismus	early therapy during RT; patient education	stretching; bite blocks; tongue depressors	physical therapy devices	onset ~ 9 weeks post-RT; early intervention essential	7,22,54
Post-treatment dental care	pre-RT extractions (7–14 days ahead); follow-up schedule	fluoride prophylaxis; caries and infection control	high-fluoride toothpaste; varnish; chlorhexidine cycles; xylitol	dental oncology referral mandatory	19,21,29,34,36,60–61
Orthodontic care	evaluate hygiene, caries risk, a systemic history	low-force mechanics; avoid mandibular treatment	–	delay for 2 years post-chemotherapy/RT; contraindicated with IV bisphosphonates	62–67
irAEs	early recognition; mucosal/saliva monitoring	systemic/topical steroids; PBM; sialogogues	prednisone; dexamethasone rinse; clobetasol gel; pilocarpine	xerostomia common; lichenoid/vesiculobullous lesions; chronic	68–70

OM – oral mucositis; ORN – osteoradionecrosis; MRONJ – medication-related osteonecrosis of the jaw; irAEs – immune-related adverse events; LLLT – low-level laser therapy; RT – radiotherapy; HA – hyaluronic acid; CPP-ACP – casein phosphopeptide–amorphous calcium phosphate; PRGF – platelet-rich growth factor; PDL – periodontal ligament.

on the prevention and management of acute oral complications during active treatment, with limited guidance on structured dental follow-up after treatment completion. Consequently, practical recommendations for the prevention and management of chronic oral sequelae in cancer survivors remain largely underdeveloped.^{72,73}

In contrast, the NCCN Head and Neck Cancer guidelines integrate oral health into survivorship care, particularly for patients treated with radiotherapy. They emphasize regular dental evaluations, intensified preventive strategies, and the careful planning of invasive dental procedures

in previously irradiated tissues, thereby recognizing the persistent and cumulative nature of treatment-related oral morbidity.⁷⁴

This discrepancy reflects a broader gap between the guidelines addressing acute treatment-related toxicity and the long-term oral healthcare needs of cancer survivors, underscoring the need for dedicated, survivorship-focused dental care recommendations to support coordinated, long-term oral health management. The comparison of key recommendations across major guideline frameworks is summarized in Table 9.

Table 9. Comparison of the key recommendations across major guideline frameworks

Condition/topic	CDON guidelines	MASCC/ISOO guidelines	NCCN (Head & Neck) guidelines
Primary scope	dental management prior to RT (extractions, timing, ORN risk reduction) ⁶⁰	OM prevention and management, including PBM ^{72,73}	comprehensive head and neck cancer care, including survivorship oral health ⁷⁴
OM – general approach	indirectly addressed through pre-RT dental care optimization ⁶⁰	central focus; recommendations depend on clinical settings and evidence strength ^{72,73}	addressed within supportive care; less detailed than MASCC/ISOO ⁷⁴
Chlorhexidine for OM prevention	outside the main scope ⁶⁰	not recommended for routine OM prevention; may be used for plaque control ^{13,72}	discussed mainly for oral hygiene, not OM prevention ⁷⁴
Palifermin in OM	outside the scope ⁶⁰	supported in selected clinical settings; not for universal use ^{7,72}	may be considered depending on settings; not standard for all patients ⁷⁴
PBM	outside the scope ⁶⁰	specifically recommended for OM prevention and management ⁷³	may be considered depending on institutional practice ⁷⁴
Xerostomia/hyposalivation	indirectly addressed as a caries risk factor ⁶⁰	not the primary focus; indirect references only ⁷²	explicitly addressed in survivorship care and long-term follow-up ^{71,74}
ORN prevention (pre-RT extractions)	the core recommendation with extraction thresholds ⁶⁰	outside the scope ^{72,73}	emphasize the careful planning of invasive procedures post-RT ^{50,74}
ORN management/HBOT	not the central focus; evidence variable ⁶⁰	outside the scope ^{72,73}	may be considered in selected cases; evidence inconsistent ^{50,74}

CDON – Canadian Dental Oncology Network; MASCC/ISOO – Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology; NCCN – National Comprehensive Cancer Network.

Taken together, these documents further highlight the absence of a unified, interdisciplinary, long-term oral care protocol for patients treated for head and neck cancer. Moreover, substantial gaps persist in the existing evidence base for dental care in oncology patients, including the limited availability of high-quality studies on PBM and the low level of the evidence supporting complementary interventions, such as acupuncture or omega-3 acid supplementation. The effectiveness of xerostomia treatment (e.g., pilocarpine and bethanechol) is only moderate, and the lack of standardized dosing protocols makes comparisons difficult. Regenerative therapies using stem cells are still in early research stages, and AI models require larger, clinically validated studies. Clear evidence is also lacking for the efficacy of HBOT, TENS, and other supportive methods. Data on managing dysgeusia (e.g., zinc supplementation), preventing radiation-induced caries (probiotics, plant oils, fluoride regimens) and treating oral candidiasis remain insufficient. The management of ORN and MRONJ likewise requires more robust comparative studies. Additionally, research linking the microbiota status to treatment-related complications is scarce, and many preventive recommendations rely on low-quality evidence.

Future research should aim to establish standardized, evidence-based dental care protocols specifically tailored to patients undergoing oncologic treatment. Priority areas include evaluating the cost-effectiveness of preventive interventions, determining the long-term outcomes of salivary gland preservation strategies, and assessing how different oral care approaches influence treatment tolerance and post-treatment recovery. Further studies are also needed to refine minimally invasive techniques, optimize radiotherapy planning to protect oral structures,

and explore novel agents that support mucosal healing and salivary function. Strengthening the evidence base in these domains will help guide more consistent clinical practice, and ultimately improve the QoL of cancer patients.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Zhang J, Ke Y, Chen C, et al. HPV cancer burden by anatomical site, country, and region in 2022. *Sci Rep.* 2025;15(1):21048. doi:10.1038/s41598-025-06700-8
2. Dai R, Zhang Y, Zou H, Li H, Zhang S. Increased burden of lip, oral, and pharyngeal cancer in adolescents and young adults from 1990 to 2021. *BMC Oral Health.* 2025;25(1):758. doi:10.1186/s12903-025-06166-0

3. Proc P, Mlynarski W, Hardan L, Bourgi R, Cuevas-Suárez CE, Lukomska-Szymanska M. Prevalence of tooth agenesis in young cancer patients: A systematic review and meta-analysis. *Dent Med Probl.* 2025;62(4):739–750. doi:10.17219/dmp/176033
4. Bertl K, Savvidis P, Kukla EB, et al. Including dental professionals in the multidisciplinary treatment team of head and neck cancer patients improves long-term oral health status. *Clin Oral Investig.* 2022;26(3):2937–2948. doi:10.1007/s00784-021-04276-x
5. Banthia R, Jain P, Jain AK, Belludi SA, Agarwal N, Patidar M. Evaluation of the association between periodontal disease and total cancer risk: A cross-sectional study. *Dent Med Probl.* 2024;61(6):843–850. doi:10.17219/dmp/175001
6. Ammajan RR, Joseph R, Rajeev R, Choudhary K, Vidhyadharan K. Assessment of periodontal changes in patients undergoing radiotherapy for head and neck malignancy: A hospital-based study. *J Cancer Res Ther.* 2013;9(4):630–637. doi:10.4103/0973-1482.126461
7. Wong HM. Oral complications and management strategies for patients undergoing cancer therapy. *ScientificWorldJournal.* 2014;2014:581795. doi:10.1155/2014/581795
8. Fonsêca TC, Jural LA, Marañón-Vásquez GA. Global prevalence of human papillomavirus-related oral and oropharyngeal squamous cell carcinomas: A systematic review and meta-analysis. *Clin Oral Investig.* 2023;28(1):62. doi:10.1007/s00784-023-05425-0
9. Lu Y, Xie Z, Luo G, et al. Global burden of oropharyngeal cancer attributable to human papillomavirus by anatomical subsite and geographic region. *Cancer Epidemiol.* 2022;78:102140. doi:10.1016/j.canep.2022.102140
10. Damgacioglu H, Sonawane K, Zhu Y, et al. Oropharyngeal cancer incidence and mortality trends in all 50 states in the US, 2001–2017. *JAMA Otolaryngol Head Neck Surg.* 2022;148(2):155–165. doi:10.1001/jamaoto.2021.3567
11. Lechner M, Liu J, Masterson L, Fenton TR. HPV-associated oropharyngeal cancer: Epidemiology, molecular biology and clinical management. *Nat Rev Clin Oncol.* 2022;19(5):306–327. doi:10.1038/s41571-022-00603-7
12. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol.* 2011;29(32):4294–4301. doi:10.1200/JCO.2011.36.4596
13. McGuire DB, Fulton JS, Park J, et al.; Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO). Systematic review of basic oral care for the management of oral mucositis in cancer patients. *Support Care Cancer.* 2013;21(11):3165–3177. doi:10.1007/s00520-013-1942-0
14. Hartnett E. Integrating oral health throughout cancer care. *Clin J Oncol Nurs.* 2015;19(5):615–619. doi:10.1188/15.CJON.615-619
15. 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
16. Sayed ME, Mugri MH, Almasri MA, et al. Role of stem cells in augmenting dental implant osseointegration: A systematic review. *Coatings.* 2021;11(9):1035. doi:10.3390/coatings11091035
17. Printz C. Chemotherapy-induced taste changes affect nutrition, quality of life. *CA Cancer J Clin.* 2025;75(4):271–273. doi:10.3322/caac.70022
18. Matsuda Y, Jayasinghe RD, Zhong H, Arakawa S, Kanno T. Oral health management and rehabilitation for patients with oral cancer: A narrative review. *Healthcare (Basel).* 2022;10(5):960. doi:10.3390/healthcare10050960
19. Beristain-Colorado MdP, Castro-Gutiérrez MEM, Torres-Rosas R, et al. Application of neural networks for the detection of oral cancer: A systematic review. *Dent Med Probl.* 2024;61(1):121–128. doi:10.17219/dmp/159871
20. Chan J, Filippi A, Filippi C. Clinical guidance for maintaining oral hygiene in patients undergoing chemotherapy or radiation therapy: A scoping review. *Swiss Dent J.* 2023;133(6):368–379. doi:10.61872/sdj-2023-06-01
21. Parra-Rojas S, Velázquez-Cayón RT, Borges-Gil A, Mejías-Torres JL, Cassol-Spanemberg J. Oral complications and management strategies for cancer patients: Principles of supportive oncology in dentistry. *Curr Oncol Rep.* 2024;26(4):391–399. doi:10.1007/s11912-024-01518-5
22. Devi MS, Ahmed A. Oral manifestation of post cancer therapy. *J Dent Spec.* 2021;9(2):53–56. doi:10.18231/j.jds.2021.014
23. Padure A, Horhat R, Talpos-Niculescu IC, et al. Oral mucositis in adult cancer patients undergoing chemotherapy: Six-month on-treatment follow-up. *J Clin Med.* 2024;13(19):5723. doi:10.3390/jcm13195723
24. Hajisalem T, Ghaffary S, Nejati B, et al. Effect of *Achillea millefolium* mouthwash on oral mucositis induced by chemotherapy in AML patients. *Jundishapur J Nat Pharm Prod.* 2019;14(1):e14077. doi:10.5812/jjnpp.14077
25. Napeñas JJ, Brennan MT, Bahrani-Mougeot FK, Fox PC, Lockhart PB. Relationship between mucositis and changes in oral microflora during cancer chemotherapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007;103(1):48–59. doi:10.1016/j.tripleo.2005.12.016
26. de Barros da Cunha SR, Mendes Ramos PA, Aló Nesrallah AC, Parahyba CJ, Fregnani ER, Corrêa Aranha AC. The effects of ionizing radiation on the oral cavity. *J Contemp Dent Pract.* 2015;16(8):679–687. doi:10.5005/jp-journals-10024-1740
27. Roldan CJ, Huh B, Song J, et al. Methylene blue for intractable pain from oral mucositis related to cancer treatment: A randomized phase 2 clinical trial. *BMC Med.* 2022;20(1):377. doi:10.1186/s12916-022-02579-8
28. Leenstra JL, Miller RC, Qin R, et al. Dopepin rinse versus placebo in the treatment of acute oral mucositis pain in patients receiving head and neck radiotherapy with or without chemotherapy: A phase III, randomized, double-blind trial (NCCTG-N09C6 [Alliance]). *J Clin Oncol.* 2014;32(15):1571–1577. doi:10.1200/JCO.2013.53.2630
29. Borowski G, Bakula P, Zasadzńska M, et al. Oral mucositis – characteristics and management of a common complication of cancer treatment [in Polish]. *Palliat Med.* 2024;16(4):221–230. doi:10.5114/pm.2024.142027
30. Jasiński M, Maciejewska M, Brodziak A, et al. Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation. *Sci Rep.* 2021;11(1):22507. doi:10.1038/s41598-021-02002-x
31. Dambrós BF, Kobus RA, Horta de Lima RC, et al. Effect of adapted ice cream on nutritional status and oral mucositis in cancer patients undergoing chemotherapy: Protocol study for a randomized controlled trial. *Clin Nutr ESPEN.* 2025;66:160–168. doi:10.1016/j.clnesp.2025.01.037
32. Doctor R, Padhya T, Mifsud M, Nickel C. A systematic review of approaches to dental care in head and neck cancer patients. *Oral Oncol Rep.* 2024;9:100205. doi:10.1016/j.oor.2024.100205
33. Meurman JH, Grönroos L. Oral and dental health care of oral cancer patients: Hyposalivation, caries and infections. *Oral Oncol.* 2010;46(6):464–467. doi:10.1016/j.oraloncology.2010.02.025
34. Kajornwongwattana W, Sanguansin N, Songsak T, Vuddhakanok S, Thanakun S. Comparative mucosal wetting capacity of novel and commercial saliva substitute formulations: An in vitro study. *Clin Cosmet Investig Dent.* 2025;17:159–167. doi:10.2147/CCIDE.S514583
35. Hosseini MS, Sanaie S, Mahmoodpoor A, et al. Cancer treatment-related xerostomia: Basics, therapeutics, and future perspectives. *Eur J Med Res.* 2024;29(1):571. doi:10.1186/s40001-024-02167-x
36. Czerzyńska M, Orłow P, Choromańska M. Adverse reactions of radiotherapy for head and neck cancers. Treatment of radiation reactions in the oral cavity [in Polish]. *Pediatr Med Rodz.* 2017;13(1):53–62. doi:10.15557/PiMR.2017.0005
37. Britto Barros JV, de Lima Barros SA, Carvalho de Almeida W, de Sousa Pereira RM. World overview of xerostomia therapeutics: Clinical trials analysis. *IOSR J Dent Med Sci.* 2020;19(1):59–62. doi:10.9790/0853-1901045962
38. Kapourani A, Kontogiannopoulos KN, Manioudaki AE, et al. A review on xerostomia and its various management strategies: The role of advanced polymeric materials in the treatment approaches. *Polymers (Basel).* 2022;14(5):850. doi:10.3390/polym14050850
39. Kapourani A, Kontogiannopoulos KN, Barmplexis P. A review on the role of pilocarpine on the management of xerostomia and the importance of the topical administration systems development. *Pharmaceuticals (Basel).* 2022;15(6):762. doi:10.3390/ph15060762
40. Solarska A, Żylicz Z. Recent advances in the prevention and treatment of post-radiotherapy xerostomia in patients with head and neck cancer. *Palliat Med Pract.* 2023;17(1):39–47. doi:10.5603/PMPL.a2022.0026
41. Austin W, Hdeib M, Fraser P, et al. Oral lubrication, xerostomia, and advanced macromolecular lubricants for treatment of dry mouth. *Lubricants.* 2024;12(4):126. doi:10.3390/lubricants12040126

42. Ludwar L, Mannel H, Hamacher S, Noack MJ, Barbe AG. Oil pulling to relieve medication-induced xerostomia: A randomized, single-blind, crossover trial. *Oral Dis*. 2022;28(2):373–383. doi:10.1111/odi.13752
43. Rupe C, Basco A, Gioio G, et al. Sodium-hyaluronate mouthwash on radiotherapy-induced xerostomia: A randomised clinical trial. *Support Care Cancer*. 2023;31(12):644. doi:10.1007/s00520-023-08090-x
44. Assy Z, Jager DH, Brand HS, Bikker FJ. Salivary film thickness and MUC5B levels at various intra-oral surfaces. *Clin Oral Investig*. 2023;27(2):859–869. doi:10.1007/s00784-022-04626-3
45. Hu J, Andablo-Reyes E, Mighell A, Pavitt S, Sarkar A (2021) Dry mouth diagnosis and saliva substitutes – a review from a textural perspective. *J Texture Stud*. 2021;52(1):141–156. doi:10.1111/jtxs.12575
46. Pedersen A, Sørensen CE, Proctor GB, Carpenter GH. Salivary functions in mastication, taste and textural perception, swallowing and initial digestion. *Oral Dis*. 2018;24(8):1399–1416. doi:10.1111/odi.12867
47. Kawashita Y, Soutome S, Umeda M, Saito T. Oral management strategies for radiotherapy of head and neck cancer. *Jpn Dent Sci Rev*. 2020;56(1):62–67. doi:10.1016/j.jdsr.2020.02.001
48. Agare GI, Chidike Ezeorba TP, Michael DC, Agbam E, Aghoja OC, Alalor CA. Zinc supplementation for mitigating oral mucositis in head and neck cancer patients undergoing radiotherapy and chemo-radiotherapy – a systematic review. *Clin Nutr ESPEN*. 2025;67:8–24. doi:10.1016/j.clnesp.2025.02.011
49. Minervini G, Shivakumar S, Ronsivalle V, Franco R, Cicciù M, Marra-podi MM. Microbiological aspects of cancer progression: A systematic review conducted according to the PRISMA 2020 guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. *Dent Med Probl*. 2024;61(5):739–746. doi:10.17219/dmp/183712
50. Sroussi HY, Epstein JB, Bensadoun RJ, et al. Common oral complications of head and neck cancer radiation therapy: Mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. *Cancer Med*. 2017;6(12):2918–2931. doi:10.1002/cam4.1221
51. Irie MS, Mendes EM, Borges JS, Osuna LG, Rabelo GD, Soares PB. Periodontal therapy for patients before and after radiotherapy: A review of the literature and topics of interest for clinicians. *Med Oral Patol Oral Cir Bucal*. 2018;23(5):e524–e530. doi:10.4317/medoral.22474
52. Bhandari S, Soni BW, Jamwal A, Ghoshal S. Oral and dental care before radiotherapy: Guidelines and development of a time-bound protocol. *Indian J Cancer*. 2022;59(2):159–169. doi:10.4103/ijc.IJC_871_20
53. Al-Manei K, Sobkowiak MJ, Nagadia RH, Heymann R, Chen MS, Özenci V. Mycobiota profile of oral fungal infections in head and neck cancer patients receiving radiotherapy: A 6-year retrospective MALDI-TOF mass spectrometry study. *Oral Oncol*. 2023;146:106556. doi:10.1016/j.oraloncology.2023.106556
54. Rathee M, Bhorla M, Dua M. Multidisciplinary oral management in cancer therapy. Part III: Post cancer treatment. *Int J Sci Basic Appl Res*. 2015;22(2):412–417. <https://gssrr.org/JournalOfBasicAndApplied/article/view/2498>.
55. Lalla RV, Latortue MC, Hong CH, et al.; Fungal Infections Section, Oral Care Study Group, Multinational Association of Supportive Care in Cancer (MASCC)/International Society of Oral Oncology (ISOO). A systematic review of oral fungal infections in patients receiving cancer therapy. *Support Care Cancer*. 2010;18(8):985–992. doi:10.1007/s00520-010-0892-z
56. Albuquerque RA, Morais VL, Sobral AP. Odontologic protocol of attendance the pediatric oncology patients: Review of literature [in Portuguese]. *Rev Odontol UNESP*. 2007;36(3):275–280.
57. Beth-Tasdogan NH, Mayer B, Hussein H, Zolk O. Interventions for managing medication-related osteonecrosis of the jaw. *Cochrane Database Syst Rev*. 2017;10(10):CD012432. doi:10.1002/14651858.CD012432.pub2
58. Mücke T, Deppe H, Hein J, et al. Prevention of bisphosphonate-related osteonecrosis of the jaws in patients with prostate cancer treated with zoledronic acid – a prospective study over 6 years. *J Craniomaxillofac Surg*. 2016;44(10):1689–1693. doi:10.1016/j.jcms.2016.07.026
59. Mozzati M, Arata V, Gallesio G. Tooth extraction in osteoporotic patients taking oral bisphosphonates. *Osteoporos Int*. 2013;24(5):1707–1712. doi:10.1007/s00198-012-2239-8
60. Watson E, Mojdami ZD, Oladega A, Hope A, Glogauer M; Canadian Dental Oncology Network Consensus Group. Clinical practice guidelines for dental management prior to radiation for head and neck cancer. *Oral Oncol*. 2021;123:105604. doi:10.1016/j.oraloncology.2021.105604
61. Pouloupoulos A, Papadopoulos P, Andreadis D. Chemotherapy: Oral side effects and dental interventions – a review of the literature. *Stomatological Dis Sci*. 2017;1:35–49. doi:10.20517/2573-0002.2017.03
62. Wishney M. Potential risks of orthodontic therapy: A critical review and conceptual framework. *Aust Dent J*. 2017;62(Suppl 1):86–96. doi:10.1111/adj.12486
63. Gawade PL, Hudson MM, Kaste SC. A systematic review of dental late effects in survivors of childhood cancer. *Pediatr Blood Cancer*. 2014;61(3):407–416. doi:10.1002/pbc.24842
64. Lotwala RB, Greenlee GM, Ott SM, Hall SH, Huang GJ. Bisphosphonates as a risk factor for adverse orthodontic outcomes: A retrospective cohort study. *Am J Orthod Dentofacial Orthop*. 2012;142(5):625–634.e3. doi:10.1016/j.ajodo.2012.05.019
65. Boyer É, Robert G, Gandemer V, Bonnaure-Mallet M. Orthodontic strategies in pediatric oncology. *J Dentofacial Anom Orthod*. 2017;20(1):104. doi:10.1051/odfen/2016035
66. Michalak I, Kuśmierczyk D, Bluj-Komarnitka K, Rayad S, Zadurska M. Radiological imaging and orthodontic treatment in the case of growing patients after oncological treatment: Case reports. *Dent Med Probl*. 2019;56(2):209–215. doi:10.17219/dmp/105958
67. Klarić Sever E, Tarle A, Soče M, Grego T. Direct radiotherapy-induced effects on dental hard tissue in combination with bleaching procedure. *Front Dent Med*. 2021;2:714400. doi:10.3389/fdmed.2021.714400
68. Elad S, Yarom N, Zadik Y. Immunotherapy-related oral adverse effects: Immediate sequelae, chronicity and secondary cancer. *Cancers (Basel)*. 2023;15(19):4781. doi:10.3390/cancers15194781
69. Nicolatou-Galitis O, Psyrri A, Tsoukalas N, et al. Oral toxicities in cancer patients, who receive immunotherapy: A case series of 24 patients. *Oral*. 2023;3(1):123–133. doi:10.3390/oral3010011
70. Xu Y, Wen N, Sonis ST, Villa A. Oral side effects of immune checkpoint inhibitor therapy (ICIT): An analysis of 4683 patients receiving ICIT for malignancies at Massachusetts General Hospital, Brigham and Women's Hospital, and the Dana-Farber Cancer Institute, 2011 to 2019. *Cancer*. 2012;127(11):1796–1804. doi:10.1002/cncr.33436
71. Samim F, Epstein JB, Zumsteg ZS, Ho AS, Barasch A. Oral and dental health in head and neck cancer survivors. *Cancers Head Neck*. 2016;1:14. doi:10.1186/s41199-016-0015-8
72. Elad S, Cheng KKF, Lalla RV, et al.; Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer*. 2020;126(19):4423–4431. doi:10.1002/cncr.33100
73. Zadik Y, Arany PR, Fregnani ER, et al.; Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO). Systematic review of photobiomodulation for the management of oral mucositis in cancer patients and clinical practice guidelines. *Support Care Cancer*. 2019;27(10):3969–3983. doi:10.1007/s00520-019-04890-2
74. Dimitrios Colevas A, Cmelak AJ, Pfister DG, et al. NCCN Guidelines® insights: Head and Neck Cancers, version 2.2025. *J Natl Compr Canc Netw*. 2025;23(2):2–11. doi:10.6004/jnccn.2025.0007

Minimally invasive indirect adhesive restorations for complex cavities: A literature review and comprehensive guide for clinical success

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Abstract

A complex cavity is so named because it results from damage to the enamel–dentin complex, and such cavities can cause patients considerable difficulties in daily life. The reconstruction of damaged areas with synthetic restorative materials requires careful consideration of the unique functional properties of natural enamel and dentin, whose inherent characteristics remain superior to those of any synthetic restorative material currently available. The objective of this article was to organize the available information on indirect adhesive restorations for complex cavities, with particular emphasis on the factors and techniques that require consideration during clinical procedures.

An electronic search was performed in MEDLINE/PubMed, the Web of Science and Google Scholar. The articles included in this study comprised randomized controlled trials (RCTs), clinical trials, clinical reports, reviews, and laboratory studies.

The amount of the remaining natural tooth structure directly affects the longevity of the restored tooth. The remarkable properties of natural enamel and dentin have long been recognized, and bondable restorative materials have therefore been continuously developed with the aim of preserving these natural tissues and reinforcing the remaining tooth structure through effective adhesion. Even when a large portion of the tooth structure has been destroyed, conservative treatment can often still be performed, allowing the benefits of these advanced techniques to be achieved. All aspects of the procedure need to be considered, including material selection, cavity design and preparation, cavity and restoration surface preparation, and the cementation procedure. Additional techniques, including immediate dentin sealing (IDS) and the controversial deep margin elevation technique, are also addressed in this article.

Keywords: indirect adhesive restorations, bondable restorative materials, non-retentive adhesive restorations, complex cavity, minimally invasive restorations

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Highlights

- Minimally invasive indirect adhesive restorations provide a conservative approach for reconstructing large areas of damaged tooth structure.
- Complex cavities that are difficult to restore directly can be effectively managed with bonded indirect restorations.
- Preserving intact tooth structure allows for a non-retentive cavity design without the need for extensive mechanical retention.

Introduction

A common dental problem that brings patients to the dentist is the presence of a large cavity. Large cavities are often associated with troublesome food retention, uncomfortable tooth sensitivity or a precarious sharp edge. A large cavity can result from a variety of causes, such as active dentin caries beneath fragile enamel, secondary caries beneath a previous restoration, or a partial fracture of the natural tooth structure.^{1,2}

A large cavity often involves 2 tooth surfaces, and then it is referred to as a compound cavity. When the damage is extensive and involves 3 or more surfaces, it is referred to as a complex cavity.³ The restoration of a complex cavity is complicated by the fact that it often involves subgingival margins, including gingival overgrowth, or a cavity extending close to the pulpal tissue (Fig. 1). In addition to the mechanical aspect of withstanding chewing forces, this situation also involves the biological aspect of ensuring that the tooth can function properly during mastication. There is also an esthetic aspect, as all patients naturally desire a natural-looking restoration.⁴

Materials for dental restorations have been intentionally developed to mimic the properties of the natural tooth

structure for reconstruction. However, enamel and dentin are often destroyed simultaneously, and both need to be replaced, even though their properties are quite different. Enamel is brittle and stiff, with a high elastic modulus of approx. 80 GPa, whereas dentin is more flexible, with an elastic modulus of approx. 20 GPa.⁵ This represents a highly effective combination created by nature, in which the strong outer shell of enamel and the resilient inner layer of dentin work together to prevent enamel from shattering when the tooth is subjected to fatigue loading.⁶ To date, there are still no restorative materials that can adequately substitute for the natural enamel–dentin combination when it is damaged.

Conservative dental treatment, or conservative dentistry, is an intellectual concept aimed at preserving as much of the unique natural tooth structure as possible, since the properties of all artificial materials fall short of those of the original tooth structure. Unbondable restorative materials require the otherwise unnecessary removal of a sound tooth structure to create a retentive form that holds the restoration in place through macromechanical retention.⁷ Consequently, the remaining tooth structure becomes more susceptible to fracture, as unbondable materials cannot replace the natural reinforcement provided by the tooth structure that has been removed. Thus, removing any part of the tooth for the purpose of creating macromechanical retention inherently weakens the remaining natural tooth structure.^{7–9} In contrast, by definition, bondable materials adhere to an intact tooth structure through micromechanical retention. For this reason, the intact portion of the tooth should be preserved as much as possible to provide a large tooth surface area for effective adhesion.¹⁰ These bondable materials can also reinforce the remaining tooth structure, thereby reducing the risk of subsequent fracture.^{8,9,11}

A comprehensive search for the existing reviews on dental adhesive indirect restorations was conducted using MEDLINE/PubMed, the Web of Science and Google Scholar. This search revealed that most reviews focused exclusively on either cavity preparation techniques or the treatment of cavity surfaces before cementation. However, comprehensive reviews integrating all relevant procedures and the rationale behind them are lacking. This review aims to address this gap by collecting and organizing the available information on dental adhesive indirect restorations,

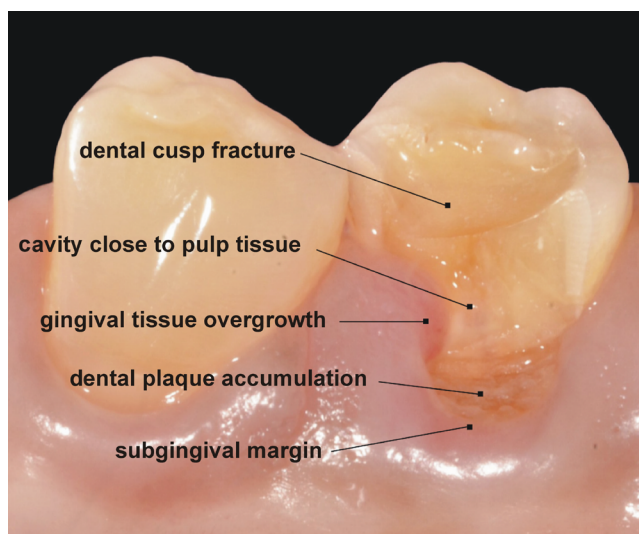


Fig. 1. Common characteristics of complex cavities

The broken enamel–dentin structure often extends close to the pulpal tissue. Cusps are frequently involved in the cavity, as is gingival overgrowth.

with particular emphasis on the factors and techniques that need to be considered during the clinical process, as these can significantly affect the success of treatment.

Methods

An electronic database search, with no date restrictions, was performed using MEDLINE/PubMed, the Web of Science and Google Scholar. The articles included in this study comprise randomized controlled trials (RCTs), clinical trials, clinical reports, reviews, and laboratory studies. Only articles published in English were included. Titles and abstracts were screened as the initial screening step. The selected articles were retrieved, read in full, and then included in this review. An additional search for relevant information was performed by reviewing the references of the selected articles.

Bondable restorative materials

There are essentially 2 types of bondable materials used with the natural tooth structure: glass ionomer cement (GIC); and dental adhesives.¹² Glass ionomer cement initially establishes hydrogen bonds with the tooth through the free carboxyl groups of the cement binding to the bound water on the tooth surface.¹³ These hydrogen

bonds are gradually replaced by the ionic bonds formed between the carboxylate groups of poly(acrylic acid) in GIC and the calcium ions of hydroxyapatite, which also results in the formation of an ion-exchange layer at the tooth–GIC interface.^{14,15} Dental adhesives are currently the most widely used bondable materials and provide reliable micro-mechanical adhesion to the tooth structure.¹⁶ Dental adhesives are used in 2 main ways – for direct and indirect restorations. Direct restorations are made of conventional resin-based materials, including resin composites, compomers and giomers, which bond directly to the dental adhesive. In contrast, indirect restorations are made of materials such as metal, ceramic, resin, or hybrid ceramic, and require an intermediate layer of resin cement to establish a bond with the dental adhesive. Thus, dental adhesives are versatile materials that can bond to almost all types of dental restorative materials (Fig. 2).

When attempting to restore a complex cavity using the direct technique, it is difficult to create good contours of the proximal surfaces and the precise occlusal anatomy corresponding to the opposing tooth. Direct restorations are also time-consuming, and they provide good functionality for only a medium term, 3–5 years, in a complex cavity.^{17–19} They frequently encounter bond degradation, leading to marginal leakage, broken restorative materials, or ineffective restoration anatomy and contour.^{20,21} For long-term success, bonded indirect partial restorations are suggested as a better alternative.

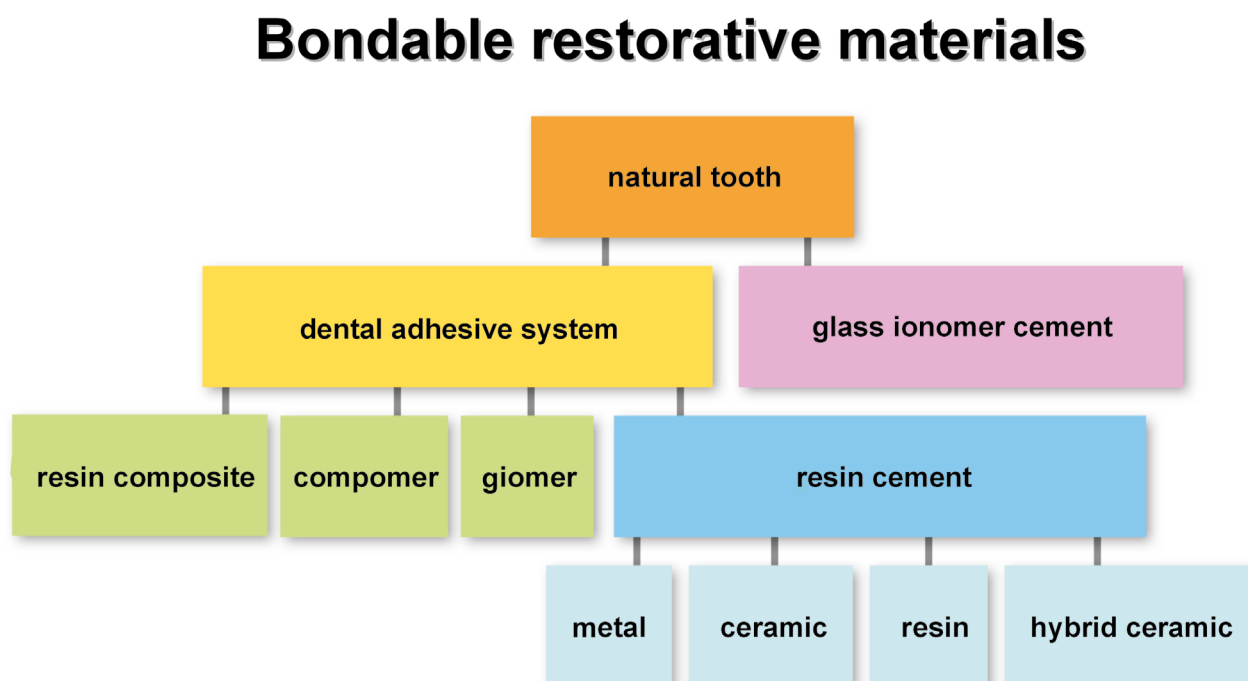


Fig. 2. Dental adhesives and glass ionomer cement (GIC) as the principal bondable restorative materials used in dentistry

Dental adhesives are remarkably versatile, since they function as intermediate materials for joining almost all kinds of other dental materials to the tooth structure.

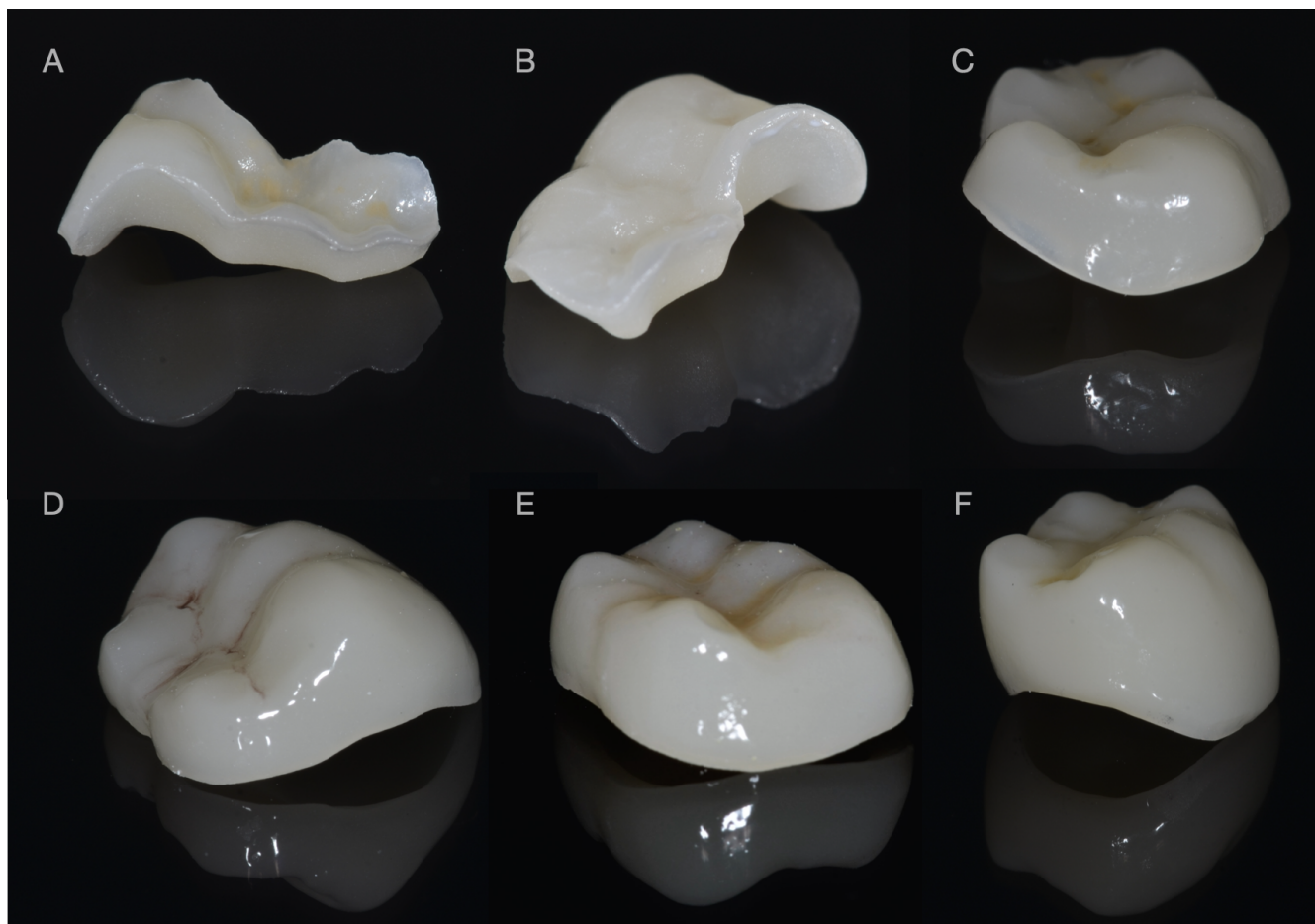


Fig. 3. Various designs of bonded partial indirect restorations

A – inlay; B – onlay; C – overlay; D – overlay-veneer; and E – long-wrap overlay, compared with the traditional crown (F).

These more durable restorations are considered minimally invasive for large defects in posterior teeth.^{1,22–24} The amount of the remaining intact natural tooth structure influences the survival of the restored tooth by helping to prevent catastrophic failure.^{2,25} Bonded indirect partial restorations can also overcome some of the limitations of direct restorations. Indirect restorations with a cement thickness of less than 200 μm generate less polymerization stress than direct resin composite restorations with a thickness of 2 mm.²⁶ They also provide more accurate proximal contacts and occlusal relationships with the neighboring teeth as a result of more convenient extra-oral fabrication. Bonded indirect partial restorations offer an opportunity to preserve and strengthen the remaining natural tooth structure, and Fig. 3 shows various designs typically used to achieve this.^{1,25,27,28} The synergistic bond between bondable restorative materials and the natural tooth structure, mediated by adhesive resin-based cement, plays a crucial role.^{2,25,27,29}

Today, resin cements are by far the most commonly used adhesive cements. Adhesive cements must bond to a variety of substrates, including enamel, dentin, ceramic, metal, and resin.³⁰ Resin cements can be classified according to their polymerization mechanism as light-cured,

chemically cured or dual-cured, or according to their adhesive strategy as total-etch, self-etch or self-adhesive.^{31,32} The ability of light to penetrate all areas of the cement and activate its photo-initiators is vital to the longevity of light-cured resin cement; therefore, thin, translucent restorations are recommended when using light-cured resin cement. Light-cured resin cement has greater color stability than chemically cured or dual-cured resin cements, and provides a convenient working time before setting.³¹ Dual-cured resin cement is polymerized by both chemical and light activation. This type of cement is indicated when the restoration is thick or opaque, such that light cannot adequately penetrate it. However, dual-cured resin cement still requires light activation to achieve a high degree of polymerization.^{33,34}

Restorative materials for non-retentive indirect restorations

This review investigates dental materials commonly used for tooth-colored bonded indirect restorations, as shown in Fig. 4. These materials include indirect resin composite, polymer-infiltrated ceramic network (PICN),

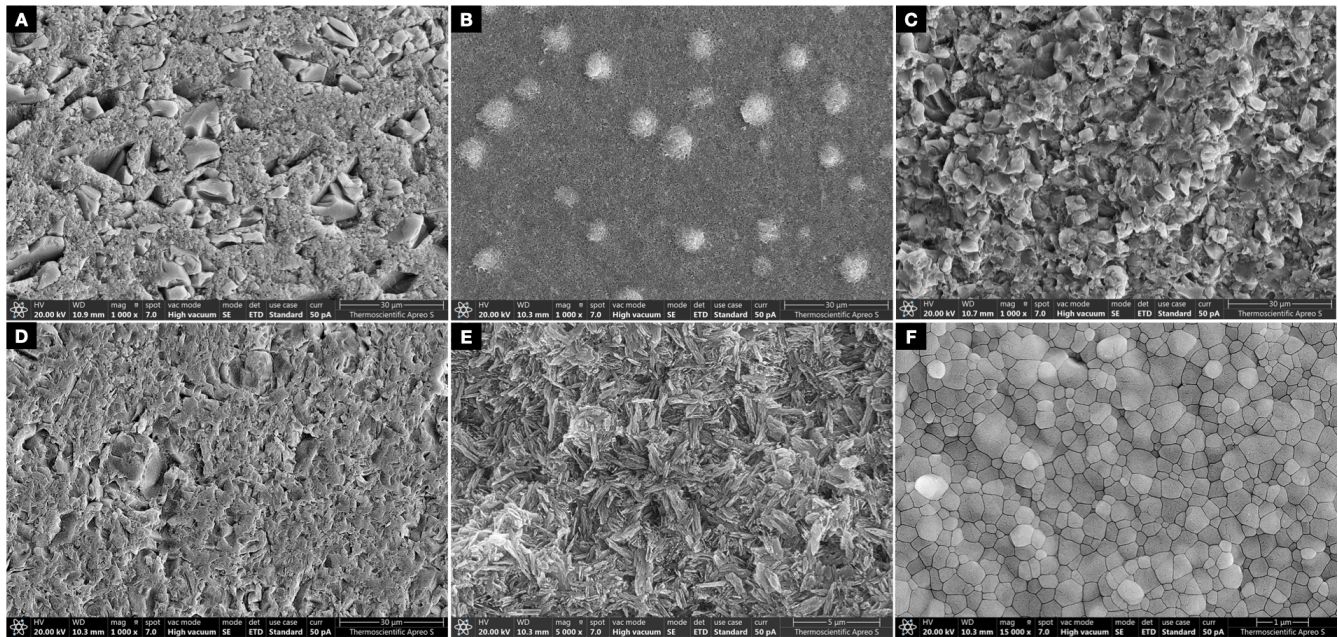


Fig. 4. Scanning electron microscopy (SEM) images of different restorative materials

A – nanohybrid resin composite; B – indirect resin composite; C – polymer-infiltrated ceramic network (PICN); D – feldspathic ceramic; E – lithium disilicate glass ceramic; F – zirconia.

feldspathic ceramic, lithium disilicate glass ceramic, and zirconia. The retention of bonded indirect restorations relies on adhesive bonding to the tooth structure, and the quality of the bond is critical to a long-term success.³⁵ Among tooth-colored bonded indirect restorative materials, etchable ceramics, such as feldspathic ceramics, leucite-reinforced glass ceramics and lithium disilicate glass ceramics, provide satisfactory bond strength to the natural tooth structure.^{22,30,36} However, feldspathic ceramics (flexural strength of 60–70 MPa; fracture toughness of $0.70\text{--}1.30\text{ MPa}\cdot\text{m}^{1/2}$)^{37,38} and leucite-reinforced glass ceramics (flexural strength of 107–160 MPa; fracture toughness of $1.28\text{--}1.38\text{ MPa}\cdot\text{m}^{1/2}$)^{39,40} exhibit a higher frequency of fracture than stronger lithium disilicate glass ceramics (flexural strength of 370–460 MPa; fracture toughness of $2.80\text{--}3.50\text{ MPa}\cdot\text{m}^{1/2}$).^{23,41,42} Therefore, highly etchable lithium disilicate glass ceramics are recommended for restoring complex posterior cavities using the bonded indirect technique.⁴³

Lithium disilicate glass ceramic

Lithium disilicate glass ceramic provides high adhesive strength to the tooth structure through both micromechanical and chemical bonding mechanisms. Microroughness is created on the intaglio surface of this etchable ceramic by applying 4.5% hydrofluoric acid for 20 s.⁴⁴ However, aggressive treatment with either a higher concentration or prolonged application of hydrofluoric acid can negatively affect mechanical performance, resulting in reductions in flexural strength and bonding potential, and can also compromise

the long-term success of the restoration, particularly in low-thickness restorations.^{45–48} After creating an irregular surface, silane is applied to promote a chemical reaction that forms strong siloxane linkages between the silica in the ceramic and the methacrylate groups in resin-based materials.^{49,50}

Self-etching ceramic primers were developed as a single-component alternative to the hydrofluoric acid and silane treatment described above. The principal advantage of self-etching ceramic primers is that they simplify the technique and reduce technique sensitivity by decreasing the number of clinical steps.⁵¹ The self-etching ceramic primer is applied to the intaglio surface of the glass-based ceramic material for 20 s using an agitating microbrush, and is then allowed to react for an additional 40 s. When the bond strength achieved with self-etching ceramic primer treatment is compared with that of the conventional procedure (hydrofluoric acid followed by silane), the conventional procedure provides a higher bond strength across all tested ceramic types, including leucite-based ceramic, lithium disilicate glass ceramic, zirconia-reinforced lithium disilicate glass ceramic, and hybrid ceramic.⁵² In addition, the bond strength of the materials treated with the self-etching ceramic primer decreases significantly after simulated aging.^{52,53}

The currently available lithium disilicate glass ceramics have different crystal structures. Rod-shaped and platelet-shaped crystals are common characteristics, but each material contains a different proportion of these 2 crystal morphologies.⁵⁴ After heat treatment, lithium disilicate glass ceramic exhibits reduced porosity and increased density due to the tight interlocking of the disilicate crystals.

The mismatch in thermal expansion coefficients between the lithium disilicate crystals and the glassy matrix generates compressive stress around the crystals, thereby impeding subsequent crack propagation within the material.⁴² Regarding friction and wear, lithium disilicate glass ceramic has abrasiveness similar to that of enamel.⁵⁵ It also exhibits excellent biocompatibility, including a favorable soft-tissue response, low plaque retention, and good adhesion and proliferation of human epithelial cells and gingival fibroblasts when the ceramic surfaces are properly polished.^{56,57}

Indirect resin composite

Indirect resin composite restorations represent improvement over direct restorations in terms of physical properties, such as hardness, wear resistance, color stability, polymerization shrinkage, and biocompatibility, as they are subjected to heat, visible-spectrum light, vacuum, and pressure during fabrication (flexural strength of 120–160 MPa; fracture toughness of 0.80–1.20 MPa·m^{1/2}; modulus of elasticity of 8.5–12.0 GPa).^{58,59} They also have a higher percentage of filler particles (75–92% by weight) than direct resin composite materials (50–65% by weight).^{58,60,61} Although these procedures produce restorations that are smaller than the prepared cavities because of polymerization shrinkage, the shrinkage is still less than that of direct resin composites. The resulting spaces are ultimately compensated for by the cementation layer.⁵⁸ Indirect resin composites have lower resistance to compressive stress than ceramics, but they have greater tensile strength and better stress distribution.⁵⁸ Therefore, they are still used for the restoration of large defects.⁶² Common indications for indirect resin composites include long-term semi-permanent restorations, such as large restorations in young patients or in teeth undergoing orthodontic treatment.

Indirect resin composite undergoes polymerization with a high degree of conversion, leaving few unreacted monomers available for bonding to the adhesive resin.⁶³ Therefore, indirect resin composite requires mechanical surface preparation. Creating a microrough surface on the indirect resin composite by sandblasting is recommended.⁶⁴ Before applying the adhesive resin, the mechanical bond to the microrough surface can be enhanced by establishing a chemical bond through the application of a silane coupling agent. The silane coupling agent binds to the silica-based fillers in the indirect resin composite and also increases the wettability of its surface.⁶⁵

The intraoral repair of indirect resin composite restorations with resin-based materials is less complicated than the repair of ceramic restorations because of the compatibility between the materials.⁶⁶ The substrate, indirect resin composite, is prepared in the same manner by sandblasting, followed by a direct restoration using a dental adhesive and a resin composite.^{67,68}

Hybrid ceramic

Hybrid ceramic materials are commercially available as polymer-infiltrated ceramic networks (PICNs), consisting of an 86% interconnected silicate glass-ceramic skeleton, with leucite as the major phase and zirconia as the minor phase, and 14% resin. Some microcracks have been found between the different phases of this material.⁶⁹ When the fracture toughness of PICN is compared with that of other tooth-colored restorative materials, PICN, at 1.5 MPa·m^{1/2}, has a lower fracture toughness than almost all types of ceramics, except feldspathic ceramic, which has a fracture toughness of approx. 1 MPa·m^{1/2}. In comparison, lithium disilicate glass ceramic has a fracture toughness of 2.37 MPa·m^{1/2}, while zirconia has a fracture toughness of 4.94 MPa·m^{1/2}.⁷⁰ Polymer-infiltrated ceramic networks and indirect resin composites have desirable low abrasiveness toward the opposing teeth.^{71,72} To create surface irregularities for bonding, the application of 4.5% hydrofluoric acid for 60 s is recommended, as silicate glass ceramic is the major component of PICN and forms its framework.⁷³

Polymer-infiltrated ceramic network has a lower elastic modulus than other ceramic materials. Materials with a low elastic modulus tend to absorb stress and distribute it through the deformation of the restoration rather than transferring it to the underlying resin cement and tooth structure.^{74,75} These low-elastic-modulus materials have demonstrated a higher rate of failure, such as displacement or fracture. Nevertheless, they have also been shown to protect the remaining natural tooth structure, particularly against catastrophic fractures.^{76,77}

Edge chipping is an inherent disadvantage of glass-ceramic materials. The development of PICN has improved this limitation of glass ceramics. However, the edge-chipping resistance of PICN (120 N/mm) remains lower than that of nanohybrid resin composites (275 N/mm), feldspathic ceramics (179 N/mm), and leucite-reinforced glass ceramics (169 N/mm).⁷¹

Zirconia

The zirconia used in this study was yttria-stabilized zirconia (YSZ), a biocompatible material widely used as a biomaterial. Yttria-stabilized zirconia can be classified according to its yttria content. Yttria stabilizes the tetragonal phase of zirconia at room temperature, reduces the coefficient of thermal expansion, and affects the grain size of zirconia.⁷⁸ The classification is often expressed as the mole percentage of yttria in tetragonal zirconia polycrystal (TZP).^{79,80} Three-mol% yttria-stabilized tetragonal zirconia polycrystal (3Y-TZP) is an early-developed form of YSZ with excellent mechanical properties (flexural strength of 1,200–1,500 MPa; fracture toughness of 3.50–4.50 MPa·m^{1/2}; modulus of elasticity of 210.0 GPa), but relatively low translucency. An increase in the yttria

content results in the zirconia material containing both tetragonal and cubic phases, classified as partially stabilized zirconia (PSZ). Eight-mol% yttria-stabilized tetragonal zirconia polycrystal (8Y-TZP) has a stable cubic phase at room temperature, and is commonly referred to as cubic stabilized zirconia (CSZ).^{79,80}

There are 3 main phases of zirconia: monoclinic at room temperature; tetragonal above approx. 1,170°C; and cubic above approx. 2,370°C. The mechanical properties of the monoclinic phase are relatively poor, whereas those of the tetragonal phase are superior. Incorporating dopants, such as yttria, into the starting powder can partially stabilize the tetragonal phase at room temperature, resulting in improved strength and fracture resistance. Under external stress, the metastable tetragonal phase can transform back to the monoclinic phase. This stress-induced transformation is accompanied by the expansion of the zirconia crystals and alterations in their shape, which generates compressive stress around the transformed regions and help impede crack propagation. These changes in the crystal structure increase the energy-absorption capacity and resistance to damage of the material, a phenomenon known as transformation toughening.^{81,82}

Although stabilization with yttria and transformation toughening reduce the risk of fracture in zirconia, the material remains susceptible to an aging process known as low-temperature degradation. The mechanical properties, particularly strength, of aged zirconia decrease as a result of the spontaneous transformation of the tetragonal phase into the monoclinic phase. During usage, the zirconia lattice develops multiple oxygen vacancies associated with the substitution of trivalent yttrium ions for tetravalent zirconium ions. These oxygen vacancies facilitate the penetration of water into the zirconia lattice.⁸³ The interaction with water destabilizes the tetragonal phase and induces its transformation into the monoclinic phase. This transformation is accompanied by an increase in grain volume, which generates stress at the zirconia surface, and causes grain uplift and the dislodgement of individual grains. Microcracks subsequently develop, allowing further penetration of water into the deeper regions of the material. As the transformation progresses into the bulk of the material, these microcracks can propagate and eventually lead to fracture. In contrast, the cubic phase does not undergo the same stress-induced tetragonal-to-monoclinic transformation; therefore, zirconia with a higher cubic-phase content is less susceptible to low-temperature degradation.^{84,85}

Zirconia contains no silica in its composition, and therefore cannot be effectively chemically etched, making chemical adhesion alone insufficient for achieving a strong bond.⁸⁶ The principal method used to enhance the bonding ability of zirconia is the creation of microroughness on the intaglio surface by airborne-particle abrasion with alumina, followed by the application of a phosphate methacrylate monomer, particularly 10-methacryloyloxydecyl

dihydrogen phosphate (MDP), to the treated surface to promote chemical bonding.^{87,88} An alternative technique is tribochemical silica coating followed by the application of silane. However, tribochemical silica coating has been questioned, as the silica layer formed on the surface may be unstable over time.⁸⁸

Similar to other restorative materials bonded using adhesive techniques, zirconia exhibits a higher bond strength when bonded to tooth enamel than when bonded to dentin.⁸⁹ However, the bond strength of zirconia is significantly lower than that of glass-based ceramics, particularly when bonded to dentin, which constitutes much of the substrate in complex cavities.^{44,90–94} Although zirconia has greater fracture resistance than silica-based ceramics, its bonding potential to dentin is limited. For minimally invasive indirect adhesive restorations of complex cavities, tooth preparation commonly results in a non-retentive cavity predominantly involving dentin. Consequently, zirconia is not recommended for non-retentive cavity preparations that rely primarily on adhesion to dentin.

Preparation design for the bonded indirect restorations of complex cavities

General features of the cavity

Catastrophic fractures, whether partial or complete, are the main cause of failure in the ceramic restorations of posterior teeth.⁹⁵ The preparation design has a significant influence on stress distribution within the restoration when it is subjected to occlusal loading. A proper preparation design can reduce mechanical stress, and thereby decrease the risk of restoration fracture.^{96,97} Although indirect resin composites are less brittle than ceramics, the principles of tooth preparation are the same for both materials.

After the removal of defects, the cavity commonly presents with intradentin undercuts and enamel that is unsupported by dentin. Unsupported enamel is no longer considered usable and should be removed initially to allow the adequate visualization of the underlying dentin undercuts. However, all intact, sound enamel without cracks or demineralized areas should be preserved as much as possible, since enamel provides an excellent bonding substrate for adhesive systems. Undercuts within the intact dentin should be blocked out with a bondable resin composite, thereby creating an appropriate path of insertion for the restoration (Fig. 5).^{2,98–101}

The most important physical feature of the prepared cavity is that all internal edges and line angles must be rounded. A smooth, rounded transition should be created from the pulpal wall to the para-pulpal walls, and from the para-pulpal walls to the approximal cavity walls. Sharp

transitions between the cavity walls should be avoided because, as can generate wedging forces between the cement and the restoration, potentially increasing the risk of fracture.⁹⁵

Parallel walls are not required for adhesive bonding, since retention is micromechanical on the surface of enamel and dentin.⁹⁵ The degree of divergence of the cavity walls affects the susceptibility of ceramics to fracture. When the cavity walls have a large degree of divergence, the restored tooth is more vulnerable to fracture. The cavity walls should not diverge more than 10° (Fig. 5).¹⁰²

Even though the thickness of the restoration has no substantial effect on its fracture resistance when using the adhesive technique, the thickness of a brittle material should not be neglected when it is used in a stress-bearing area.¹⁰³ Different types of materials from different manufacturers require different ideal thickness for function: lithium disilicate ceramic – 1.5 mm (min. 1.0 mm); PICN – 1.5 mm (min. 1.0 mm); 3Y-TZP – 1.0 mm (min. 0.5 mm); 4Y-TZP and 5Y-TZP – 1.5 mm (min. 1.0 mm). Commonly, the thinnest region of a posterior restoration is at the fissure areas, which should have a minimum thickness of 1.5 mm (Fig. 5).¹⁰⁴ If there is insufficient clearance space in the fissure area to receive the restoration, a round instrument is recommended to deepen the fissure area.

It has been shown that a flat pulpal wall is not required for adhesive restorations.⁹⁵

If the preparation design involves an intracoronal cavity, the width of the cavity should be at least 2.5 mm, and the thickness of the remaining cusp should be no less than 2 mm (Fig. 5).^{2,95} A compromised tooth, whether endodontically treated or cracked, should have a thickness of the remaining wall of no less than 3 mm.^{105,106} If the preparation cannot meet this requirement, the cusp of the affected wall should be shortened. The proximal margin should be extended without contacting the adjacent tooth. This approach enables clear identification of the margin with either a conventional impression or a digital scan, and facilitates the removal of excess adhesive cement and the finishing of the margin after the restoration is delivered.¹⁰⁷

The efficacy of the bond between the restorative material and the remaining tooth structure is vital to the longevity of the restoration. Phosphoric acid-etched enamel provides significantly greater shear bond strength (50–90 MPa) than dentin (45–70 MPa).¹⁰⁸ It is recommended to preserve as much intact enamel as possible, as it is more stable over time than dentin.¹⁰⁹ This is because enamel is not subject to the enzymatic degradation associated with dentin, which is mediated by matrix metalloproteinases (MMPs) and cysteine cathepsins (CCs).^{110,111} The restoration

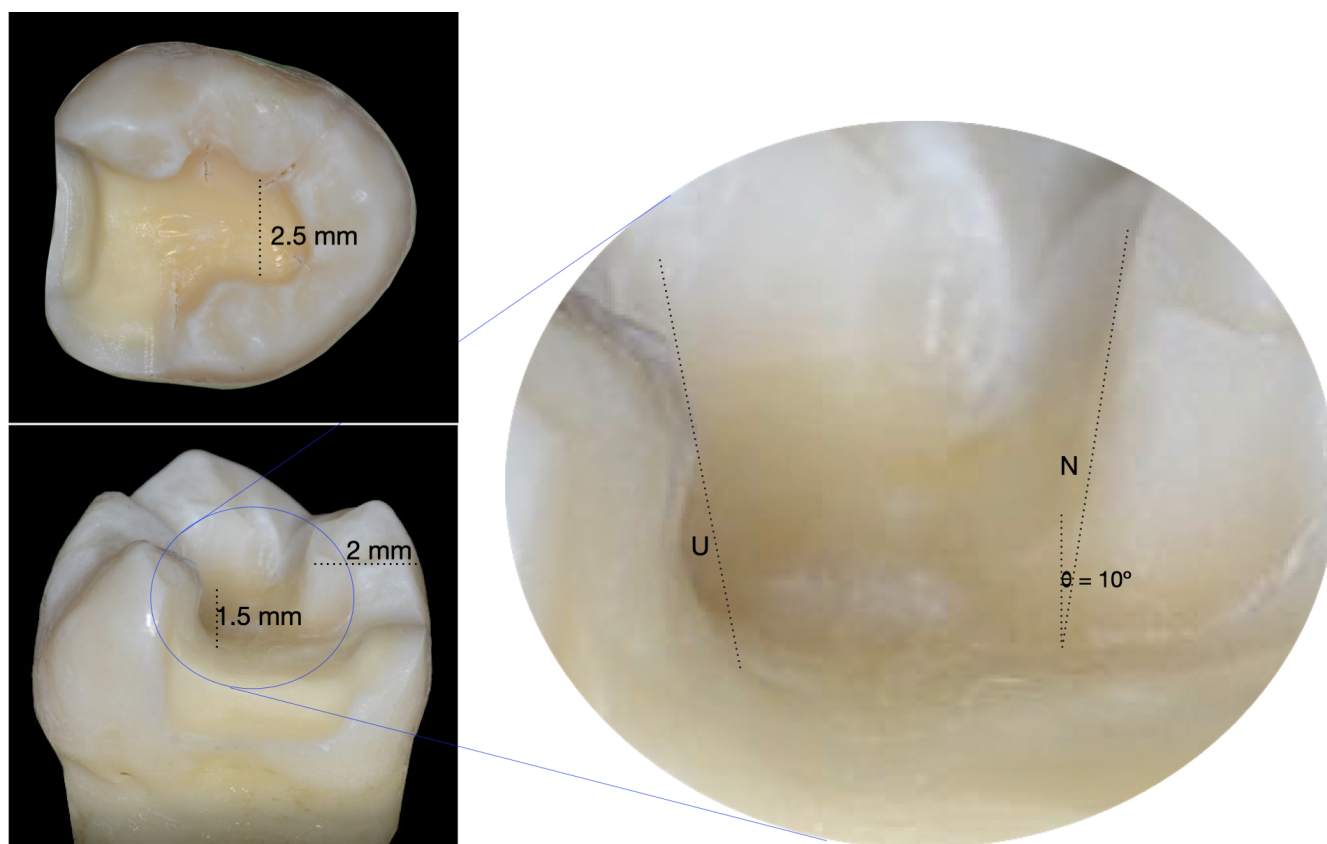


Fig. 5. Tooth cavity preparation

When restored with bondable materials, an intracoronal cavity must have a minimum width of 2.5 mm and a minimum depth of 1.5 mm. The minimum thickness of the remaining cusp should be 2 mm. The intradentin undercuts (U) must be blocked out with a bondable resin composite, in contrast to the non-undercut area (N), which should have a divergence (θ) of approx. 10° .

margins are among the most critical areas, as they are particularly susceptible to dental caries, which is a common cause of indirect restoration failure. A non-beveled preparation should be used at the margins of ceramic and resin-based restorations, since adequate material thickness contributes to their strength.¹¹² Therefore, a continuous enamel margin without a bevel and a preparation margin angle of 90° is recommended to maximize bonding effectiveness and marginal durability, thereby contributing to the longevity of the restoration.^{95,97}

Margin preparation design

Placing the preparation margin on enamel is a reliable approach for ensuring effective bonding with the dental adhesive system. In addition to its effect on bond strength, the marginal design influences the clinical success of the restoration in several other ways. The preparation margin must be sufficiently strong to withstand chewing forces and sufficiently smooth to allow proper seating of the restoration, and facilitate cleaning and plaque removal. Importantly, the restoration margin should fit as closely as possible to the preparation finishing line to minimize the amount of exposed cement, and thereby reduce microleakage and the risk of secondary caries.

When the remaining wall thickness of a compromised tooth is less than 3 mm, the cusp associated with the thin wall should be reduced.^{2,95,105,106} Importantly, it is recommended that compromised teeth, including endodontically treated and cracked teeth, have all cusps restored and receive restorations with full occlusal coverage.^{113–115} The required restoration thickness in a reduced-cusp area depends on the type of material used. Lithium disilicate glass ceramics should be at least 1.5-millimeter thick, whereas feldspathic ceramics and leucite-reinforced glass ceramics should be at least 2-millimeter thick in reduced-cusp areas (Fig. 6).¹⁰⁵

A shoulder margin preparation with a rounded internal line angle (a radial shoulder) is recommended for resin composite or ceramic restorations. A shoulder margin is a common margin design that can be placed at any location on the prepared tooth and has a width of approx. 1 mm (max. 1.5 mm). A bevel preparation (contra-bevel preparation) is an alternative margin design for areas of cuspal reduction when an esthetic transition between the tooth and the restoration is desired.¹⁰⁶ A bevel preparation with an inclination of 45° or greater is performed to avoid unsupported enamel and provide an adequate bulk of the restorative material at the margin (Fig. 6).²⁵

The height of the axial walls determines the path of insertion of the restoration, and contributes to its stability and reliability during delivery. The height of the axial walls has no effect on the fracture resistance of the restoration.²⁵ However, an axial wall height of at least 2 mm is recommended for compromised teeth to strengthen the remaining tooth structure by providing a ferrule effect (Fig. 6).¹¹⁶

The biologically oriented preparation technique (BOPT) was introduced because gingival recession is one of the most common complications associated with restorations in proximity to the periodontal tissues. This procedure is intended to enhance periodontal tissue stability and promote the coronal migration of the marginal gingival tissue.¹¹⁷ The technique combines tooth preparation without a defined finishing line with the immediate placement of a provisional restoration. A vertical tooth preparation is performed to eliminate the anatomical emergence profile without creating a finishing line.¹¹⁸ Simultaneously, the gingival sulcus is de-epithelialized (gingivage), allowing tissue regeneration.¹¹⁹ The provisional restoration must have an emergence profile that accurately mimics that of the natural tooth. The immediate placement of the provisional restoration helps preserve the biological space, the cervical contour of the tooth, and the blood clot formed following the de-epithelialization of the gingival sulcus. Moreover, the provisional restoration accelerates the tissue-healing process, allowing the surrounding tissues to adapt their form and position to the new restoration profile. The thickened gingival tissue subsequently remodels over time to conform to the definitive restoration.¹²⁰

Deep margin elevation (coronal margin relocation)

Deep margin elevation is the process of repositioning a subgingival finishing line to a supragingival margin by applying directly bondable restorative materials beneath the margin of an indirect restoration.^{121,122} Several benefits can result from deep margin elevation. For example, it facilitates access to otherwise difficult to visualize areas during impression taking, intraoral scanning and restoration delivery.^{123–126} The deep margin elevation technique is generally performed in conjunction with immediate dentin sealing (IDS) in the same area to reduce marginal leakage and enhance bond strength (Fig. 7).¹²⁷

In the deep margin elevation procedure, dental adhesives and resin composite materials are used to elevate the prepared margin by no more than 2 mm. Glycerin gel coating is recommended during the final polymerization step, followed by finishing and polishing. A bitewing radiograph should then be taken to evaluate the quality and adaptation of the resin composite elevation.¹²³

In some situations, deep margin elevation can substitute for surgical crown lengthening, thereby avoiding potential anatomical complications, such as furcation exposure or the loss of periodontal attachment.¹²⁸ However, the decision to use deep margin elevation as an alternative depends on the individual patient, as the invasion of the biological width has also been reported following this procedure.^{129,130} Surgical crown lengthening is generally required when the distance between the restoration margin and the alveolar bone crest is less than 2 mm. When this distance is greater than 2 mm, deep margin

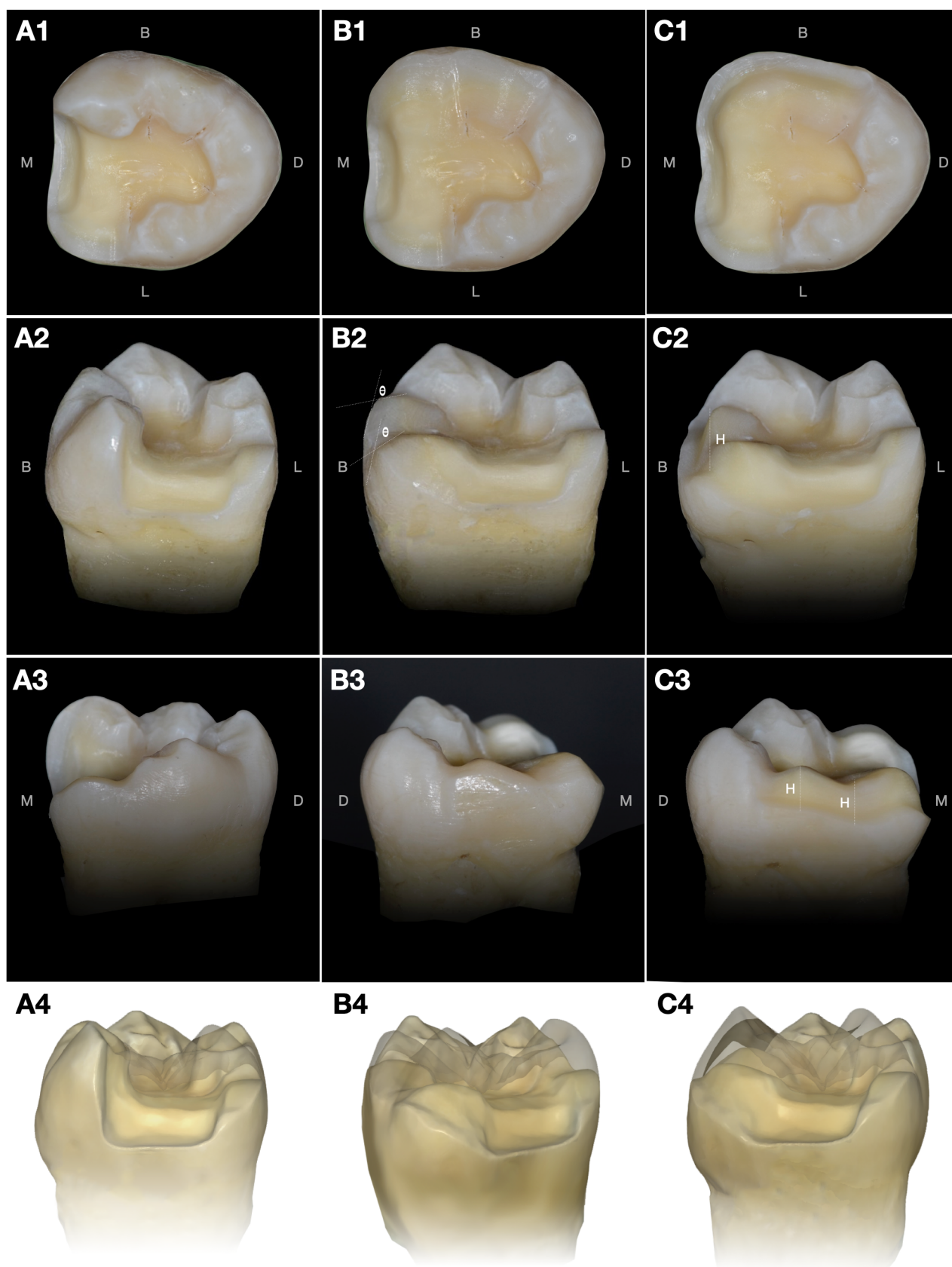


Fig. 6. Various margin preparation designs

A1–A4 – shoulder margin; B1–B4 – contra-bevel margin; C1–C4 – wrap-around-shoulder margin.

For the contra-bevel margin, the inclination (θ) of the bevel preparation should be at least 45° to prevent unsupported enamel and provide an adequate bulk of the restorative material at the margin. Wrapping the shoulder margin around the tooth creates an axial wall. The height of this axial wall (H) contributes to restoration stability, establishes the path of insertion and functions as a ferrule (M – mesial surface; D – distal surface; B – buccal surface; L – lingual surface).

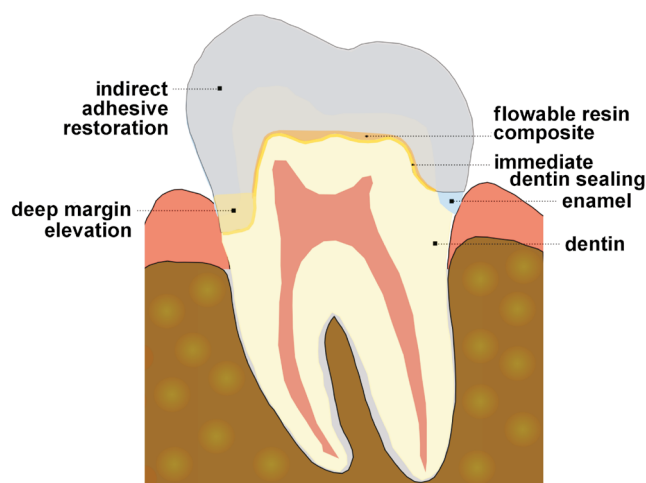


Fig. 7. Immediate dentin sealing (IDS)

The IDS procedure is recommended for all freshly cut dentin. A difficult-to-access subgingival area can be managed with the deep margin elevation procedure during IDS, using the same adhesive system.

A flowable resin composite can also be applied over the IDS adhesive to block small undercuts and enhance the integrity of the adhesive interface through its hydrophobic properties.

elevation can be performed, as sufficient space remains to preserve connective tissue attachment. When the distance is less than this threshold, surgical crown lengthening is required to create adequate space for connective tissue attachment.¹³¹

Even conservative treatment using an adhesive restoration can sometimes require a preparation with a subgingival margin extending onto the root dentin. This is because the proximal margin is frequently located beyond the cemento-enamel junction (CEJ) as a result of extensive dental caries. This location can make the restoration preparation and cementation procedures difficult, so deep margin elevation is commonly recommended in such circumstances (Fig. 7). However, this area lacks enamel, and dentin and cementum do not provide bonding as effectively as enamel.^{100,122,132} Thus, although many studies have found no difference in the overall marginal integrity of restorations with or without deep margin elevation, some studies have demonstrated more favorable outcomes with deep margin elevation in other aspects, such as sealing ability, bond strength and fracture resistance.^{124–126,133–138} Currently, there is insufficient evidence to support the routine use of this technique, since its clinical outcomes can be unpredictable. Nevertheless, deep margin elevation can be a useful alternative when surgical crown lengthening is contraindicated or presents anatomical challenges, such as furcation involvement, root proximity or root concavity.¹³⁹

Immediate dentin sealing

The traditional procedure for indirect restorations requires at least 2 appointments. During the 1st appointment, the tooth is prepared, followed by an immediate

conventional impression or digital scan, and temporary luting of a provisional restoration. During the 2nd appointment, the definitive restoration is cemented onto the tooth. Specifically, the adhesive resin used during the cementation procedure is applied to dentin that is no longer freshly cut, as the freshly cut dentin was exposed to both the impression material and the temporary luting cement during the 1st appointment. This process is therefore referred to as delayed dentin sealing (DDS).^{140–142} Residues from the impression material and the temporary luting cement can remain on the dentin surface and infiltrate the dentinal tubules. This can interfere with hybridization by the adhesive resin and reduce bond strength.¹⁴²

The IDS procedure involves applying an adhesive resin to freshly cut dentin immediately after tooth preparation (Fig. 7). Although a wide variety of adhesive systems can be used for IDS, only three-step etch-and-rinse and two-step self-etch adhesive systems have been shown to improve the bond strength between freshly cut dentin and the restoration by creating a stable hybrid layer.^{141,143} In contrast, when two-step etch-and-rinse or one-step self-etch adhesive systems are used for IDS, they do not provide advantages over the DDS technique.¹⁴⁴ Ideally, the adhesive resin should bond to the uncontaminated dentin to create an effective hybrid layer.^{143,145} The IDS procedure allows the adhesive resin to penetrate deeply into dentin and polymerize before cementation, resulting in a well-developed hybrid layer. This differs from conventional adhesive cementation, in which the adhesive resin is applied when the restoration is seated to avoid incomplete seating of the restoration.^{146,147} During seating, pressure can cause the collagen fibers within dentin to collapse, resulting in an imperfect hybrid layer and a lower bond strength as compared to the separate polymerization of the adhesive resin and the resin cement.^{148–151} A well-developed hybrid layer can protect against bacterial infiltration and dentin sensitivity while enhancing bond strength.^{142,152,153} Effective IDS can also reduce microleakage through the gaps between dentin and the restoration, and promote better adaptation of the restoration to dentin.^{145,148,154}

Either a three-step etch-and-rinse adhesive system or a two-step self-etch adhesive system is recommended for IDS. Moreover, an adhesive resin containing fillers provides a uniform film thickness of approx. 88 μm , which is advantageous for creating a high-quality hybrid layer.¹⁵⁵ If an unfilled adhesive resin must be used, a flowable resin composite is particularly recommended. When a flowable resin composite is applied over the thin film of an adhesive resin, the hydrophobic properties of both resins improve the integrity of the adhesive interface and make it more receptive to resin cement, particularly chemical- or dual-cured cements, thereby avoiding adhesion incompatibility.¹⁵⁶

When performing IDS, the adhesive resin must undergo a second polymerization under glycerin gel, which functions as an air-blocking agent, and thereby reduces

the oxygen-inhibited layer. This step is followed by rinsing with an air-water spray. Importantly, any excess adhesive resin on enamel should be removed with a dental bur to complete the IDS procedure.¹⁴³ Although glycerin is used to reduce the thickness of the oxygen-inhibited layer, an approx. 40-micrometer layer can still remain on the surface.^{157,158} The hydrophilic monomer hydroxyethyl methacrylate (HEMA), which is a component of adhesive resins, is also present in the oxygen-inhibited layer. This can cause the residual inhibition of the polymerization of elastomeric impression materials.¹⁵⁹ The platinum salt that functions as the polymerization catalyst in vinyl polysiloxane can be scavenged by and react with the oxygen-inhibited layer, resulting in the inhibition of vinyl polysiloxane polymerization.¹⁵⁸ Polyether impression materials, which are hydrophilic and have greater stiffness and lower tear resistance than vinyl polysiloxane, contain cationic initiators that can react with monomer free radicals in the oxygen-inhibited layer. This can result in ionic copolymerization, allowing the superficial surface of the polyether to adhere to the adhesive resin surface and causing the superficial layer of the impression to tear easily during removal.¹⁵⁹

The oxygen-inhibited layer of IDS can also affect the provisional restoration. The resin-based materials used for the provisional restoration can bond to the adhesive resin of IDS during fabrication through the oxygen-inhibited layer.¹⁶⁰ Therefore, the oxygen-inhibited layer should be removed with either pumice or ethanol before taking the impression and fabricating the bis-acryl provisional restoration under a heavy separating medium.^{159,161}

At the appointment for the delivery of the definitive restoration, the cavity surface should be air-abraded,¹⁴³ after which the appropriate adhesive technique for the resin cement can be performed. It is important to note that various adhesives used for IDS and/or definitive cementation may contain acidic monomers, and the interactions between different acidic monomers can affect bond strength. For example, a low shear bond strength has been reported when phosphate methacrylate interacts with carboxylic methacrylate.¹⁶² Even when 2 adhesives contain phosphate methacrylate monomers, the bond strength between them can be low, since they contain different types of phosphate functional monomers.¹⁶² To avoid compromised bond strength caused by the interactions between acidic monomers, the safest approach is to avoid combining different types of acidic monomers.¹⁶² When two adhesives contain the same acidic monomer, this interaction is not a concern.

Durability of the bonded indirect restorations of complex cavities

The fracture resistance of a large complex cavity is significantly reduced by the loss of a substantial portion of the tooth structure. After the placement of a bonded restoration, the fracture resistance substantially increases.²

Although a bonded restoration can reinforce the remaining tooth structure, the durability of the adhesive bond remains a concern. The flexure of the tooth during chewing contributes to the mechanical degradation of the adhesive interface, which occurs in combination with hydrolytic and enzymatic degradation.¹⁶³ The subsequent loss of adhesion, marginal leakage, and an increased flexure of the cavity walls can ultimately lead to the fracture of the remaining tooth structure.¹⁶⁴ Although the bond strength to enamel is greater and more reliable than that to dentin, the fatigue resistance of the enamel bond is substantially lower than that of the dentin bond.^{164–166} Enamel fatigue failure often initiates and propagates within enamel as a crack, without adhesive failure, in the form of enamel cohesive failure.¹⁶⁴

The fracture of an etchable ceramic restoration used as a bonded indirect restoration in a complex cavity is a particular concern in patients who clench or grind their teeth (bruxism). The failure rate of lithium disilicate glass ceramic restorations in patients with bruxism has been found to be not significantly different from that in patients without bruxism. However, feldspathic ceramics and leucite-reinforced glass ceramics have been associated with higher failure rates in patients with bruxism.^{167–169}

The thickness of lithium disilicate glass ceramic restorations affects the tensile stress distribution within the restoration. Thin restorations develop higher tensile stress, which increases their risk of fracture.¹⁷⁰ The thickness of the cement layer also influences stress at the adhesive interface. As cement layer thickness increases, greater stress is generated at the adhesive interface as a result of the polymerization shrinkage of the adhesive resin cement.¹⁷¹ Thus, both the thickness of the restoration and the thickness of the cement layer can influence the longevity of the restoration.

The degree of conversion of the adhesive resin cement is another important factor that should be considered. The effects of the translucency and thickness of lithium disilicate glass ceramic restorations on the degree of conversion of light-cured adhesive resin cement have been investigated. The translucency of the restorations had no significant effect on the degree of conversion, whereas lithium disilicate glass ceramic restorations thicker than 1 mm significantly reduced the degree of conversion of the adhesive resin cement.¹⁷² Since posterior restorations made of lithium disilicate glass ceramic generally require a minimum thickness of 1.5 mm,^{2,25,173} dual-cured or chemically cured adhesive resin cement is recommended instead of light-cured resin cement. In this way, adequate polymerization can be achieved despite the thickness of the restoration.

Conclusions

Minimally invasive indirect adhesive restoration is a conservative procedure for reconstructing large areas of destroyed natural tooth structure. This technique is recommended for complex cavities that are difficult to restore

directly with conventional fillings. Since the technique allows the intact natural tooth structure to be preserved as much as possible, the cavity is prepared in a non-retentive form and does not require the creation of a major mechanical retentive form.

Ethics approval and consent to participate

Not applicable.

Data availability

All the data generated and/or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

- Wayakanon K. Partially coverage restoration: An esthetically conservative treatment for a complex cavity restoration. *Open J Stomatol.* 2017;7(4):234–241. doi:10.4236/ojst.2017.74017
- Thainimit I, Totiam P, Wayakanon K. Fracture resistance of remaining buccal cusps in maxillary premolar ceramic onlay restorations. *Oper Dent.* 2019;45(4):624–631. doi:10.2341/19-251-L
- Summitt JB, Robbins JW, Hilton TJ, Schwartz RS, eds. *Fundamentals of Operative Dentistry: A Contemporary Approach*. 3rd ed. Batavia, IL: Quintessence Publishing; 2006.
- Totou D, Naka O, Mehta SB, Banerji S. Esthetic, mechanical, and biological outcomes of various implant abutments for single-tooth replacement in the anterior region: A systematic review of the literature. *Int J Implant Dent.* 2021;7(1):85. doi:10.1186/s40729-021-00370-7
- Mahoney E, Holt A, Swain M, Kilpatrick N. The hardness and modulus of elasticity of primary molar teeth: An ultra-micro-indentation study. *J Dent.* 2000;28(8):589–594. doi:10.1016/s0300-5712(00)00043-9
- Chun K, Choi H, Lee J. Comparison of the mechanical property and role between enamel and dentin in the human teeth. *J Dent Biomech.* 2014;5:1758736014520809. doi:10.1177/1758736014520809
- Robbins JW, Burgess JO, Summitt JB. Retention and resistance features for complex amalgam restorations. *J Am Dent Assoc.* 1989;118(4):437–442. doi:10.14219/jada.archive.1989.0189
- Deshpande SR, Gaddalay SL, Damade YN, Khanvilkar UD, Chaudhari AS, Anala V. Reinforcing the cervical dentin with bonded materials to improve fracture resistance of endodontically treated roots. *J Conserv Dent.* 2022;25(2):179–184. doi:10.4103/jcd.jcd_609_21
- Arola D, Galles LA, Sarubin MF. A comparison of the mechanical behavior of posterior teeth with amalgam and composite MOD restorations. *J Dent.* 2001;29(1):63–73. doi:10.1016/s0300-5712(00)00036-1
- Vaidyanathan TK, Vaidyanathan J. Recent advances in the theory and mechanism of adhesive resin bonding to dentin: A critical review. *J Biomed Mater Res B Appl Biomater.* 2009;88(2):558–578. doi:10.1002/jbm.b.31253
- Watts DC, El Mowafy OM, Grant AA. Fracture resistance of lower molars with Class 1 composite and amalgam restorations. *Dent Mater.* 1987;3(5):261–264. doi:10.1016/S0109-5641(87)80084-2
- Kochotwuttinont S, Wayakanon K. Evaluating the failure of resin-based materials on the proximal cervical dentin. *Open Dent J.* 2020;14:631–640. doi:10.2174/1874210602014010631
- Wood D, Hill R. Structure-property relationships in ionomer glasses. *Clin Mater.* 1991;7(4):301–312. doi:10.1016/0267-6605(91)90074-P
- Beech DR. Improvement in the adhesion of polyacrylate cements to human dentine. *Br Dent J.* 1973;135(10):442–445. doi:10.1038/sj.bdj.4803103
- Ngo HC, Mount G, Mc Intyre J, Tuisuva J, Von Doussa RJ. Chemical exchange between glass-ionomer restorations and residual carious dentine in permanent molars: An in vivo study. *J Dent.* 2006;34(8):608–613. doi:10.1016/j.jdent.2005.12.012
- Buonocore MG, Matsui A, Gwinnett AJ. Penetration of resin dental materials into enamel surfaces with reference to bonding. *Arch Oral Biol.* 1968;13(1):61–70. doi:10.1016/0003-9969(68)90037-x
- Fennis WM, Kujis RH, Roeters FJ, Creuquers NH, Kreulen CM. Randomized control trial of composite cuspal restorations: Five-year results. *J Dent Res.* 2014;93(1):36–41. doi:10.1177/0022034513510946
- Laegreid T, Gjerdet NR, Johansson AK. Extensive composite molar restorations: 3 years clinical evaluation. *Acta Odontol Scand.* 2012;70(4):344–352. doi:10.3109/00016357.2011.603355
- Scholtanus JD, Ozcan M. Clinical longevity of extensive direct composite restorations in amalgam replacement: Up to 3.5 years follow-up. *J Dent.* 2014;42(11):1404–1410. doi:10.1016/j.jdent.2014.06.008
- Opdam NJ, Van de Sande FH, Bronkhorst E, et al. Longevity of posterior composite restorations: A systematic review and meta-analysis. *J Dent Res.* 2014;93(10):943–949. doi:10.1177/0022034514544217
- Van de Sande FH, Opdam NJ, Da Rosa Rodolpho PA, Correa MB, Demarco FF, Cenci MS. Patient risk factors' influence on survival of posterior composites. *J Dent Res.* 2013;92(7 Suppl):785–835. doi:10.1177/0022034513484337
- Belli R, Petschelt A, Hofner B, Hajtó J, Scherrer SS, Lohbauer U. Fracture rates and lifetime estimations of CAD/CAM all-ceramic restorations. *J Dent Res.* 2016;95(1):67–73. doi:10.1177/0022034515608187
- Beier US, Kapferer I, Burtscher D, Giesinger JM, Dumfahrt H. Clinical performance of all-ceramic inlay and onlay restorations in posterior teeth. *Int J Prosthodont.* 2012;25(4):395–402. PMID:22720292.
- Federlin M, Hiller KA, Schmalz G. Controlled, prospective clinical split-mouth study of cast gold vs. ceramic partial crowns: 5.5 year results. *Am J Dent.* 2010;23(3):161–167. PMID:20718214.
- Channarong W, Lohawiboonkij N, Jaleyasuthumkul P, Ketpan K, Duangrattananapathip N, Wayakanon K. Fracture resistance of bonded ceramic overlay restorations prepared in various designs. *Sci Rep.* 2022;12(1):16599–16608. doi:10.1038/s41598-022-21167-7
- Yamamoto T, Nakamura Y, Nishide A, Kubota Y, Momoi Y. Contraction stresses in direct and indirect composite restorations compared by crack analysis. *J Adhes Dent.* 2013;15(1):47–54. doi:10.3290/j.jad.a28171
- Van Dijken JW, Hasselrot L, Ormin A, Olofsson AL. Restorations with extensive dentin/enamel-bonded ceramic coverage. A 5-year follow-up. *Eur J Oral Sci.* 2001;109(4):222–229. doi:10.1034/j.1600-0722.2001.00063.x
- Bremer BD, Geurtsen W. Molar fracture resistance after adhesive restoration with ceramic inlays or resin-based composites. *Am J Dent.* 2001;14(4):216–220. PMID:11699740.
- Bustamante-Hernández N, Montiel-Company JM, Bellot-Arcis C, et al. Clinical behavior of ceramic, hybrid and composite onlays. A systematic review and meta-analysis. *Int J Environ Res Public Health.* 2020;17(20):7582. doi:10.3390/ijerph17207582
- Ferracane JL, Stansbury JW, Burke FJ. Self-adhesive resin cements – chemistry, properties and clinical considerations. *J Oral Rehabil.* 2011;38(4):295–314. doi:10.1111/j.1365-2842.2010.02148.x
- Simon JF, Darnell LA. Considerations for proper selection of dental cements. *Compend Contin Educ Dent.* 2012;33(1):28–38. PMID:22432174.
- Burgess JO, Ghuman T, Cakir D. Self-adhesive resin cements. *J Esthet Restor Dent.* 2010;22(6):412–419. doi:10.1111/j.1708-8240.2010.00378.x
- Hasegawa EA, Boyer DB, Chan DC. Hardening of dual-cured cements under composite resin inlays. *J Prosthet Dent.* 1991;66(2):187–192. doi:10.1016/s0022-3913(05)80046-0
- El-Badrawy WA, El-Mowafy OM. Chemical versus dual curing of resin inlay cements. *J Prosthet Dent.* 1995;73(6):515–524. doi:10.1016/s0022-3913(05)80109-x

35. Tian T, Tsoi JKH, Matinlinna JP, Burrow MF. Aspects of bonding between resin luting cements and glass ceramic materials. *Dent Mater*. 2014; 30(7):e147–e162. doi:10.1016/j.dental.2014.01.017
36. Clausen JO, Tara MA, Kern M. Dynamic fatigue and fracture resistance of non-retentive all-ceramic full-coverage molar restorations. Influence of ceramic material and preparation design. *Dent Mater*. 2010;26(6):533–538. doi:10.1016/j.dental.2010.01.011
37. Giordano 2nd RA, Campbell S, Pober R. Flexural strength of feldspathic porcelain treated with ion exchange, overglaze, and polishing. *J Prosthet Dent*. 1994;71(5):468–472. doi:10.1016/0022-3913(94)90184-8
38. Ghiaz K, Vasanthakumar, Kamalanathan D, et al. Evaluation of hardness and fracture toughness of feldspathic porcelain by various surface finishing techniques. *J Clin Diagn Res*. 2022;16(6):ZC01–ZC07. doi:10.7860/JCDR/2022/53002.16469
39. Cattell MJ, Chadwick TC, Knowles JC, Clarke RL, Lynch E. Flexural strength optimisation of a leucite reinforced glass ceramic. *Dent Mater*. 2001;17(1):21–33. doi:10.1016/s0109-5641(00)00046-4
40. Byeon SM, Song JJ. Mechanical properties and microstructure of the leucite-reinforced glass-ceramics for dental CAD/CAM. *J Dent Hyg Sci*. 2018;18(1):42–49. doi:10.17135/jdhs.2018.18.1.42
41. Morimoto S, Rebello de Sampaio FB, Braga MM, Sesma N, Özcan M. Survival rate of resin and ceramic inlays, onlays, and overlays: A systematic review and meta-analysis. *J Dent Res*. 2016;95(9):985–994. doi:10.1177/0022034516652848
42. Zarone F, Di Mauro MI, Ausiello P, Ruggiero G, Sorrentino R. Current status on lithium disilicate and zirconia: A narrative review. *BMC Oral Health*. 2019;19(1):134. doi:10.1186/s12903-019-0838-x
43. Luciano M, Francesca Z, Michela S, Tommaso M, Massimo A. Lithium disilicate posterior overlays: Clinical and biomechanical features. *Clin Oral Investig*. 2020;24(2):841–848. doi:10.1007/s00784-019-02972-3
44. 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
45. Murillo-Gómez F, Palma-Dibb RG, De Goes MF. Effect of acid etching on tridimensional microstructure of etchable CAD/CAM materials. *Dent Mater*. 2018;34(6):944–955. doi:10.1016/j.dental.2018.03.013
46. Bajraktarova-Valijakova E, Grozdanov A, Guguvcevski L, et al. Acid etching as surface treatment method for luting of glass-ceramic restorations, part 1: Acids, application protocol and etching effectiveness. *Open Access Maced J Med Sci*. 2018;6(3):568–573. doi:10.3889/oam-jms.2018.147
47. Prochnow C, Venturini AB, Guilardi LF, et al. Hydrofluoric acid concentrations: Effect on the cyclic load-to-failure of machined lithium disilicate restorations. *Dent Mater*. 2018;34(9):e255–e263. doi:10.1016/j.dental.2018.06.028
48. Sundfeld D, Muniz Palaiol AR, Piovesan Fugolin AP, et al. The effect of hydrofluoric acid and resin cement formulation on the bond strength to lithium disilicate ceramic. *Braz Oral Res*. 2018;32:e43. doi:10.1590/1807-3107bor-2018.vol32.0043
49. Swank HM, Motyka NC, Bailey CW, Vandewalle KS. Bond strength of resin cement to ceramic with simplified primers and pretreatment solutions. *Gen Dent*. 2018;66(5):33–37. PMID:30188854.
50. Neis CA, Guimarães Albuquerque NL, De Souza Albuquerque I, et al. Surface treatments for repair of feldspathic, leucite - and lithium disilicate-reinforced glass ceramics using composite resin. *Braz Dent J*. 2015;26(2):152–155. doi:10.1590/0103-6440201302447
51. El-Damanhoury HM, Gaintantzopoulou MD. Self-etching ceramic primer versus hydrofluoric acid etching: Etching efficacy and bonding performance. *J Prosthodont Res*. 2018;62(1):75–83. doi:10.1016/j.jpor.2017.06.002
52. Tribst JPM, Diamantino PJS, De Freitas MR, et al. Effect of active application of self-etching ceramic primer on the long-term bond strength of different dental CAD/CAM materials. *J Clin Exp Dent*. 2021; 13(11):e1089–e1095. doi:10.4317/jced.58723
53. Donmez MB, Okutan Y, Yucel MT. Effect of prolonged application of single-step self-etching primer and hydrofluoric acid on the surface roughness and shear bond strength of CAD/CAM materials. *Eur J Oral Sci*. 2020;128(6):542–549. doi:10.1111/eos.12747
54. Li D, Guo JW, Wang XS, Zhang SF, He L. Effects of crystal size on the mechanical properties of a lithium disilicate glass-ceramic. *Mater Sci Eng A*. 2016;669:332–339. doi:10.1016/j.msea.2016.05.068
55. Lee A, Swain M, He L, Lyons K. Wear behavior of human enamel against lithium disilicate glass ceramic and type III gold. *J Prosthet Dent*. 2014;112(6):1399–1405. doi:10.1016/j.prosdent.2014.08.002
56. Forster A, Ungvári K, Györgyey Á, Kukovecz Á, Turzó K, Nagy K. Human epithelial tissue culture study on restorative materials. *J Dent*. 2014; 42(1):7–14. doi:10.1016/j.jdent.2013.11.008
57. Tetè S, Zizzari VL, Borelli B, et al. Proliferation and adhesion capability of human gingival fibroblasts onto zirconia, lithium disilicate and feldspathic veneering ceramic in vitro. *Dent Mater J*. 2014;33(1):7–15. doi:10.4012/dmj.2013-185
58. Nandini S. Indirect resin composites. *J Conserv Dent*. 2010;13(4):184–194. doi:10.4103/0972-0707.73377
59. Quinn JB, Quinn GD. Material properties and fractography of an indirect dental resin composite. *Dent Mater*. 2010;26(6):589–599. doi:10.1016/j.dental.2010.02.008
60. Fahi Jr. N, Ritter AV. Composite veneers: The direct-indirect technique revisited. *J Esthet Restor Dent*. 2021;33(1):7–19. doi:10.1111/jerd.12696
61. Alzraikat H, Burrow MF, Maghairah GA, Taha NA. Nanofilled resin composite properties and clinical performance: A review. *Oper Dent*. 2018;43(4):E173–E190. doi:10.2341/17-208-T
62. Huth KC, Chen HY, Mehl A, Hickel R, Manhart J. Clinical study of indirect composite resin inlays in posterior stress-bearing cavities placed by dental students: Results after 4 years. *J Dent*. 2011;39(7):478–488. doi:10.1016/j.jdent.2011.04.005
63. Bagis YH, Rueggeberg FA. The effect of post-cure heating on residual, unreacted monomer in a commercial resin composite. *Dent Mater*. 2000;16(4):244–247. doi:10.1016/s0109-5641(00)00006-3
64. Spitznagel FA, Horvath SD, Guess PC, Blats MB. Resin bond to indirect composite and new ceramic/polymer materials: A review of the literature. *J Esthet Restor Dent*. 2014;26(6):382–393. doi:10.1111/jerd.12100
65. D'Arcangelo C, Vanini L. Effect of three surface treatments on the adhesive properties of indirect composite restorations. *J Adhes Dent*. 2007;9(3):319–326. PMID:17655072.
66. Bayraktar Y, Arslan M, Demirtag Z. Repair bond strength and surface topography of resin-ceramic and ceramic restorative blocks treated by laser and conventional surface treatments. *Microsc Res Tech*. 2021; 84(6):1145–1154. doi:10.1002/jemt.23672
67. Sismanoglu S, Yildirim-Bilmez Z, Erten-Taysi A, Ercal P. Influence of different surface treatments and universal adhesives on the repair of CAD-CAM composite resins: An in vitro study. *J Prosthet Dent*. 2020; 124(2):238.e1–238.e9. doi:10.1016/j.prosdent.2020.02.029
68. Tatar N, Ural C. Repair success of two innovative hybrid materials as a function of different surface treatments. *Int J Prosthodont*. 2018; 31(3):267–270. doi:10.11607/ijp.5581
69. Della Bona A, Corazza PH, Zhang Y. Characterization of a polymer-infiltrated ceramic-network material. *Dent Mater*. 2014;30(5):564–569. doi:10.1016/j.dental.2014.02.019
70. Coldea A, Swain MV, Thiel N. In-vitro strength degradation of dental ceramics and novel PICN material by sharp indentation. *J Mech Behav Biomed Mater*. 2013;26:34–42. doi:10.1016/j.jmbbm.2013.05.004
71. Argyrou R, Thompson GA, Cho SH, Berzins DW. Edge chipping resistance and flexural strength of polymer infiltrated ceramic network and resin nanoceramic restorative materials. *J Prosthet Dent*. 2016; 116(3):397–403. doi:10.1016/j.prosdent.2016.02.014
72. Coldea A, Fischer J, Swain MV, Thiel N. Damage tolerance of indirect restorative materials (including PICN) after simulated bur adjustments. *Dent Mater*. 2015;31(6):684–694. doi:10.1016/j.dental.2015.03.007
73. Fouquet V, Lachard F, Abdel-Gawad S, Dursun E, Attal JP, François P. Shear bond strength of a direct resin composite to CAD-CAM composite blocks: Relative contribution of micromechanical and chemical block surface treatment. *Materials (Basel)*. 2022;15(14):5018–5033. doi:10.3390/ma15145018
74. Dejak B, Młotkowski A, Romanowicz M. Finite element analysis of stresses in molars during clenching and mastication. *J Prosthet Dent*. 2003;90(6):591–597. doi:10.1016/j.prosdent.2003.08.009
75. Magne P, Belser UC. Porcelain versus composite inlays/onlays: Effects of mechanical loads on stress distribution, adhesion, and crown flexure. *Int J Periodontics Restorative Dent*. 2003;23(6):543–555. PMID:14703758.
76. Magne P. Virtual prototyping of adhesively restored, endodontically treated molars. *J Prosthet Dent*. 2010;103(6):343–351. doi:10.1016/S0022-3913(10)60074-1

77. De Carvalho Ramos N, Bastos Campos TM, De La Paz IS, et al. Microstructure characterization and SCG of newly engineered dental ceramics. *Dent Mater*. 2016;32(7):870–878. doi:10.1016/j.dental.2016.03.018
78. Zhang Y, Lawn BR. Novel zirconia materials in dentistry. *J Dent Res*. 2018;97(2):140–147. doi:10.1177/0022034517737483
79. Miyazaki T, Nakamura T, Matsumura H, Ban S, Kobayashi T. Current status of zirconia restoration. *J Prosthodont Res*. 2013;57(4):236–261. doi:10.1016/j.jpor.2013.09.001
80. Calvache Arcila LV, De Carvalho Ramos N, Bastos Campos TM, et al. Mechanical behavior and microstructural characterization of different zirconia polycrystals in different thicknesses. *J Adv Prosthodont*. 2021;13(6):385–395. doi:10.4047/jap.2021.13.6.385
81. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent Mater*. 2011;27(1):17–28. doi:10.1016/j.dental.2010.10.023
82. Kelly JR, Denry I. Stabilized zirconia as a structural ceramic: An overview. *Dent Mater*. 2008;24(3):289–298. doi:10.1016/j.dental.2007.05.005
83. Chevalier J, Gremillard L, Deville S. Low-temperature degradation of zirconia and implications for biomedical implants. *Annu Rev Mater Res*. 2007;37:1–32. doi:10.1146/annurev.matsci.37.052506.084250
84. Lawson S. Environmental degradation of zirconia ceramics. *J Eur Ceram Soc*. 1995;15(6):485–502. doi:10.1016/0955-2219(95)00035-5
85. Deville S, Chevalier J, Gremillard L. Influence of surface finish and residual stresses on the ageing sensitivity of biomedical grade zirconia. *Biomaterials*. 2006;27(10):2186–2192. doi:10.1016/j.biomaterials.2005.11.021
86. Rinke S, Fischer C. Range of indications for translucent zirconia modifications: Clinical and technical aspects. *Quintessence Int*. 2013;44(8):557–566. doi:10.3290/j.qi.a29937
87. Kern M. Bonding to oxide ceramics – laboratory testing versus clinical outcome. *Dent Mater*. 2015;31(1):8–14. doi:10.1016/j.dental.2014.06.007
88. Quigley NP, Loo DSS, Choy C, Ha WN. Clinical efficacy of methods for bonding to zirconia: A systematic review. *J Prosthet Dent*. 2021;125(2):231–240. doi:10.1016/j.prosdent.2019.12.017
89. Al-Zordk W, Ali Al. Retention strength of zirconia occlusal veneer restoration. Effect of dental bonding surface and cement type. *Int J Esthet Dent*. 2023;18(3):292–308. PMID:37462381.
90. Sagen MA, Vos L, Dahl JE, Ronold HJ. Shear bond strength of resin bonded zirconia and lithium disilicate – effect of surface treatment of ceramics and dentin. *Biomater Investig Dent*. 2022;9(1):10–19. doi:10.1080/26415275.2022.2038177
91. El Zayat SA, Younis JF, Wahsh MM. Bond strength of translucent zirconia and lithium-disilicate glass ceramic to dentin using different surface treatments. *Int J Dent Oral Health*. 2020;6(2).
92. Mohamed M, Farghaly E. Comparison of the shear bond strength of translucent zirconia and lithium disilicate ceramic following immediate dentin sealing. *Egypt Dent J*. 2022;68(2):1713–1720. doi:10.21608/edj.2022.122023.1984
93. 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 [published online as ahead of print on December 31, 2021]. *Dent Med Probl*. doi:10.17219/dmp/135652
94. Sarmento HR, Campos F, Sousa RS, et al. Influence of air-particle deposition protocols on the surface topography and adhesion of resin cement to zirconia. *Acta Odontol Scand*. 2014;72(5):346–353. doi:10.3109/00016357.2013.837958
95. Ahlers MO, Mörig G, Blunck U, Hajtó J, Pröbster L, Frankenberger R. Guidelines for the preparation of CAD/CAM ceramic inlays and partial crowns. *Int J Comput Dent*. 2009;12(4):309–325. PMID:20108869.
96. Arnetzl GV, Arnetzl G. Biomechanical examination of inlay geometries – is there a basic biomechanical principle? *Int J Comput Dent*. 2009;12(2):119–130. PMID:19413268.
97. Arnetzl GV, Arnetzl G. Design of preparations for all-ceramic inlay materials. *Int J Comput Dent*. 2006;9(4):289–298. PMID:17343243.
98. Otto T. Up to 27-years clinical long-term results of chairside Cerec 1 CAD/CAM inlays and onlays. *Int J Comput Dent*. 2017;20(3):315–329. PMID:28852748.
99. Swift Jr. EJ, Perdigão J, Heymann HO. Bonding to enamel and dentin: A brief history and state of the art, 1995. *Quintessence Int*. 1995;26(2):95–110. PMID:7568728.
100. Cardoso MV, De Almeida Neves A, Mine A, et al. Current aspects on bonding effectiveness and stability in adhesive dentistry. *Aust Dent J*. 2011;56(Suppl 1):31–44. doi:10.1111/j.1834-7819.2011.01294.x
101. Van Meerbeek B, De Munck J, Yoshida Y, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. *Oper Dent*. 2003;28(3):215–235. PMID:12760693.
102. Esquivel-Upshaw JF, Anusavice KJ, Yang MC, Lee RB. Fracture resistance of all-ceramic and metal-ceramic inlays. *Int J Prosthodont*. 2001;14(2):109–114. PMID:11843445.
103. Bakeman EM, Rego N, Chaiyubutr Y, Kois JC. Influence of ceramic thickness and ceramic materials on fracture resistance of posterior partial coverage restorations. *Oper Dent*. 2015;40(2):211–217. doi:10.2341/12-459-L
104. Banks RG. Conservative posterior ceramic restorations: A literature review. *J Prosthet Dent*. 1990;63(6):619–626. doi:10.1016/0022-3913(90)90316-5
105. Veneziani M. Posterior indirect adhesive restorations: updated indications and the morphology driven preparation technique. *Int J Esthet Dent*. 2017;12(2):204–230. PMID:28653051.
106. Ferraris F. Posterior indirect adhesive restorations (PIAR): Preparation designs and adhesion clinical protocol. *Int J Esthet Dent*. 2017;12(4):482–502. PMID:28983533.
107. Fradeani M, Barducci G. Versatility of IPS Empress restorations. Part II: Veneers, inlays, and onlays. *J Esthet Dent*. 1996;8(4):170–176. doi:10.1111/j.1708-8240.1996.tb00421.x
108. Hajizadeh H, Nemati-Karimoo A, Nasseh A, Rahmanpour N. Evaluating the shear bond strength of enamel and dentin with or without etching: A comparative study between dimethacrylate-based and silorane-based adhesives. *J Clin Exp Dent*. 2015;7(5):e563–e568. doi:10.4317/jced.52322
109. Gurel G, Morimoto S, Calamita MA, Coachman C, Sesma N. Clinical performance of porcelain laminate veneers: Outcomes of the aesthetic pre-evaluative temporary (APT) technique. *Int J Periodontics Restorative Dent*. 2012;32(6):625–635. PMID:23057051.
110. Pashley DH, Tay FR, Yiu C, et al. Collagen degradation by host-derived enzymes during aging. *J Dent Res*. 2004;83(3):216–221. doi:10.1177/154405910408300306
111. Tjäderhane L, Nascimento FD, Breschi L, et al. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. *Dent Mater*. 2013;29(1):116–135. doi:10.1016/j.dental.2012.08.004
112. Krejci I, Lutz F, Fülleman J. Tooth-colored inlays/overlays. Tooth-colored adhesive inlays and overlays: Materials, principles and classification [in French, German]. *Schweiz Monatsschr Zahnmed*. 1992;102(1):72–83. PMID:1738830.
113. Adolph G, Zehnder M, Bachmann LM, Göhring TN. Direct resin composite restorations in vital versus root-filled posterior teeth: A controlled comparative long-term follow-up. *Oper Dent*. 2007;32(5):437–442. doi:10.2341/06-147
114. Lin CL, Chang YH, Pai CA. Evaluation of failure risks in ceramic restorations for endodontically treated premolar with MOD preparation. *Dent Mater*. 2011;27(5):431–438. doi:10.1016/j.dental.2010.10.026
115. Bitter K, Meyer-Lueckel H, Fotiadis N, et al. Influence of endodontic treatment, post insertion, and ceramic restoration on the fracture resistance of maxillary premolars. *Int Endod J*. 2010;43(6):469–477. doi:10.1111/j.1365-2591.2010.01701.x
116. Hoopes W, Cushen S, DuVall N, Wajdowicz M, Brewster J, Roberts H. Failure load effect of molar axial wall height with CAD/CAM ceramic crowns with moderate occlusal convergence. *J Esthet Restor Dent*. 2018;30(3):249–253. doi:10.1111/jerd.12366
117. Loi I, Di Felice A. Biologically oriented preparation technique (BOPT): A new approach for prosthetic restoration of periodontically healthy teeth. *Eur J Esthet Dent*. 2013;8(1):10–23. PMID:23390618.
118. Serra-Pastor B, Loi I, Fons-Font A, Solá-Ruiz MF, Agustin-Panadero R. Periodontal and prosthetic outcomes on teeth prepared with biologically oriented preparation technique: A 4-year follow-up prospective clinical study. *J Prosthodont Res*. 2019;63(4):415–420. doi:10.1016/j.jpor.2019.03.006
119. Tupac RG, Neacy K. A comparison of cord gingival displacement with the gingitage technique. *J Prosthet Dent*. 1981;46(5):509–515. doi:10.1016/0022-3913(81)90239-0
120. Carnevale G, Sterrantino SF, Di Febo G. Soft and hard tissue wound healing following tooth preparation to the alveolar crest. *Int J Periodontics Restorative Dent*. 1983;3(6):36–53. PMID:6584413.

121. Dietschi D, Spreafico R. Current clinical concepts for adhesive cementation of tooth-colored posterior restorations. *Pract Periodontics Aesthet Dent*. 1998;10(1):47–54. PMID:9582662.
122. Juloski J, Köken S, Ferrari M. Cervical margin relocation in indirect adhesive restorations: A literature review. *J Prosthodont Res*. 2018;62(3):273–280. doi:10.1016/j.jpor.2017.09.005
123. Kielbassa AM, Philipp F. Restoring proximal cavities of molars using the proximal box elevation technique: Systematic review and report of a case. *Quintessence Int*. 2015;46(9):751–764. doi:10.3290/j.qi.a34459
124. Da Silva Gonçalves D, Cura M, Ceballos L, Fuentes MV. Influence of proximal box elevation on bond strength of composite inlays. *Clin Oral Investig*. 2017;21(1):247–254. doi:10.1007/s00784-016-1782-7
125. Zaruba M, Göhring TN, Wegehaupt FJ, Attin T. Influence of a proximal margin elevation technique on marginal adaptation of ceramic inlays. *Acta Odontol Scand*. 2013;71(2):317–324. doi:10.3109/00016357.2012.680905
126. Juloski J, Köken S, Ferrari M. No correlation between two methodological approaches applied to evaluate cervical margin relocation. *Dent Mater J*. 2020;39(4):624–632. doi:10.4012/dmj.2018-410
127. Magne P. IDS: Immediate dentin sealing (IDS) for tooth preparations. *J Adhes Dent*. 2014;16(6):594. doi:10.3290/j.jad.a33324
128. Sarfati A, Tirlet G. Deep margin elevation versus crown lengthening: Biologic width revisited. *Int J Esthet Dent*. 2018;13(3):334–356. PMID:30073217.
129. Veneziani M. Adhesive restorations in the posterior area with subgingival cervical margins: New classification and differentiated treatment approach. *Eur J Esthet Dent*. 2010;5(1):50–76. PMID:20305873.
130. Ferrari M, Köken S, Grandini S, Cagidiaco EF, Joda T, Discepoli. Influence of cervical margin relocation (CMR) on periodontal health: 12-month results of a controlled trial. *J Dent*. 2018;69:70–76. doi:10.1016/j.jdent.2017.10.008
131. Castelo-Baz P, Argibay-Lorenzo O, Munoz F, et al. Periodontal response to a tricalcium silicate material or resin composite placed in close contact to the supracrestal tissue attachment: A histomorphometric comparative study. *Clin Oral Investig*. 2021;25(10):5743–5753. doi:10.1007/s00784-021-03876-x
132. Spreafico R, Marchesi G, Turco G, et al. Evaluation of the in vitro effects of cervical marginal relocation using composite resins on the marginal quality of CAD/CAM crowns. *J Adhes Dent*. 2016;18(4):355–362. doi:10.3290/j.jad.a36514
133. Müller V, Friedl KH, Friedl K, Hahnel S, Handel G, Lang R. Influence of proximal box elevation technique on marginal integrity of adhesively luted Cerec inlays. *Clin Oral Investig*. 2017;21(2):607–612. doi:10.1007/s00784-016-1927-8
134. Köken S, Juloski J, Sorrentino R, Grandini S, Ferrari M. Marginal sealing of relocated cervical margins of mesio-occluso-distal overlays. *J Oral Sci*. 2018;60(3):460–468. doi:10.2334/josnusd.17-0331
135. Marchesi G, Spreafico R, Frassetto A, et al. Cervical margin-relocation of CAD/CAM lithium disilicate ceramic crown using resin-composite. *Dent Mater*. 2014;30(Suppl 1):e14. doi:10.1016/j.dental.2014.08.029
136. Vertolli TJ, Martinsen BD, Hanson CM, Howard RS, Kooistra S, Ye L. Effect of deep margin elevation on CAD/CAM-fabricated ceramic inlays. *Oper Dent*. 2020;45(6):608–617. doi:10.2341/18-315-L
137. Zhang H, Li H, Cong Q, Zhang Z, Du A, Wang Y. Effect of proximal box elevation on fracture resistance and microleakage of premolars restored with ceramic endocrowns. *PLoS One*. 2021;16(5):e0252269. doi:10.1371/journal.pone.0252269
138. Frankenberger R, Hehn J, Hajtő J, et al. Effect of proximal box elevation with resin composite on marginal quality of ceramic inlays in vitro. *Clin Oral Investig*. 2013;17(1):177–183. doi:10.1007/s00784-012-0677-5
139. Dadlanca-Blanco AB, Blanco-Carrión J, Martín-Biedma B, Varela-Patiño P, Bello-Castro A, Castelo-Baz P. Management of large class II lesions in molars: How to restore and when to perform surgical crown lengthening? *Restor Dent Endod*. 2017;42(3):240–252. doi:10.5395/rde.2017.42.3.240
140. Swift Jr. E.J. Critical appraisal: Immediate dentin sealing for indirect bonds restorations. *J Esthet Restor Dent*. 2009;21(1):62–67. doi:10.1111/j.1708-8240.2008.00232.x
141. Harden L, Devoto W, Bourgi R, et al. Immediate dentin sealing for adhesive cementation of indirect restorations: A Systematic review and meta-analysis. *Gels*. 2022;8(3):175. doi:10.3390/gels8030175
142. Paul SJ, Schärer P. Effect of provisional cements on the bond strength of various adhesive bonding systems on dentine. *J Oral Rehabil*. 1997;24(1):8–14. doi:10.1046/j.1365-2842.1997.00484.x
143. Samartzis TK, Papalexopoulos D, Sarafianou A, Kourtis S. Immediate dentin sealing: A literature review. *Clin Cosmet Investig Dent*. 2021;13:233–256. doi:10.2147/CCIDE.S307939
144. Magne P, So WS, Cascione D. Immediate dentin sealing supports delayed restoration placement. *J Prosthet Dent*. 2007;98(3):166–174. doi:10.1016/S0022-3913(07)60052-3
145. Elbishari H, Elsubeihi ES, Alkhouljah T, Elsubeihi HE. Substantial in-vitro and emerging clinical evidence supporting immediate dentin sealing. *Jpn Dent Sci Rev*. 2021;57:101–110. doi:10.1016/j.jdsr.2021.05.004
146. Choi YS, Choi IH. An effect of immediate dentin sealing on the shear bond strength of resin cement to porcelain restoration. *J Adv Prosthodont*. 2010;2(2):39–45. doi:10.4047/jap.2010.2.2.39
147. Gresnigt MM, Cune MS, De Roos JG, Özcan M. Effect of immediate and delayed dentin sealing on the fracture strength, failure type and Weibull characteristics of lithium disilicate laminate veneers. *Dent Mater*. 2016;32(4):e73–e81. doi:10.1016/j.dental.2016.01.001
148. Magne P, Douglas WH. Porcelain veneers: Dentin bonding optimization and biomimetic recovery of the crown. *Int J Prosthodont*. 1999;12(2):111–121. PMID:10371912.
149. Dietschi D, Herzfeld D. In vitro evaluation of marginal and internal adaptation of class II resin composite restorations after thermal and occlusal stressing. *Eur J Oral Sci*. 1998;106(6):1033–1042. doi:10.1046/j.0909-8836.1998.eos106609.x
150. Frankenberger R, Sindel J, Krämer N, Petschelt A. Dentin bond strength and marginal adaptation: Direct composite resins vs ceramic inlays. *Oper Dent*. 1999;24(3):147–155. PMID:10530276.
151. McCabe JF, Rusby S. Dentine bonding – the effect of pre-curing the bonding resin. *Br Dent J*. 1994;176(9):333–336. doi:10.1038/sj.bdj.4808447
152. Abu Nawareg MM, Zidan AZ, Zhou J, Chiba A, Tagami J, Pashley DH. Adhesive sealing of dentin surfaces in vitro: A review. *Am J Dent*. 2015;28(6):321–332. PMID:26846037. PMCID:PMC4943037.
153. Spohr AM, Borges GA, Platt JA. Thickness of immediate dentin sealing materials and its effect on the fracture load of a reinforced all-ceramic crown. *Eur J Dent*. 2013;7(4):474–483. doi:10.4103/1305-7456.120682
154. Qanungo A, Aras MA, Chitre V, Mysore A, Amin B, Daswani SR. Immediate dentin sealing for indirect bonded restorations. *J Prosthodont Res*. 2016;60(4):240–249. doi:10.1016/j.jpor.2016.04.001
155. Stavridakis MM, Krejci I, Magne P. Immediate dentin sealing of onlay preparations: Thickness of pre-cured dentin bonding agent and effect of surface cleaning. *Oper Dent*. 2005;30(6):747–757. PMID:16382598.
156. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? *J Can Dent Assoc*. 2003;69(11):726–731. PMID:14653938.
157. Rueggeberg FA, Margeson DH. The effect of oxygen inhibition on an unfilled/filled composite system. *J Dent Res*. 1990;69(10):1652–1658. doi:10.1177/00220345900690100501
158. Erickson RL. Mechanism and clinical implications of bond formation for two dentin bonding agents. *Am J Dent*. 1989;2:117–123. PMID:2517773.
159. Magne P, Nielsen B. Interactions between impression materials and immediate dentin sealing. *J Prosthet Dent*. 2009;102(5):298–305. doi:10.1016/S0022-3913(09)60178-5
160. Wayakanon P, Narakaew T, Wayakanon K. Effects of various beverages on characteristics of provisional restoration materials. *Clin Exp Dent Res*. 2024;10(2):e842. doi:10.1002/cre2.842
161. Ghiggi PC, Steiger AK, Marcondes ML, Mota EG, Burnett Jr. LH, Spohr AM. Does immediate dentin sealing influence the polymerization of impression materials? *Eur J Dent*. 2014;8(3):366–372. doi:10.4103/1305-7456.137650
162. Pheerarangsikul N, Wayakanon P, Wayakanon K. Effects of various functional monomers on adhesion between immediate dentin sealing and resin cement. *Oper Dent*. 2022;47(5):562–573. doi:10.2341/21-057-L

163. 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
164. Yahyazadehfard M, Mutluay MM, Majd H, Ryou H, Arola D. Fatigue of the resin–enamel bonded interface and the mechanisms of failure. *J Mech Behav Biomed Mater.* 2013;21:121–132. doi:10.1016/j.jmbbm.2013.02.017
165. De Munck J, Braem M, Wevers M, et al. Micro-rotary fatigue of tooth–biomaterial interfaces. *Biomaterials.* 2005;26(10):1145–1153. doi:10.1016/j.biomaterials.2004.04.013
166. Elbahie E, Beitzel D, Mutluay MM, Majd H, Yahyazadehfard M, Arola D. Durability of adhesive bonds to tooth structure involving the DEJ. *J Mech Behav Biomed Mater.* 2018;77:557–565. doi:10.1016/j.jmbbm.2017.10.001
167. Fabbri G, Zarone F, Dellificorelli G, et al. Clinical evaluation of 860 anterior and posterior lithium disilicate restorations: Retrospective study with a mean follow-up of 3 years and a maximum observational period of 6 years. *Int J Periodontics Restorative Dent.* 2014;34(2):165–177. doi:10.11607/prd.1769
168. Granell-Ruiz M, Agustín-Panadero R, Fons-Font A, Román-Rodríguez JL, Solá-Ruiz MF. Influence of bruxism on survival of porcelain laminate veneers. *Med Oral Patol Oral Cir Bucal.* 2014;19(5):e426–e432. doi:10.4317/medoral.19097
169. Smales RJ, Etemadi S. Survival of ceramic onlays placed with and without metal reinforcement. *J Prosthet Dent.* 2004;91(6):548–553. doi:10.1016/j.prosdent.2004.03.011
170. Souto Borges AL, Dos Santos Rangel Silva R, De Matos JD, et al. Table-top lithium disilicate ceramic restoration thickness effect on the stress distribution in upper premolars: 3D finite element analysis. *Int J Odontostomat.* 2021;15(4):942–947. doi:10.4067/S0718-381X2021000400942
171. Tribst JPM, Dos Santos AF, Da Cruz Santos G, et al. Effect of cement layer thickness on the immediate and long-term bond strength and residual stress between lithium disilicate glass-ceramic and human dentin. *Materials (Basel).* 2021;14(18):5153. doi:10.3390/ma14185153
172. Poca A, De Peretti Della Rocca K, Nasr K, Ducassé R, Canceill T. Effects of translucency and thickness of lithium disilicate-reinforced glass-ceramic veneers on the degree of conversion of a purely light-curing bonding resin: An in vitro study. *Polymers.* 2023;15(7):1617–1625. doi:10.3390/polym15071617
173. Špehar D, Jakovac M. Clinical evaluation of reduced-thickness monolithic lithium-disilicate crowns: One-year follow-up results. *Processes.* 2021;9(12):2119. doi:10.3390/pr9122119

Enhancement of physical and antimicrobial properties of mineral trioxide aggregate (MTA) reinforced with gold nanoparticles: An in vitro preliminary study

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Abstract

Background. Clinical use of mineral trioxide aggregate (MTA) is often associated with several challenges, including a prolonged setting time, poor handling characteristics and compromised physical properties in the presence of tissue fluids. Mineral trioxide aggregate, when used as a perforation repair or root-end filling material, needs adequate strength to withstand the forces acting on it. Recently, several researchers have attempted to overcome these challenges through the use of various additives.

Objectives. To evaluate the physical and antimicrobial properties of MTA reinforced with gold nanoparticles (AuNPs).

Material and methods. Sixteen customized putty molds (6 mm in height x 4 mm in diameter) were filled with the materials assigned to 2 groups: control group, MTA ($n = 8$); and experimental group, MTA + AuNPs ($n = 8$). Compressive strength (CS) was evaluated using a universal testing machine, while setting time was assessed using a flat-ended indenter. The antimicrobial properties of MTA and MTA + AuNPs were evaluated using the agar diffusion method against *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. The data was statistically analyzed using an unpaired *t*-test.

Results. Compressive strength was significantly higher in the MTA + AuNPs group (115.75 ± 2.22 MPa) than in the MTA group (55.00 ± 5.35 MPa) ($p < 0.001$). The MTA + AuNPs group also demonstrated a significantly shorter setting time than the MTA group (48.05 min vs. 165.00 min, respectively). The unpaired *t*-test revealed a significantly greater antimicrobial activity against all tested microorganisms in the MTA + AuNPs group than in the MTA group ($P. aeruginosa > E. coli > S. aureus > E. faecalis > C. albicans$).

Conclusions. Incorporation of AuNPs into MTA enhanced CS, shortened setting time and increased antimicrobial activity against the tested microorganisms.

Keywords: compressive strength, mineral trioxide aggregate, gold nanoparticles, setting time, antimicrobial efficacy

Highlights

- The incorporation of 1% gold nanoparticles (AuNPs) significantly increased the compressive strength of mineral trioxide aggregate (MTA).
- Gold nanoparticles significantly reduced the setting time of MTA.
- Gold nanoparticle-reinforced MTA demonstrated enhanced antimicrobial activity against all tested microorganisms.
- These findings support AuNPs as a potential additive for improving MTA properties.

Introduction

Mineral trioxide aggregate (MTA), owing to its superior biocompatibility and cementogenic properties, has shown successful outcomes when used for perforation repair, pulp capping, apexification, or as a retrograde filling material.¹ Hydration of this silicate-based biomaterial with distilled water leads to the formation of calcium silicate hydrate and calcium hydroxide in the crystalline phase of set MTA, followed by the formation of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). An extended setting time, poor handling characteristics and compromised physical characteristics in the presence of tissue fluids are the main challenges of MTA, especially when used as a perforation repair or root-end filling material, where adequate strength is required to withstand the forces acting on the material.² Recently, a number of researchers have attempted to address these issues by utilizing specific additives (calcium chloride, bioactive glass, TiO_2 particles) and by using alternative hydration liquids (calcium chloride and calcium gluconate).³

In recent years, nanotechnology has emerged as a promising approach for enhancing the physical properties of various dental materials.⁴ A greater surface-to-volume ratio and a greater quantity of atoms at their grain peripheries make nanoparticles (NPs) unique. The physical and chemical properties of materials become quite different when the size and number of atoms are greatly reduced.^{5,6} Nanoparticles have the ability to be easily arranged in various configurations due to their high surface-to-core ratio, thus making them amenable to manipulation and enabling diverse applications. Nanoparticles have found their place in various aspects of dental sciences, including the use of titanium dioxide or zirconium dioxide NPs for improving the physical properties of denture resin, the addition of bioglass, zirconia and glass ceramics to endodontic sealers, or the addition of nanosilver to root canal disinfectants, to name a few.⁷

A nanoparticle that has recently garnered significant interest is the gold nanoparticle (AuNP). Being one of the oldest dental restorative materials, gold is known for its superior antibacterial properties and high biocompatibility.⁸ When converted to an NP form, its properties are further enhanced. Gold NPs have high

chemical stability, photostability and low toxicity.⁹ The use of organic complexes of Au(I) and Au(III) ions as a mode of antibiotic delivery enhances the bactericidal effect of the antibiotics.¹⁰ Many studies have investigated the use of AuNPs in dentistry, including applications in AuNP-coated dental floss, AuNP-modified dental aligners, AuNP-stabilized dental implants, and tumor screening.^{11,12}

The present study aimed to evaluate the potential positive effects of the incorporation of AuNPs on the physical and antimicrobial properties of MTA. The null hypothesis was that the addition of AuNPs would not enhance the antimicrobial efficacy, increase the compressive strength (CS) or reduce the setting time of MTA.

Material and methods

Synthesis of citrate-capped AuNPs

Gold nanoparticles (30 nm in diameter) were produced by refluxing 0.625 mL of 1% aqueous trisodium citrate with 50 mL of 0.25 mM chloroauric acid (HAuCl_4) until the solution turned wine red. Trisodium citrate acted as both a reducing and a stabilizing agent. The resulting AuNP solution was collected and lyophilized for further use. The size of the AuNPs was confirmed by placing 2.0 μL of the AuNP solution at a concentration of 0.1 mg/mL^{-1} in purified water (Milli-Q® H_2O ; Merk Millipore, Darmstadt, Germany) on a carbon-coated grid and allowing it to dry. The sample was then examined with the use of a transmission electron microscope (Leo 912 AB; Carl Zeiss, Oberkochen, Germany) at 120 kV ($\times 40,000$ magnification).¹³

Preparation of a mixture of MTA and AuNPs

Sixteen customized cylindrical molds made of addition silicone putty, with a height of 6 mm and an internal diameter of 4 mm, were prepared (Advancing Standards Transforming Markets (ASTM) standard E384).¹⁴ Mineral trioxide aggregate (8 g) and AuNPs corresponding to 1% of the MTA weight (0.08 g) were weighed using an electronic weighing scale (Wensar, Chennai, India). The materials

were combined and mixed with a stainless-steel spatula on a glass slab using saline as the liquid phase at a powder-to-liquid ratio of 3:1. The resulting MTA–AuNP mixture constituted the experimental material.

For comparison of the physical properties, the specimens were allocated to 2 groups:

- control group: MTA alone ($n = 8$);
- experimental group: MTA containing 1% AuNPs by weight ($n = 8$).

Compressive strength testing

Four samples from each group were used for CS testing. The mixed cement was packed into the molds and stored in an incubator at 37°C for 4 days. After setting, the specimens were removed from the molds and visually inspected for voids and chipped edges.

Compressive strength was evaluated with a universal testing machine (Heico, New Delhi, India). A compressive load was applied parallel to the long axis of the molds at a crosshead speed of 1 mm/min until the fracture occurred. The maximum load required to fracture each specimen was recorded. Compressive strength was calculated using the following formula:

$$CS = \text{applied load [N]} / \text{area [mm}^2\text{]}$$

Setting time testing

Setting time was evaluated in accordance with ISO 9917-1. A flat-ended indenter measuring 1.0 mm in diameter and carrying 400 g load was carefully applied vertically to the surface of the tested material. The procedure was repeated every 60 s. The final setting time was noted when the indenter no longer produced an indentation on the material.¹⁵

Antimicrobial activity testing

Antimicrobial activity was evaluated against 4 bacterial species: *Enterococcus faecalis* (ATCC 29212); *Escherichia coli* (ATCC 25922); *Pseudomonas aeruginosa*

(ATCC 15692); and *Staphylococcus aureus* (ATCC 29213), as well as 1 fungal species, *Candida albicans* (ATCC 10231). Antimicrobial efficacy was assessed using a double-layer agar diffusion method. Plates containing fresh cultures were prepared for all microorganisms tested. Two wells, each measuring 4 mm in depth and 6 mm in diameter, were prepared in each plate using a sterile copper band and filled with the respective test materials. The plates were maintained at room temperature for 2 h to allow pre-diffusion of the materials and were subsequently incubated at 37°C for either 24 h or 72 h. The microbial inhibition zones surrounding the set materials were measured using a caliper with a precision of 0.1 mm.

Statistical analysis

An independent samples *t*-test was used to compare the mean values of the physical and antimicrobial properties between the 2 groups. A two-sided (two-tailed) test with a significance level of $\alpha = 0.05$ was used. A *p*-value <0.05 was considered statistically significant.

Results

Tables 1 and 2 present the mean and standard deviation values for the physical properties and growth inhibition zone diameters against the different microorganisms tested in the MTA and MTA + AuNPs groups. For both physical properties evaluated, the unpaired *t*-test demonstrated a significant effect of material type (with and without AuNPs) on setting time and CS. Compared with the control group, the experimental group showed significantly higher CS and lower setting time values ($p < 0.001$). The unpaired *t*-test also revealed significant differences between MTA and MTA + AuNPs in antimicrobial activity against *E. faecalis*, *C. albicans*, *E. coli*, *P. aeruginosa*, and *S. aureus* ($p < 0.001$). Overall, the antimicrobial activity ranked in descending order as: *P. aeruginosa* > *E. coli* > *S. aureus* > *E. faecalis* > *C. albicans*.

Table 1. Intergroup comparison of compressive strength (CS) and setting time

Variable	Control group (MTA)		Experimental group (MTA + AuNPs)		<i>t</i> -value	<i>p</i> -value
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>		
Compressive strength [N]	55.00	5.35	115.75	2.22	20.97	<0.001*
Setting time [min]	165.00	1.63	48.05	0.40	139.04	<0.001*

* statistically significant ($p < 0.05$, independent samples *t*-test); *M* – mean; *SD* – standard deviation; MTA – mineral trioxide aggregate; AuNPs – gold nanoparticles.

Table 2. Intergroup comparison of antimicrobial activity against the tested microorganisms

Variable	Microorganism	Incubation time	Control group (MTA)		Experimental group (MTA + AuNPs)		t-value	p-value
			M	SD	M	SD		
Zone of inhibition [mm]	<i>E. faecalis</i>	24 h	6.75	0.50	17.75	0.96	−20.37	<0.001*
		72 h	7.75	0.50	20.50	0.58	−33.39	<0.001*
	<i>C. albicans</i>	24 h	6.75	0.50	15.25	0.50	−24.04	<0.001*
		72 h	9.00	0.82	15.50	0.58	−13.00	<0.001*
	<i>E. coli</i>	72 h	8.00	0.82	25.25	0.96	−27.42	<0.001*
		24 h	7.00	0.82	27.00	0.82	−34.64	<0.001*
	<i>P. aeruginosa</i>	24 h	10.00	0.82	28.50	0.58	−37.00	<0.001*
		72 h	8.00	0.82	29.25	0.50	−44.39	<0.001*
	<i>S. aureus</i>	72 h	9.00	0.82	24.50	0.58	−31.00	<0.001*
		24 h	8.00	0.82	25.50	0.58	−35.00	<0.001*

* statistically significant ($p < 0.05$, independent samples t -test).

Discussion

The first known use of AuNPs dates back to 1971.¹⁶ Gold nanoparticles are characterized by chemical stability, compatibility and antibacterial activity, making them a potential additive to dental cements, particularly because they can retain their nanometric dimensions in the presence of an appropriate stabilizer.⁸ In the present study, AuNPs were produced using a reduction method with sodium citrate as the reducing agent. This approach is widely employed due to its versatility and the ease with which particle size and shape can be controlled. To prevent NP aggregation, citrate was used as a capping agent to stabilize the NPs.¹⁷

The physical parameters evaluated in the present study were setting time and CS, while antimicrobial efficacy was assessed against the bacterial species (*E. coli*, *E. faecalis*, *S. aureus*, *P. aeruginosa*) and the fungal species (*C. albicans*). Gram-positive bacteria are predominant in primary root canal infections, while *E. faecalis* and yeasts such as *C. albicans* are more commonly found in retreatment cases.¹⁸

High CS is a critical property of any dental restorative material, as the restoration must withstand masticatory forces that may reach 284 N.¹⁹ The results of the present study showed a significant increase (110.5%) in mean CS in the experimental group compared with the control group (115.75 $\pm$ 2.22 MPa vs. 55.00 $\pm$ 5.35 MPa, respectively; $p < 0.001$) (Table 1). High CS is particularly desirable when MTA is used as a perforation repair material, for pulp capping, or as an apical plug in apexogenesis. It has been reported that the initial CS of MTA is 40 MPa and may increase to 67 MPa within 3 weeks.¹⁹ The CS of calcium silicate-based biomaterials reflects the extent to which hydration and maturation occur during cement setting. The increase in CS observed after the incorporation of AuNPs may be attributed to accelerated hydration,

reduced cement porosity, increased density of particle interfaces, and the ability of the NPs to act against compressive forces.^{20,21}

Similar outcomes with the use of NPs have been reported in previous studies. Samiei et al. observed a substantial increase in the CS of MTA following the incorporation of 1% by weight TiO₂ NPs, while Elsaka et al. noted improved CS when glass ionomer cement (GIC) was reinforced with TiO₂ NPs.^{22,23} Reinforcement of GIC with 3% TiO₂ NPs improved CS; however, higher concentrations of 5% and 7% adversely affected surface microhardness. This effect may be attributed to the smaller particle size and larger surface area of TiO₂ NPs compared with glass particles, resulting in an insufficient amount of the polyacrylic ionomer matrix to effectively bind the increased quantity of TiO₂ NP powders and consequently weakening the interfacial bonding between the particles and the ionomer matrix. Interestingly, another study by Samiei et al. reported contrasting results, with a noteworthy reduction in the CS of MTA following the incorporation of 2% by weight Ze–Ag–Zn NPs.²⁴ These findings suggest that both NP size and the amount incorporated have an effect on the physical properties of the biomaterial. As no previous studies have evaluated the effect of AuNP incorporation on the properties of MTA, a concentration of 1% AuNPs by weight was selected based on findings obtained with other nanoparticles. Higher AuNP concentrations might adversely affect the adequacy of MTA constituents required to maintain its inherent bioactive and physical properties.

A long setting time of almost 3 h has long been considered a drawback of MTA.²⁵ This is particularly relevant when MTA is used as a root-end filling material following apical surgery or as a perforation repair material, because the unset material may be susceptible to contamination or washout. Clinically, a setting time of up to 25–30 min is desirable, as faster setting reduces the likelihood of contamination and provides greater initial strength, thereby reducing the

risk of material washout. The results of the present study showed that the mean setting time was significantly shorter in the MTA + AuNPs group than in the MTA group (48.05 ± 0.40 min (range: 47.50–48.40 min) vs. 165.00 ± 1.63 min (range: 163–167 min), respectively; $p < 0.001$).

A smaller particle size of the powder reactant provides a larger surface area per unit mass, which catalyzes the reaction. Thus, the setting reaction is influenced by the size of the NPs incorporated into the material.²⁶ The nanostructure and composition of the unreacted powder facilitate rapid hydration and the formation of a nanoporous calcium aluminate silicate hydrate microstructure, thereby promoting both rapid setting and an effective bioactive response.²⁷

Evaluation of the antimicrobial properties showed that MTA reinforced with AuNPs produced significantly larger zones of inhibition against all tested microorganisms than MTA alone. The antimicrobial activity ranked in descending order as: *P. aeruginosa* > *E. coli* > *S. aureus* > *E. faecalis* > *C. albicans*.

Previous studies have similarly demonstrated the potential of nanotechnology to improve the antimicrobial efficacy of MTA. In a study conducted by Samiei et al. in 2013, the antimicrobial properties of MTA and MTA containing silver NPs (MTA/SN) were tested against *E. faecalis*, *P. aeruginosa*, *S. aureus*, and *C. albicans*. For *E. faecalis*, *C. albicans* and *P. aeruginosa*, the microbial inhibition zones produced by MTA/SN were significantly larger than those produced by MTA alone.²⁸ The results of the present study are also consistent with those reported by Akbari et al., who observed increased antimicrobial efficacy of MTA with and without silver NPs against *E. faecalis*, *S. aureus* and *C. albicans*.²⁹

In the present study, the antimicrobial effect of AuNPs differed between gram-positive and gram-negative bacteria. The difference may be related to differences in bacterial membrane structure, particularly the thickness of the peptidoglycan layer, which is 50% higher in gram-positive than gram-negative bacteria.²⁹

The significant difference between the 2 groups may also be related to direct interactions between AuNPs and bacterial cells, potentially resulting in membrane penetration and disruption of cellular function. The high surface-to-volume ratio of NPs may enhance their antibacterial activity by promoting intracellular generation of reactive oxygen species.³⁰ Gold nanoparticles incorporated into MTA exerted antimicrobial effects by altering membrane potential and reducing adenosine triphosphate synthase activity, thereby disrupting bacterial metabolism. Another significant advantage of NPs is that, unlike antibiotics, they are less likely to promote bacterial resistance, as NPs directly interact with bacterial cell walls, leading to cell death.^{31,32}

The present investigation was limited by its in vitro design and relatively small sample size. Further research should evaluate different concentrations and particle sizes of AuNPs. A clinical study will be required to validate the results obtained in the present study.

Conclusions

The findings of the study suggest that the addition of AuNPs to MTA increases CS, reduces setting time, and enhances antimicrobial activity against the tested species. Reinforcement of MTA with AuNPs appears to improve material properties and may therefore have potential clinical relevance. Further studies evaluating different AuNP concentrations are warranted.

Ethics approval and consent to participate

Approval from the Institutional Ethics Committee was obtained prior to the commencement of the study (Ref. No. 90th ECM II BThesis/P9).

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. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. *J Endod.* 1999;25(3):197–205. doi:10.1016/S0099-2399(99)80142-3
2. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review – Part III: Clinical applications, drawbacks, and mechanism of action. *J Endod.* 2010;36(3):400–413. doi:10.1016/j.joen.2009.09.009
3. Kim J, Kim HJ, Chang SW, et al. Effect of bioactive glass addition on the physical properties of mineral trioxide aggregate. *Biomater Res.* 2021;25(1):39. doi:10.1186/s40824-021-00238-2
4. Pokrowiecki R, Pałka K, Mielczarek A. Nanomaterials in dentistry: A cornerstone or a black box? *Nanomedicine (Lond).* 2018;13(6):639–667. doi:10.2217/nnm-2017-0329
5. Azam A, Ahmed F, Arshi N, Chaman M, Naqvi AH. One step synthesis and characterization of gold nanoparticles and their antibacterial activities against *E. coli* (ATCC 25922 strain). *Int J Theor Appl Sci.* 2009;1(2):1–4. <https://www.researchtrend.net/ijtas/tas12/1%20AMEER%20AZAM.pdf>. Accessed February 20, 2024.
6. Sun Y, An C. Shaped gold and silver nanoparticles. *Front Mater Sci.* 2011;5(1):1–24. doi:10.1007/s11706-011-0100-1
7. AlKahtani RN. The implications and applications of nanotechnology in dentistry: A review. *Saudi Dent J.* 2018;30(2):107–116. doi:10.1016/j.sdentj.2018.01.002

8. Zhan X, Yan J, Tang H, Xia D, Lin H. Antibacterial properties of gold nanoparticles in the modification of medical implants: A systematic review. *Pharmaceutics*. 2022;14(12):2654. doi:10.3390/pharmaceutics14122654
9. Hwang S, Nam J, Jung S, Song J, Doh H, Kim S. Gold nanoparticle-mediated photothermal therapy: Current status and future perspective. *Nanomedicine (Lond)*. 2014;9(13):2003–2022. doi:10.2217/nnm.14.147
10. Chitra K, Annadurai G. Antibacterial activity of pH-dependent bio-synthesized silver nanoparticles against clinical pathogen. *BioMed Res Int*. 2014;2014:725165. doi:10.1155/2014/725165
11. Traitanon N, Dechkunakorn S, Tantivitayakul P, et al. Antibacterial effect of gold nanoparticles coated dental floss against cariogenic bacteria. *Key Eng Mater*. 2021;904:293–300. doi:10.4028/www.scientific.net/KEM.904.293
12. Zhang M, Liu X, Xie Y, et al. Biological safe gold nanoparticle-modified dental aligner prevents the *Porphyromonas gingivalis* biofilm formation. *ACS Omega*. 2020;5(30):18685–18692. doi:10.1021/acsomega.0c01532
13. Agarwal S, Mishra P, Shivange G, et al. Citrate-capped gold nanoparticles for the label-free detection of ubiquitin C-terminal hydrolase-1. *Analyst*. 2015;140(4):1166–1173. doi:10.1039/C4AN01935K
14. Bolhari B, Meraji N, Rezazadeh Sefideh M, Pedram P. Evaluation of the properties of mineral trioxide aggregate mixed with zinc oxide exposed to different environmental conditions. *Bioact Mater*. 2020;5(3):516–521. doi:10.1016/j.bioactmat.2020.04.001
15. ISO 9917-1. Dentistry – Water-based cements – Part 1: Powder/liquid acid-base cements. <https://cdn.standards.iteh.ai/samples/45818/68572262f2584ca686db446f0aa522f5/ISO-9917-1-2007.pdf>. Accessed September 21, 2023.
16. Faulk WP, Taylor GM. *Immunochemistry*. 1971;8(11):1081–1083. doi:10.1016/0019-2791(71)90496-4
17. Das E. Development of nano structure plasmon gold by green synthesis for fabrication of bio/chemical sensor. *Res J Recent Sci*. 2013;3:14–19.
18. Ryan K, Ray CG. *Sherris Medical Microbiology: An Introduction to Infectious Diseases*. New York, NY: McGraw Hill Medical; 2004:294–295.
19. de Abreu RAM, Pereira MD, Furtado F, Prado GPR, Mestriner Jr W, Ferreira LM. Masticatory efficiency and bite force in individuals with normal occlusion. *Arch Oral Biol*. 2014;59(10):1065–1074. doi:10.1016/j.archoralbio.2014.05.005
20. Shahi S, Ghasemi N, Rahimi S, et al. The effect of different mixing methods on the flow rate and compressive strength of mineral trioxide aggregate and calcium-enriched mixture. *Iran Endod J*. 2015;10(4):248–251. doi:10.7508/iej.2015.04.008
21. Meyers MA, Mishra A, Benson DJ. Mechanical properties of nanocrystalline materials. *Progress Mater Sci*. 2006;51(4):427–556. doi:10.1016/j.pmatsci.2005.08.003
22. Samiei M, Janani M, Asl-Aminabadi N, et al. Effect of the TiO₂ nanoparticles on the selected physical properties of mineral trioxide aggregate. *J Clin Exp Dent*. 2017;9(2):e191–e195. doi:10.4317/jced.53166
23. Elsaka SE, Hamouda IM, Swain MV. Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: Influence on physical and antibacterial properties. *J Dent*. 2011;39(9):589–598. doi:10.1016/j.jdent.2011.05.006
24. Samiei M, Ghasemi N, Asl-Aminabadi N, Divband B, Golparvar-Dashti Y, Shirazi S. Zeolite–silver–zinc nanoparticles: Biocompatibility and their effect on the compressive strength of mineral trioxide aggregate. *J Clin Exp Dent*. 2017;9(3):e356–e360. doi:10.4317/jced.53392
25. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review – Part I: Chemical, physical, and antibacterial properties. *J Endod*. 2010;36(1):16–27. doi:10.1016/j.joen.2009.09.006
26. Ha WN, Bentz DP, Kahler B, Walsh LJ. D90: The strongest contributor to setting time in mineral trioxide aggregate and Portland cement. *J Endod*. 2015;41(7):1146–1150. doi:10.1016/j.joen.2015.02.033
27. Jiménez-Sánchez MC, Segura-Egea JJ, Díaz-Cuenca A. A microstructure insight of MTA repair HP of rapid setting capacity and bioactive response. *Materials (Basel)*. 2020;13(7):1641. doi:10.3390/ma13071641
28. Samiei M, Aghazadeh M, Lotfi M, Shakoei S, Aghazadeh Z, Vahid Pakdel SM. Antimicrobial efficacy of mineral trioxide aggregate with and without silver nanoparticles. *Iran Endod J*. 2013;8(4):166–170. PMID:24171023.
29. Akbari M, Zebarjad SM, Nategh B, Rouhani A. Effect of nano silica on setting time and physical properties of mineral trioxide aggregate. *J Endod*. 2013;39(11):1448–1451. doi:10.1016/j.joen.2013.06.035
30. Shamaila S, Zafar N, Riaz S, Sharif R, Nazir J, Naseem S. Gold nanoparticles: An efficient antimicrobial agent against enteric bacterial human pathogen. *Nanomaterials (Basel)*. 2016;6(4):71. doi:10.3390/nano6040071
31. Hayden SC, Zhao G, Saha K, et al. Aggregation and interaction of cationic nanoparticles on bacterial surfaces. *J Am Chem Soc*. 2012;134(16):6920–6923. doi:10.1021/ja301167y
32. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int J Nanomedicine*. 2017;12:1227–1249. doi:10.2147/IJN.S121956

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