

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

Aldilla Miranda<sup>1,2,A–F</sup>, Ira Komara<sup>2,A,D,E,F\*</sup>, Arief Cahyanto<sup>3,4,A,D,E,F\*</sup>, Cortino Sukotjo<sup>5,A,D,E,F\*</sup>, Agus Susanto<sup>2,A,C–F</sup>

<sup>1</sup> Doctoral Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

<sup>2</sup> Department of Periodontology, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia

<sup>3</sup> Department of Clinical Sciences, College of Dentistry, Ajman University, UAE

<sup>4</sup> Centre of Medical and Bio-allied Health Sciences Research, Ajman University, UAE

<sup>5</sup> Department of Prosthodontics, School of Dental Medicine, University of Pittsburgh, USA

\*These authors contributed equally to this work.

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl.

## Address for correspondence

Aldilla Miranda

E-mail: aldilla.miranda@unpad.ac.id

## Funding sources

This work was supported by a research grant from the Indonesia Endowment Fund for Education Agency, Ministry of Finance, Indonesia, through the Riset Inovatif Produktif (RISPRO) funding scheme.

## Conflict of interest

None declared

## Acknowledgements

The authors would like to acknowledge the Research Center for Radioisotope, Radiopharmaceutical and Biodesimetry Technology, National Research and Innovation Agency (BRIN), Indonesia, for providing research facilities and support.

Received on February 27, 2025

Reviewed on April 16, 2025

Accepted on May 9, 2025

Published online on January 28, 2026

## 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 ( $\theta$ ), 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

## Cite as

Miranda A, Komara I, Cahyanto A, Sukotjo C, Susanto A. Dose-dependent effects of gamma-ray irradiation on SLA-treated titanium Grade 4: An in vitro evaluation of its physical, chemical and surface properties [published online as ahead of print on January 28, 2026]. *Dent Med Probl.* doi:10.17219/dmp/204865

## DOI

10.17219/dmp/204865

## Copyright

Copyright by Author(s)

This is an article distributed under the terms of the

Creative Commons Attribution 3.0 Unported License (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>).

## 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).<sup>1</sup> 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.<sup>2–4</sup> 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.<sup>1,2</sup> Gamma rays can sterilize devices in their final packaging at ambient temperature, making them suitable for mass production.<sup>5</sup> 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.<sup>6</sup>

Standard gamma sterilization protocols often employ doses around 25 kGy, as it meets the requirements regarding the sterility assurance level for medical devices.<sup>7</sup> However, ensuring that such radiation does not compromise the physical and chemical integrity of titanium (Ti) implants during the terminal sterilization process is critical.<sup>7</sup> 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.<sup>2</sup> Surface roughness and hydrophilicity play a crucial role in osseointegration.<sup>8,9</sup> Implants with moderate roughness ( $S_a$  1–2  $\mu\text{m}$ ) and high hydrophilicity exhibit enhanced osseointegration.<sup>1</sup> Ueno et al. demonstrated that gamma-ray irradiation enhanced the hydrophilicity of Ti surfaces, reducing the water contact angle ( $\theta$ ) to approx. 10°, which might improve osseointegration.<sup>2</sup>

Similarly, El-Bediwi et al. reported that gamma-ray exposure increased surface roughness and microstructural changes in Ti, affecting its mechanical integrity.<sup>10</sup> Research has shown that gamma-ray irradiation can disrupt the titanium oxide ( $\text{TiO}_2$ ) layer, potentially increasing Ti ion release and raising concerns about cytotoxic effects.<sup>1,11</sup> Zhou et al. further report that mechanical wear during implantation and prolonged chemical corrosion may also compromise this oxide layer.<sup>11</sup> 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.<sup>12,13</sup>

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.<sup>11,14–16</sup> 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 VDmax 15 protocols as sufficient to achieve microbial reduction to acceptable levels, provided the natural bioburden is very low.<sup>17</sup> Moreover, the ISO standards for medical device sterilization recommend dose adjustments based on bioburden assessments, rather than a fixed-dose approach.<sup>2</sup> Preliminary research on the prototype Ti implant sterilization using gamma rays identified a bio-burden threshold at a 15 kGy dose.<sup>6</sup> 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 sandblasted, large-grit, acid-etched (SLA). First, the samples were sand-blasted with alumina particles (200–300  $\mu\text{m}$ ). 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 ( $\theta$ ) 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 (AAnalyst 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  $0^\circ$ , hydrophilic for angles between  $0^\circ$  and  $90^\circ$ , hydrophobic within the  $90$ – $120^\circ$  range, ultrahydrophobic between  $120^\circ$  and  $150^\circ$ , and superhydrophobic when exceeding  $150^\circ$ . A scoring system was used to quantify these properties, with a score of 1 assigned for superhydrophilic ( $0^\circ$ ), 2 for hydrophilic ( $<90^\circ$ ), 3 for hydrophobic ( $90$ – $120^\circ$ ), 4 for ultrahydrophobic ( $120$ – $150^\circ$ ), 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  $37^\circ\text{C}$  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 ( $\eta_p^2$ ) 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 ( $536.5 \pm 5.3$  HV), whereas the values for the 15 kGy and 25 kGy groups were markedly lower at  $251.3 \pm 8.1$  HV and  $203.8 \pm 63.2$  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

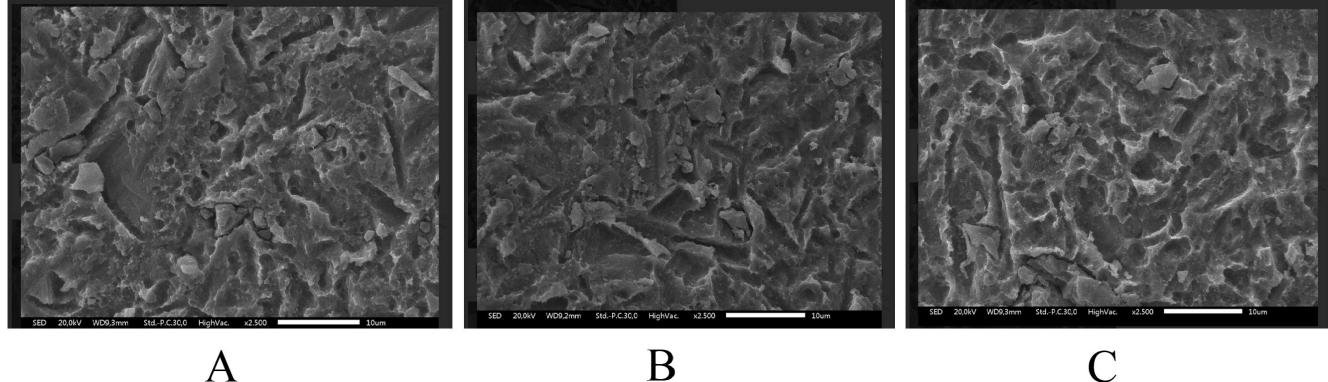
Scanning electron microscopy revealed dose-dependent surface damage on the SLA-treated Ti (Fig. 1–3).

**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 $\pm$ 5.3	–	0.0000*	0.0000*
15 kGy	251.3 $\pm$ 8.1	0.0000*	–	0.1023
25 kGy	203.8 $\pm$ 63.2	0.0000*	0.1023	–

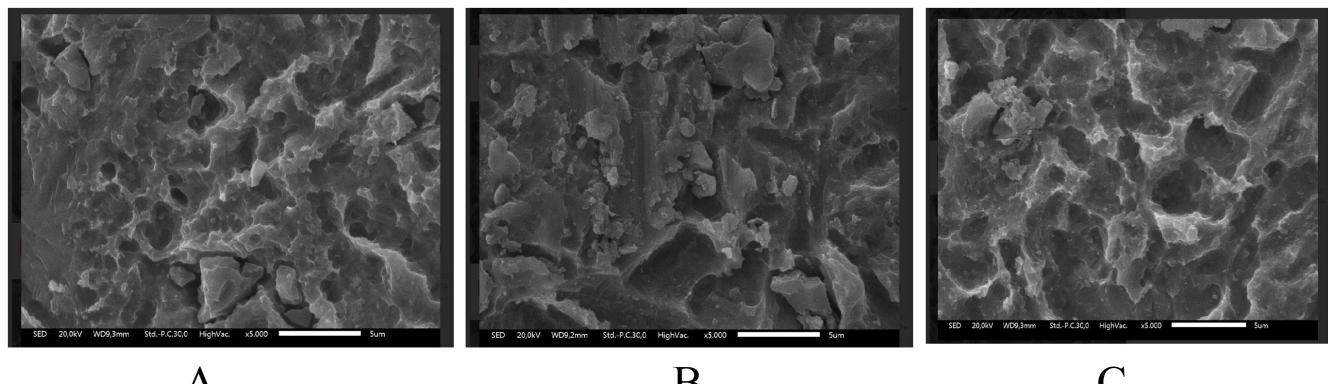
Data presented as mean  $\pm$  standard deviation ( $M \pm SD$ ).

\* statistically significant.



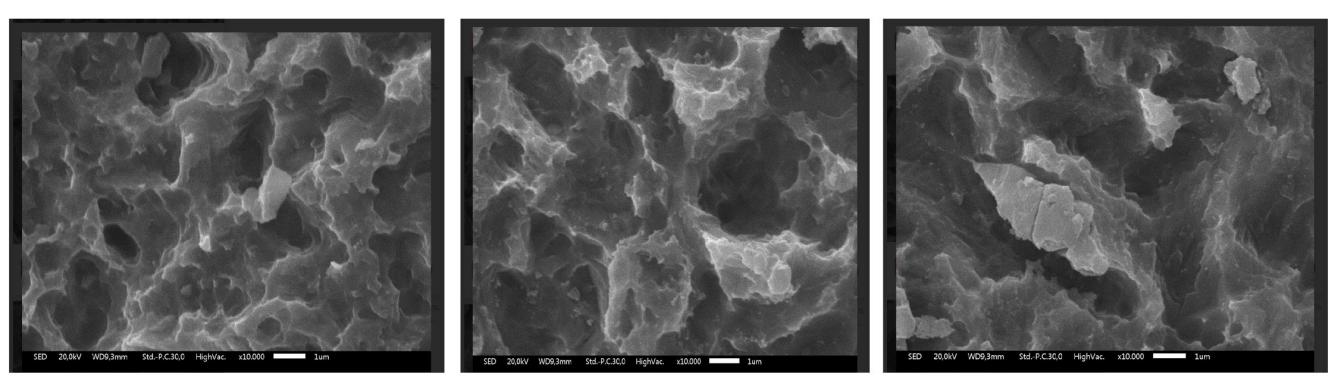
**Fig. 1.** Scanning electron microscopy (SEM) images at  $\times 2,500$  magnification

A – 0 kGy; B – 15 kGy gamma radiation; C – 25 kGy gamma radiation.



**Fig. 2.** Scanning electron microscopy (SEM) images at  $\times 5,000$  magnification

A – 0 kGy; B – 15 kGy gamma radiation; C – 25 kGy gamma radiation.



**Fig. 3.** Scanning electron microscopy (SEM) images at  $\times 10,000$  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)	p-value (Kruskal–Wallis test)
0 kGy	3.00 $\pm$ 10.50	0.0085*
15 kGy	2.00 $\pm$ 5.50	–
25 kGy	1.50 $\pm$ 3.50	–

Data presented as median  $\pm$  average rank ( $Me \pm Avg. Rank$ ).

\* 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 [ $\mu\text{g/L}$ ]	p-value (one-way ANOVA)
0 kGy	44.68 $\pm$ 2.48	<0.0001*
15 kGy	93.75 $\pm$ 16.98	–
25 kGy	218.98 $\pm$ 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 ( $536.5 \pm 5.3$  HV), and progressively lower hardness values observed at doses of 15 kGy ( $251.3 \pm 8.1$  HV) and 25 kGy ( $203.8 \pm 63.2$  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  $251.3 \pm 8.1$  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.<sup>2,18</sup> 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  $\mu\text{m}$ ) enhances cellular activity, osseointegration and bone apposition by facilitating fibrin protein adhesion and osteogenic cell attachment, and improving implant stability within the host bone.<sup>19,20</sup>

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  $\text{TiO}_2$  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.<sup>21–23</sup>

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.<sup>24,25</sup> 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.<sup>26,27</sup> Gittens et al. reported that keratinocyte proliferation on superhydrophilic surfaces improved epithelial closure.<sup>28</sup>

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.<sup>28,29</sup>

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).<sup>30,31</sup> 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 (O<sub>2</sub>) 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.<sup>2</sup> 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.<sup>2,32,33</sup> Gamma rays alter the hydrophilicity of the implant surface and disrupt the TiO<sub>2</sub> bonds, leading to ion release.<sup>34</sup> 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)-1 $\beta$  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.<sup>35–37</sup> 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.<sup>38,39</sup> 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.<sup>40,41</sup>

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.

### ORCID iDs

Aldilla Miranda  <https://orcid.org/0000-0003-1474-0053>  
 Ira Komara  <https://orcid.org/0000-0003-0144-6939>  
 Arief Cahyanto  <https://orcid.org/0000-0003-2222-5895>  
 Cortino Sukotjo  <https://orcid.org/0000-0002-2171-004X>  
 Agus Susanto  <https://orcid.org/0000-0002-8656-7061>

### References

- 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
- 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
- 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.
- 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
- Govindaraj S, Muthuraman MS. Systematic review on sterilization methods of implants and medical devices. *Int J ChemTech Res.* 2015;8(2):897–911.
- 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
- 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
- Da Silva Remídio 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
- 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
- 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.
- 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
- 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
- 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/oii.v17i1.111
- 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
- Dohshi S, Anpo M, Okuda S, Kojima T. Effect of  $\gamma$ -ray irradiation on the wettability of  $TiO_2$  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  $\gamma$ -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  $TiO_2$  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/ma1122520

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/siteselectondocuments/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, Prechtl 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