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Original Article

Dentistry Section

Antibacterial Efficacy and Antioxidant Potential of Hafnium-coated Titanium Implants: An In-vitro Assessment

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ABSTRACT

Introduction: Implant related infections and oxidative stress remain major concerns in dental and orthopaedic implantology, often leading to complications and implant failure. Titanium, though biocompatible and widely used, lacks inherent antibacterial and antioxidant capabilities. To overcome these limitations, surface coatings on biomedical implants have gained significant attention for their ability to enhance antibacterial and antioxidant properties, addressing challenges such as infection and oxidative stress.

Aim: This study evaluated the antibacterial efficacy and antioxidant potential of hafnium-coated titanium implants through in-vitro experiments.

Materials and Methods: The present in-vitro study was conducted at the Saveetha Dental College and Hospitals, Chennai, India, over a duration of three months (March to May 2024) in an in-vitro experimental set-up. Hafnium oxide nanoparticle-coated titanium micro screws (Group A; n=6) were compared with uncoated titanium screws (Group B; n=6) using Zone of Inhibition (ZOI) and bacterial viability tests against *S. mutans*, *E. faecalis*, and *C. albicans*. Antioxidant activity was assessed using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA). Independent sample t-tests were used to compare the mean values of ZOI, Colony Forming Units (CFU) counts, and DPPH scavenging percentages between the two groups.

Results: Hafnium-coated titanium implants exhibited significant antibacterial activity, with ZOI values of 13.0±0.5 mm, 24.0±0.6 mm, and 21.0±0.4 mm for *S. mutans*, *E. faecalis*, and *C. albicans*, respectively, outperforming uncoated titanium (11.0±0.4 mm, 22.0±0.5 mm, and 16.0±0.6 mm, respectively). Bacterial viability tests further confirmed the efficacy of hafnium coatings, showing reduced CFU counts (*S. mutans*: $3.0\pm0.2\times10^4$ CFU/mL; *E. faecalis*: $2.5\pm0.3\times10^4$ CFU/mL; *C. albicans*: $4.0\pm0.2\times10^4$ CFU/mL) compared to uncoated controls. While the DPPH assay revealed moderate antioxidant activity for hafnium-coated surfaces, it was lower than uncoated titanium.

Conclusion: These findings suggest that hafnium-coated titanium implants possess enhanced antibacterial properties, likely due to the inhibitory effects of hafnium ions on bacterial growth and biofilm formation. The moderate antioxidant activity indicates potential for reducing oxidative stress, improving implant biocompatibility. This study highlights the promise of hafnium coatings in developing infection-resistant and durable dental and orthopaedic implants. Further research is warranted to optimise coating performance and validate clinical applications.

Keywords: Antimicrobial properties, Biofilm inhibition, Coated implants, Free radical scavenging, Oxidative stress

INTRODUCTION

Surface coatings have emerged as a promising strategy to enhance the functional properties of biomedical implants, including antibacterial activity, corrosion resistance, and biocompatibility [1]. Metal-based coatings, such as silver, zinc, and titanium nitride, have been extensively studied for their ability to enhance the antibacterial and mechanical properties of dental implants [2,3]. However, challenges like cytotoxicity, limited long-term efficacy, and the need for multifunctional coatings that address both infection and oxidative stress remain areas of ongoing research.

Extensive research has been conducted on coatings such as silver, zinc, and cerium oxide, demonstrating their potential to mitigate implant associated infections and oxidative stress, though challenges like cytotoxicity and long-term stability remain [3-5]. Silver-coated implants have demonstrated significant antimicrobial activity against common oral pathogens like *Staphylococcus aureus* and *Escherichia coli* [4]. Similarly, zinc oxide coatings have shown promising results in reducing bacterial adhesion and biofilm formation [6]. In addition to antibacterial properties, researchers have investigated the use of antioxidant coatings, such as cerium oxide and selenium, to mitigate oxidative stress and improve implant biocompatibility [7]. The antibacterial and antioxidant potential of biomaterials is a crucial aspect of implantology, as microbial infections and oxidative stress contribute to implant failure.

Despite these advancements, many coatings face limitations, including cytotoxicity, limited long-term efficacy, and potential resistance development in bacteria. Hafnium-based coatings have gained attention in dental and medical studies for their exceptional corrosion resistance, biocompatibility, and potential to improve implant performance [8,9]. Recent research highlights their ability to enhance wear resistance and reduce bacterial adhesion [8]. This said, the studies on their combined antibacterial and antioxidant properties remain limited [10,11]. The current study explores hafnium coatings as a biomaterial alternative, providing a synthetic yet biocompatible approach to enhancing implant longevity through similar antimicrobial and antioxidant mechanisms.

Despite promising properties such as corrosion resistance and biocompatibility, there is a significant lack of comprehensive studies on the dual functionality of hafnium-metal coatings. This lacunae in the literature highlights the need for a detailed investigation into the dual functionality of hafnium-coated titanium implants. This study aimed to evaluate the antibacterial efficacy and antioxidant potential of hafnium-coated titanium implants through in-vitro experiments. This manuscript is part of a larger ongoing project aimed at developing and characterising surface-modified titanium implants with enhanced biological performance for clinical applications.

MATERIALS AND METHODS

The present experimental in-vitro study was conducted at the Department of Microbiology and the Department of Prosthodontics, Saveetha Dental College and Hospitals, Chennai, India, over a duration of three months (March to May 2024). Ethical clearance for this study was obtained from the Institutional Review Board of Saveetha University (IRB number: SRB/SDC/UG-2077/24/PROSTHO/213).

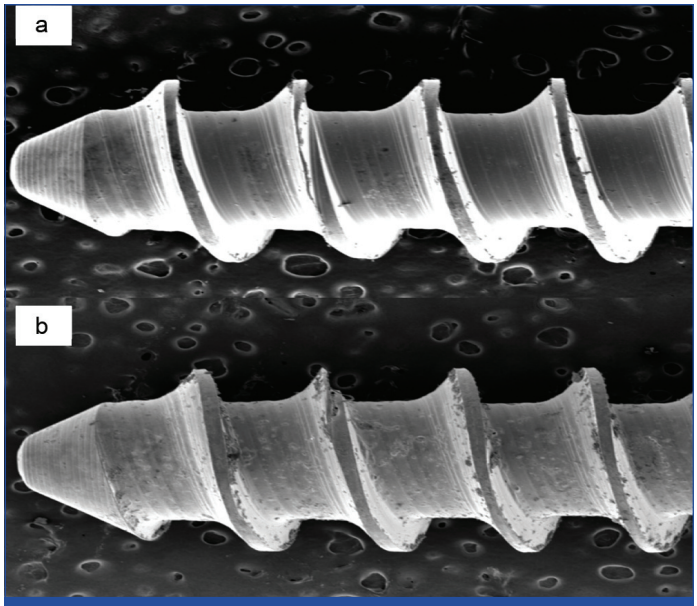
Inclusion and Exclusion criteria: The study included sterile titanium micro screws of uniform dimensions and surface characteristics, appropriate for nanoparticle coating. Only those screws that underwent successful hafnium oxide nanoparticle coating without physical defects were included in Group A, while uncoated but identical titanium screws formed Group B. Standard laboratory strains of *Streptococcus mutans, Enterococcus faecalis,* and *Candida albicans* were selected for antimicrobial testing, and high-purity reagents were used for all microbiological and antioxidant assays. Titanium screws with surface irregularities, contamination, or coating inconsistencies were excluded from the study. Microbial cultures showing impurity or deviation from expected growth patterns were also omitted. Any test samples that failed internal quality control checks during ZOI, CFU analysis, or DPPH assay were not considered for final data analysis.

Sample size estimation: The sample size for this in-vitro study was determined based on similar published experimental designs evaluating antimicrobial and antioxidant properties of coated biomaterials [12]. A total of 12 sterile titanium micro screws were selected to maintain consistency, reproducibility, and cost-efficiency of the testing process. The screws were evenly divided into two groups (n=6 per group): Group A, comprising hafnium oxide nanoparticle-coated titanium screws, and Group B, consisting of uncoated titanium screws as the control. The sample size allowed for adequate statistical comparison of outcomes such as ZOI, bacterial colony counts, and DPPH radical scavenging activity using non parametric tests due to small group size and non normal distribution.

Study Procedure

Preparation of hafnium-coated titanium implants: The titanium micro screws used in this study were sourced from Jeil Medical Corporation's Le Forte® System, Republic of Korea (6 mm×2 mm). The outer thread diameter of Implant micro screws was 1.5 mm. To achieve a smooth and uniform surface, the screws were polished sequentially using silicon carbide emery sheets of 400, 600, 800, and 1000 grit. After polishing, they were ultrasonically cleaned in deionised water to remove any residual particles or impurities. For Group A coating, the cleaned screws were treated with a 2% hafnium sol, prepared using hafnium oxide nanoparticles (Nano Research Elements™, Haryana, India). To ensure optimal coating quality, the screws were rinsed multiple times after treatment and dried in a hot air oven at 50°C to enhance adhesion. In parallel, using previously used technique of sonification, 200 mg of hafnium oxide nanoparticles were dispersed in double-distilled water and sonicated to create a homogeneous suspension, a critical step for achieving uniform nanoparticle distribution. A direct current power source was then applied to the hafnium oxide suspension to facilitate the coating process on the titanium screws [13]. The coated screws were then subjected to testing [Table/Fig-1].

Antibacterial efficacy and antioxidant potential assessment: The antibacterial activity was assessed against common pathogens, including *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans* using ZOI and bacterial viability tests. Subsequently, hafnium coated and uncoated implants are placed on agar plates inoculated with bacterial strains, and the area of clear zones where no bacterial growth was observed was noted as ZOI, to assess antibacterial activity [14]. Furthermore, the implants are exposed to bacterial suspensions, and the number of viable bacteria adhering to the surfaces was quantified using CFU counts. The Positive Control (PC), a known antibacterial agent, chlorhexidine and negative control (N-Coat) with no antibacterial activity, sterile distilled water were used.



[Table/Fig-1]: The figure presents scanning electron microscope images showcasing samples from both groups involved in the study: (a) uncoated titanium micro screws; (b) hafnium oxide nanoparticle-coated titanium micro screws.

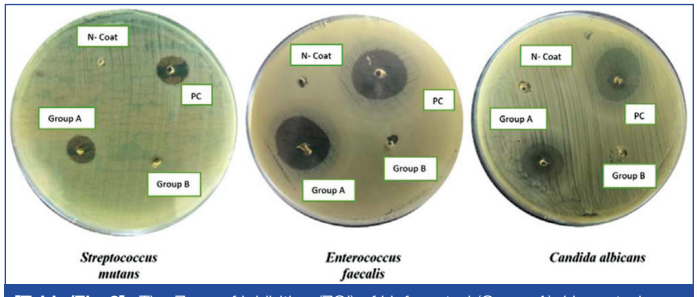
The antioxidant activity of hafnium-coated and uncoated implant screws was evaluated by measuring their ability to neutralise DPPH radicals. The reduction in DPPH absorbance was quantified using spectrophotometry [15,16]. The screws were exposed to a 0.1 mM DPPH solution, and the reduction in DPPH absorbance was measured using spectrophotometry at 517 nm after a 30-minute incubation period. The decrease in absorbance indicates the extent of radical scavenging by the samples. As a PC, ascorbic acid (vitamin C) was used, a well-known antioxidant with strong radical scavenging properties, reaching near 100% [17].

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). Mann-Whitney U was used to compare the mean values of ZOI, CFU counts, and Kruskal Wallis test was used to analyse DPPH scavenging percentages between the two groups along with PC. A p-value of <0.05 was considered statistically significant.

RESULTS

Antibacterial activity: The ZOI tests showed clear zones around hafnium-coated implants (Haf- Coated) for *S. mutans*, *E. faecalis* and *C. albicans* and the uncoated titanium (Ctl) showed measurable zones of inhibition. However, these values were lower than the PC and slightly higher than negative control (N-Coat) with no antibacterial activity, sterile distilled water [Table/Fig-2]. Bacterial



[Table/Fig-2]: The Zone of Inhibition (ZOI) of Haf-coated (Group A), Uncoated titanium (Group B), positive (PC) and negative controls (N-Coat) in agar culture plates for *S. mutans*, *E. faecalis* and *C. albicans*.

viability tests showed that hafnium-coated titanium demonstrated a significant reduction in *S. mutans*, *E. faecalis* and *C. albicans* CFU counts compared to uncoated titanium. PC represents a strong antibacterial/antifungal agent, showing very low CFU counts and N-Coat represents no antibacterial/antifungal activity, with high CFU counts [Table/Fig-3]. The Mann-Whitney U test for ZOI values showed a U statistic=36.0 and a p-value=0.0022, indicating a statistically significant difference between the hafnium-coated screws (Group A) and uncoated screws (Group B).The Mann–Whitney U test also revealed a statistically significant difference in bacterial viability between the groups (U=0.0, p=0.0049), indicating that hafnium-coated titanium screws exhibited significantly greater antibacterial activity than uncoated titanium screws [Table/Fig-4].

Category	Zone of Inhibition (ZOI) (in mm)			Bacterial viability (in CFU/mL)		
	S. mutans	*E. faecalis*	*C. albicans*	*S. mutans*	*E. faecalis*	*C. albicans*
Group A	12.0±0.50	23.0±0.51	21.0±0.45	$3.0±0.2×10^4$	$2.5±0.3×10^4$	$4.0±0.2×10^4$
Group B	2.0±0.45	4.0±0.53	2.0±0.62	$8.5±0.2×10^5$	$7.2±0.3×10^5$	$6.8±0.2×10^5$
PC	13.0±0.01	24.0±0.01	22.0±0.02	$1.2±0.01×10^3$	$1.0±0.02×10^3$	$1.5±0.01×10^3$
N-Coat	0	0	0	$1.1±0.01×10^6$	$9.8±0.02×10^5$	$9.9±0.02×10^5$

[Table/Fig-3]: Zone of Inhibition (ZOI) and bacterial viability of *S. mutans*, *E. faecalis* and *C. albicans*.
PC: Positive control, N-coat: Negative control

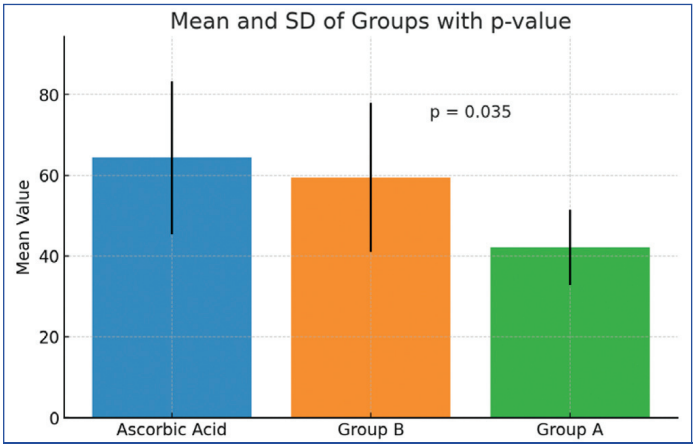
Antioxidant activity: The Haf-Coated samples demonstrates DPPH radical scavenging activity lower than that of ascorbic acid and uncoated samples (p=0.035) [Table/Fig-5]. But the activity indicates that the hafnium coating contributes to some antioxidant properties [Table/Fig-6].

Parameters	Zone of Inhibition (ZOI)			Bacterial viability		
	S. mutans	*E. faecalis*	*C. albicans*	*S. mutans*	*E. faecalis*	*C. albicans*
U value	36.0	36.0	36.0	0.0	0.0	0.0
p-value	0.0012[a]	0.0028[a]	0.0011[a]	0.0049[a]	0.0054[a]	0.0048[a]

[Table/Fig-4]: Statistical analysis of Zone of Inhibition (ZOI) and bacterial cell viability using Mann-Whitney U test.
[a]Statistically significant at p<0.05

Statistics	Ascorbic acid	Group A	Group B	p-value
Mean (%DPPH scavenging activity)	64.33	42.2	59.5	0.035[b]
Standard Deviation	18.94	9.29	18.45	

[Table/Fig-5]: Statistical analysis of DPPH scavenging assay using Kruskal Wallis test.
[b]Statistically significant at p<0.05



[Table/Fig-6]: Bar graph representing %DPPH scavenging activity of Ascorbic Acid (blue), Uncoated titanium (orange) and Haf-Coated (green).

DISCUSSION

The study indicated that hafnium-coated titanium implants possess antibacterial and antioxidant properties. The hafnium-coated titanium implants were tested and showed ZOI values closer to or exceeding the PC, this would indicate that the hafnium coating significantly enhances antibacterial activity compared to uncoated titanium. They demonstrate promising broad-spectrum antibacterial activity with low toxicity, particularly against oral pathogens like *S. mutans, E. faecalis, and C. albicans.* The antioxidant properties suggest that hafnium can reduce oxidative stress, potentially improving the longevity and biocompatibility of implants but not as much as uncoated implants. These findings were promising for the development of improved dental and orthopaedic implants that are resistant to infection and oxidative damage.

Previous studies have evaluated the antibacterial efficacy and cytocompatibility of titanium implants coated with silver, chitosan, and metallic nanoparticles against common pathogens associated with implant-related infections [17-19]. While silver and copper coatings are highly effective antimicrobials, they carry a higher risk of cytotoxicity and show limited antioxidant potential [20]. Zinc oxide offers strong antibacterial action with moderate safety [21], and titanium dioxide is effective only under UV light, limiting its in-vivo use [22]. These provide some antioxidant benefits through Reactive Oxygen Species (ROS) modulation and can be pro-oxidant at higher concentrations. Graphene oxide shows potent antimicrobial activity and enhances cell adhesion, making it a strong candidate for multifunctional coatings [23,24]. Comparatively, as per the findings of the present study, hafnium oxide offered a balanced antioxidant effect, supporting implant biocompatibility without compromising antimicrobial efficacy.

The validity of antibacterial activity tests, like ZOI and CFU, is well-established for evaluating the efficacy of antimicrobial agents against specific pathogens like *Streptococcus mutans* (a key pathogen in dental caries), *Enterococcus faecalis* (associated with endodontic infections), and *Candida albicans* (a common fungal pathogen) [25]. This method is particularly useful for comparing the relative potency of different coatings or surface treatments [26,27]. The antibacterial effects of silver nanoparticles, chitosan, and graphene oxide have been widely explored for their ability to enhance implant surfaces against peri-implant pathogens [28]. On the other hand, the CFU/mL assay offers a quantitative assessment by counting viable bacterial or fungal cells after exposure to the test material, providing direct evidence of antimicrobial activity [25,29]. Together, these tests offer complementary insights: highlighting the inhibitory potential and confirming the reduction in microbial viability. These methods ensure a comprehensive evaluation of antibacterial and antifungal properties, making them reliable tools for assessing the efficacy of hafnium-coated titanium implants or similar materials. In the present study therefore, hafnium coatings have demonstrated positive response in disrupting bacterial cell viability and biofilm formation, making them promising candidates for implant surface modifications.

The DPPH assay is a widely accepted and validated method for evaluating the quantitative measure of antioxidant activity [30,31].

This is crucial for assessing the material's potential to mitigate oxidative stress caused by ROS in biological environments. Oxidative stress is a significant factor in implant failure, as it can lead to inflammation, tissue damage, and reduced biocompatibility [32,33]. By demonstrating the ability to scavenge free radicals, this assay validates the antioxidant efficacy of the coating, suggesting its potential to enhance implant longevity and performance. This assay is cost-effective, reproducible, and straightforward, making it a preferred choice for preliminary screening of antioxidant properties [34,35]. In this study, DPPH assay application in evaluating hafnium-coated titanium implants show that the coating has some ability to reduce oxidative stress, which is critical for improving the integration, functionality and optimising coating formulations of implants in-vivo. While the antioxidant activity of hafnium-coated titanium was moderate, its radical scavenging effect is consistent.

Limitation(s)

Although this study provided valuable insights, it is important to acknowledge its limitations, including the in-vitro nature of the experiments, which may not fully replicate in-vivo conditions, and the need for further validation through clinical trials to confirm the findings. Another limitation was that the study focused on a limited number of bacterial/fungal strains, which may not represent the full spectrum of microbial interactions in real-world applications. The future scope of this study includes conducting in-vivo experiments to validate the findings in a biological environment and exploring the long-term performance of hafnium-coated implants in clinical settings. Additionally, further research could investigate the mechanisms of action and optimise coating techniques for enhanced antibacterial and antioxidant properties.

CONCLUSION(S)

In conclusion, hafnium-coated titanium implants demonstrate enhanced antibacterial efficacy, significantly inhibiting the growth of S. mutans, *E. faecalis*, and *C. albicans* compared to uncoated titanium. The coating also exhibits decreased antioxidant activity than the uncoated group, suggesting its low potential to mitigate oxidative stress. These properties highlight the promise of hafnium-coated implants in reducing infection risks and improving biocompatibility for dental and orthopaedic applications. Further studies are needed to optimise the coating's performance and validate its clinical benefits. This advancement could pave the way for more durable and infection-resistant implant materials.

REFERENCES

[1] Xuereb M, Camilleri J, Attard N. Systematic review of current dental implant coating materials and novel coating techniques. Int J Prosthodont. 2015;28(1):51-59.

[2] Nguyen-Tri P, Rtimi S, Plamondon CO. Nanomaterials-Based Coatings: Fundamentals and Applications. Elsevier; 2019.

[3] Castillejo A, Martínez G, Delgado-Pujol EJ, Villalobo E, Carrillo F, Casado-Jurado D, et al. Enhanced porous titanium biofunctionalization based on novel silver nanoparticles and nanohydroxyapatite chitosan coatings. Int J Biol Macromol. 2025;299:139846.

[4] Paiwand S, Schäfer S, Kopp A, Beikler T, Fiedler I, Gosau M, et al. Antibacterial potential of silver and zinc loaded plasma-electrolytic oxidation coatings for dental titanium implants. Int J Implant Dent. 2025;11(1):12.

[5] Gopi D, Murugan N, Ramya S, Shinyjoy E, Kavitha L. Retraction: Ball flower like manganese, strontium substituted hydroxyapatite/cerium oxide dual coatings on the AZ91 Mg alloy with improved bioactive and corrosion resistance properties for implant applications. RSC Adv. 2023;13(15):10016.

[6] Zhou J, Wang H, Virtanen S, Witek L, Dong H, Thanassi D, et al. Hybrid zinc oxide nanocoating on titanium implants: Controlled drug release for enhanced antibacterial and osteogenic performance in infectious conditions. Acta Biomater. 2024;189:589-604.

[7] You M, Li K, Xie Y, Huang L, Zheng X. The effects of cerium valence states at cerium oxide coatings on the responses of bone mesenchymal stem cells and macrophages. Biol Trace Elem Res. 2017;179(2):259-70.

[8] Abdullin IS, Mironov MM, Garipova GI. Bactericidal and biologically stable coatings for medical implants and instruments. Med Tekh. 2004;(4):20-22.

[9] Nanbakhsh K, Van Gompel M, Ritasalo R, Gollhardt A, Horváth D, Tóth K, et al. An in-vivo biostability evaluation of ALD and Parylene-ALD multilayers as micro-packaging solutions for small single-chip implants. Small. 2025;2025;e2410141.

[10] Yousef A, Akhtyamov I, Shakirova F, Zubairova L, Gatina E, Aliev Cle. Effect of hafnium and titanium coated implants on several blood biochemical markers after osteosynthesis in rabbits. Int J Clin Exp Med. 2014;7(10):3473-77.

[11] Romanov D, Sosnin K, Pronin S, Konovalov S, Moskovskii S, Gromov V, et al. Electroexplosive hafnium coating on titanium implant modified by nitrogen ions and electron beam processing. Surface and Coatings Technology. 2021;409:126895.

[12] Orlo E, Nerín C, Lavorgna M, Wrona M, Russo C, Stanzione M, et al. Antioxidant activity of coatings containing eugenol for flexible aluminium foils to preserve food shelf-life. Food Packaging and Shelf Life. 2023;39:101145.

[13] Brodnjak UV. Influence of ultrasonic treatment on properties of bio-based coated paper. Progress in Organic Coatings. 2017;103:93-100.

[14] Roshanak S, Yarabbi H, Movaffagh J, Shahidi F. Fabrication and characterization of Buforin I-Loaded electrospun chitosan/polyethylene oxide nanofibrous membranes with antimicrobial activity for food packing applications. Polymers (Basel). 2025;17(4):549.

[15] de Sousa Silva M, Passos JS, Daré RG, Nunes JR, Adriani PP, Lopes LB. Microemulsions improve the cutaneous co-localization of lipoic acid and quercetin and antioxidant effects in cutaneous cells and tissue. AAPS PharmSciTech. 2025;26(3):70.

[16] Eldurini S, Abd El-Hady BM, Shafaa MW, Gad AAM, Tolba E. A multicompartment vascular implant of electrospun wintergreen oil/polycaprolactone fibers coated with poly(ethylene oxide). Biomed J. 2021;44(5):589-97.

[17] Verma S, Mahapatra A, Gupta K, Dewan H, Raju M, Prasad DA. Assessment of the antibacterial properties and cytocompatibility of implant coatings incorporating antimicrobial agents: An in-vitro study. J Pharm Bioallied Sci. 2024;16(Suppl 4):S3607-S3609.

[18] Shahzadi S, Zafar N, Sharif R. Antibacterial activity of metallic nanoparticles. Bact Pathog Antibact Control. 2018;51(10.5772).

[19] Harris LG, Mead L, Müller-Oberländer E, Richards RG. Bacteria and cell cytocompatibility studies on coated medical grade titanium surfaces. J Biomed Mater Res A. 2006;78(1):50-58.

[20] Babu SM, Ittiachen L. Biogenic silver nanoparticle-coated low density polyethylene film with antibacterial and antioxidant properties. J Coat Technol Res. 2024;21(4):1409-20.

[21] Gökmen GG, Mirsafi FS, Leißner T, Akan T, Mishra YK, Kışla D. Zinc oxide nanomaterials: Safeguarding food quality and sustainability. Compr Rev Food Sci Food Saf. 2024;23(6):e70051.

[22] Abend A, Sperger D, Diaz DA, Guo R, Reul R, Wu SJ. Industry's perspective on challenges assessing the in vivo impact of removing titanium dioxide (TiO_2) from drug products. J Pharm Sci. 2024;113(11):3119-22.

[23] Vishaka S, Safiya SN, Binigha M, Carmelin DS, Sravanthy PG, Snega R, et al. Evaluation of antibacterial, antioxidant, anti-inflammatory and anticancer efficacy of titanium-doped graphene oxide nanoparticles. Cureus. 2024;16(1):e51737.

[24] Rajaraman V, Rajeshkumar S, Nallaswamy D, Ganapathy D. Cytotoxic effect and antimicrobial activity of chitosan nanoparticles and hafnium metal based composite: Two sides of the same coin- An in vitro study. J Pharm Res Int. 2020;32(19):122-31.

[25] Dathan PC, Nallaswamy D, Rajeshkumar S, Joseph S, Ismail S, Rashid N. Evaluation of anti-inflammatory, antioxidant and antimicrobial activity of Pomegranate Peel Extract: An in-vitro study. J Clin Diagn Res. 2024;18(6):ZC01-08.

[26] Govindaraju L, Jeevanandan G, Veeraraghavan VP, El-Sherbiny M, Binshafi GB, Alkharji SK, et al. An in-vitro analysis of the antimicrobial efficacy of a novel obturating material for primary teeth. Journal International Dental Medical Research. 2024;17(1):110-14.

[27] Omar A, Nadworny P. Review: Antimicrobial efficacy validation using in vitro and in vivo testing methods. Adv Drug Deliv Rev. 2017;112:61-68.

[28] Biju D, Arumugam P, Kannan S, Yadalam PK, Ronsivalle V, Cicciù M, 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):627-32.

[29] Shen Y, Stojicic S, Haapasalo M. Bacterial viability in starved and revitalized biofilms: Comparison of viability staining and direct culture. J Endod. 2010;36(11):1820-23.

[30] Xie J, Schaich KM. Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. J Agric Food Chem. 2014;62(19):4251-60.

[31] Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci. 2021;22(7):3380.

[32] Rajasekar A, Varghese SS. Comparison of malondialdehyde levels among patients with sandblasted acid-etched and anodized surface dental implants: A prospective clinical study. J Long Term Eff Med Implants. 2024;34(3):01-07.

[33] Wang J, Hu C, Ma X, Zhang Y, Zhang X, Hong X, et al. The role of oxidative stress biomarkers in the development of peri-implant disease: A systematic review and meta-analysis. J Dent. 2024;2024:105026.

[34] Dharini S, Pandiar D, Rajeshkumar S, Krishnan RP. Evaluation of anti-inflammatory and antioxidant properties of persea americana and syzygium aromaticum-based herbal mouthwash formulation: An in-vitro study. J Clin Diagn Res. 2024;18(10):ZF01-ZF05.

[35] Darshinidevi T, Suresh V, Sivaperumal P, Dilipan E. Synthesis of silver nanoparticles from Cymodocea rotundata leaf extract and their biological activities. Cureus. 2023;15(11):e49316.

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