



RESEARCH ARTICLE

DEVELOPMENT AND CHARACTERIZATION OF SELENIUM CONVERSION COATING ON TITANIUM DENTAL IMPLANTS FOR IMPROVED OSSEOINTEGRATION

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ABSTRACT

Background: With the increase in the incidence of peri-implant complications, the development of implant surface coatings focused on improving osseointegration is the need of the hour. The aim of this study was the development and characterization of selenium conversion coatings on titanium dental implants, representing an innovative approach to enhancing osseointegration.

Materials and methods: A 0.1 M solution of sodium selenite was prepared by dissolving it in deionized water, with the pH adjusted to 4 using phosphoric acid. Titanium implants were cleaned, etched in 10% sodium hydroxide, and immersed in the selenium solution for 1 hour with agitation. The coated implants were rinsed, dried, and characterized with Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), Fourier Transform Infrared Spectroscopy (FTIR), biocompatibility, and corrosion analysis. Student's independent t-test was performed to compare the biocompatibility of the selenium conversion coating-based titanium implants (Group A) versus bare titanium implants (Group B) at 24, 48, 72, 96, and 120 hours.

Results: Material characterization revealed successful development of selenium conversion coating on titanium implant surface that appeared as a generalized rough surface with spherical agglomerates. The coating proved its biocompatibility with greatest percentage of cell viability noted at 24 hours with a p-value of 0.653. No statistically significant difference was noted among both Groups A and B in relation to the biocompatibility. Corrosion analysis revealed the coating to be thermodynamically stable with good corrosion resistance properties.

Conclusion: The developed selenium conversion coating has proven its potential for exploration as a dependable implant surface coating. Additionally, extended in vivo studies are needed to validate its clinical effectiveness.

Keywords: Selenium; peri-implantitis; osseointegration; dental implants

INTRODUCTION

Edentulism remains a prevalent condition globally, with significant implications on an individual's health and well-being.¹ The rehabilitation of edentulous patients is essential for improving their quality of life and reducing the burden of oral diseases on public health systems. As the global population ages, addressing edentulism and enhancing access to rehabilitative dental care will be increasingly important in promoting healthy aging and overall well-being.

Dental implants have revolutionized the field of restorative dentistry, emerging as the preferred treatment option for edentulism.² Over the past few decades, dental implants have increasingly supplanted traditional options, such as removable dentures and fixed bridges, due to their superior functionality, longevity, and ability to enhance the quality of life for patients. This shift reflects advances in implant technology, surgical techniques, and a growing body of evidence supporting the efficacy of implants in providing long-term oral health benefits. Advances in implant technology have also made the procedure more accessible to a broader range of patients. Techniques such as guided implant surgery, immediate loading, and the development of mini-implants have expanded the indications for implants, allowing even those with limited bone density or other complicating factors to benefit from this treatment.³

However, with the increase in dental implant placements worldwide, there is also an increase in the prevalence of peri-implant diseases and complications, which also greatly impacts the long-term success of the implants.⁴ The focus has now shifted towards understanding and managing the complications around dental implants. Peri-implant diseases arise in the peri-implant space as a reaction to the peri-implant bacteria, leading to increased probing depths, suppuration, progressive bone loss, and mobility of the implant.⁵ The primary etiologic factor for these biologic complications is the formation of gram-negative anaerobic pathogenic bacteria-based biofilm over the implant surface.⁶ The rate of bacterial biofilm formation over the implant surface is further compounded by various factors such as the implant design, implant surface characteristics, poor oral hygiene maintenance by the patient, previous history of periodontal disease, a hyperactive host immune response, presence of systemic risk factors like diabetes and bone disorders, smoking.⁷ Moreover, the inadequately planned implant placements leading to increased cases of mechanical failures and advanced

diagnostic abilities for early detection of per-implant diseases may also have contributed to the rise in the cases of per-implant diseases.

Recent developments in material science have enabled the creation of dental implant surface coatings that not only enhance osseointegration but also exhibit antimicrobial properties and promote healing.⁸ The various mechanisms that have been developed to enhance osseointegration can be broadly divided into enhancing surface roughness, increasing wettability, providing chemical bioactivity, and antimicrobial coatings.^{9,10} From sandblasted and acid-etched surfaces, nanostructured implant surfaces, and bioactive glass coatings to antimicrobial peptide coatings and drug-eluting, coatings are being explored to provide a more stable osseointegration with minimal post-operative complications.¹¹

Recently, conversion coatings have gained significant attention in biomedical engineering.¹² These coatings chemically modify the surface of a metal through a reaction between the metal substrate and a chemical solution, resulting in the formation of a protective layer. They have been shown to improve their performance by enhancing corrosion resistance, wear protection, and adhesion properties. They have been broadly classified as phosphate conversion coatings, chromate conversion coatings, anodized coatings, oxide coatings, and elemental conversion coatings. Various elements like gadolinium, and strontium have been explored as elemental conversion coatings owing to their inherent antimicrobial and antioxidant properties. Multiple strategies like chemical immersion technique, electrochemical deposition, and plasma-assisted techniques are available for the development of conversion techniques. They have been postulated to provide improved corrosion resistance and enhanced osseointegration with dental implants.

In the present study, a selenium conversion coating was developed on the titanium implant surface. Studies have proven the antimicrobial, antibiofilm, antioxidant, and anti-inflammatory properties of selenium in various biomedical applications.¹³⁻¹⁵ Though selenium has been explored extensively for its antimicrobial and antioxidant properties, selenium as a conversion coating on implants has not yet been explored. This coating may provide a mechanically strong implant surface coating along with antimicrobial and osseoinductive properties. The objective of present study was to develop, characterize, and analyze the surface, chemical, biocompatibility, and corrosion-resistant properties of selenium conversion coatings on titanium dental implants.

MATERIALS AND METHODS

Approval from the Institute's Scientific Review Board was obtained for conduction of the study. Commercially pure titanium implants of grade 2 variety of size 20*15*2 mm were procured from Ti Anode Fabricators Pvt Ltd, Chennai, India. The MG-63 cell line was obtained from the National Centre for Cell Science, Pune, India. All media, supplements, and cell staining reagents were sourced from HiMedia Laboratories Private Limited, Thane, India.

Preparation of selenium solution: To prepare the selenium conversion coating solution, sodium selenite was dissolved in deionized water to achieve a concentration of 0.1 M, the pH of which was arrived at 4 using a small amount of phosphoric acid. The low pH enhances the deposition process by ensuring the titanium surface interacts effectively with selenium ions.

Development of selenium conversion coating on titanium implants: To begin with, the titanium implants were thoroughly cleaned and degreased by ultrasonication in ethanol for 10 minutes to remove oils or contaminants, following which they were rinsed with deionized water and air-dried. The implants were then immersed in 10% sodium hydroxide for 30 minutes at room temperature. This step was done to acid etch the implant surface for the production of a rough surface that would be conducive to the adhesion of the coating. The implants were then thoroughly rinsed with distilled water to remove the etchant and were ultrasonicated in distilled water for 10 minutes to remove residual chemicals.

For the deposition of selenium conversion coating, the treated titanium implants were immersed in the prepared selenium solution for a duration of 1 hour. The solution was allowed for gentle continuous agitation with a magnetic stirrer at 40 rpm to ensure uniform exposure to selenium ions. Following the coating process, the implants were withdrawn from the solution and washed with deionized water to remove any surplus selenium ions. They were then allowed to air dry in a clean environment. The developed selenium conversion coated titanium implant surfaces were then characterized to analyze the surface, chemical composition, biocompatibility, antimicrobial activity, and corrosion analysis.

Material characterization: The surface properties of a titanium coating were meticulously examined using advanced characterization techniques to ensure a

comprehensive analysis. A Field Emission Scanning Electron Microscope (FE-SEM, JSM IT800, JEOL, Tokyo, Japan) was employed to evaluate surface morphology and structural details. A thin platinum layer was sputter-coated onto the sample for 30 seconds to enhance conductivity and prevent charging issues during imaging. The FE-SEM was operated at an accelerating voltage of 3 kV, achieving high-resolution images with a spatial resolution of 10 micrometers.

To complement the structural analysis, Energy-dispersive X-ray (EDX) spectroscopy was utilized to determine the chemical composition of the material. This approach provided detailed insights into the elemental distribution on the titanium-coated surface.

Additionally, the vibrational properties of the material were characterized using Fourier Transform Infrared Spectroscopy (FTIR) with a Bruker Alpha II instrument. This technique enabled the identification of functional groups and vibrational modes, shedding light on the chemical interactions and material properties.

These combined methodologies offered a robust and multidimensional understanding of the titanium coating's physical and chemical attributes, ensuring a detailed and reliable evaluation for further applications.

Biocompatibility analysis:

The study assessed the biocompatibility, cell morphology, and growth patterns of implant coatings using MG-63 cells. Selenium conversion coated titanium implants comprised Group A while uncoated titanium implants were used as the control group comprised Group B. Dulbecco's Modified Eagle's Medium (DMEM) was prepared and supplemented with essential growth factors and nutrients. Cryopreserved MG-63 cells were cultured until they reached 80% confluency, then seeded onto coated titanium implants at a density of 10,000cells/cm².

Fluorescence staining techniques were employed to analyze cellular behaviour, including attachment, spreading, and proliferation. Rhodamine B and Acridine Orange were used for single-dye staining, while combined staining methods were applied for more comprehensive insights. The imaging process preserved the morphology of the stained cells, allowing for accurate evaluation.

For detailed visualization, fixed and stained cells were transferred onto glass slides, with DAPI mounting medium used for nuclear counterstaining. Image J software was used to process the acquired images and

analyze cellular activity. The number of living and dead cells was counted to compute cell viability, a critical indicator of biocompatibility. Assessments were conducted at various time points to monitor changes in cell viability over time.

Corrosion analysis: The coated titanium implants were placed in the electrochemical cell as the working electrode, ensuring that the implant surface area was exposed to the electrolyte, phosphate buffer saline with a pH of 7.4. Under open circuit potential, the titanium implants were allowed to stabilize in the electrolyte for 30–60 minutes, with no external current applied. Impedance measurements were conducted using an amplitude of ± 10 mV. Impedance spectra were taken at frequencies ranging from 0.01 Hz to 200 kHz. The potentiodynamic polarization was scanned at a rate of 1 mV/s.

Statistical analysis: The data was analyzed using IBM SPSS Statistics for Windows, Version 23.0 (released in 2015 by IBM Corp., Armonk, NY, USA). An unpaired t-test was utilized to compare the biocompatibility of selenium-coated titanium implants to the bare titanium implants, with p-values < 0.05 indicating statistical significance.

RESULTS

Material characterization: The SEM analysis (Figure 1) revealed a surface that showed a generalized rough surface with selenium nanoparticles as spherical aggregates. The coating appeared as a uniformly spread layer of selenium with no agglutination of selenium ions which showed the optimized nature of the coating. No significant defects or contaminants were observed, indicating a well-prepared surface that is likely to enhance the implant's corrosion resistance and biocompatibility. The rough uneven coating on the implant surface created by the conversion coating would aid in adhesion, differentiation and migration of cells over the coating. This would ultimately decrease the osseointegrative period allowing for enhance osteoblast attachment and bone deposition. EDX analysis (Figure 2) revealed titanium and oxygen as the predominant component at 83.6wt% and 13.9% respectively. It also showed the presence of selenium at 2.5wt% which proved the successful coating of selenium conversion coating on titanium. FTIR analysis (Figure 3 and Table 1) revealed predominant waves at 860, 1419 which depict the presence of selenium ions and the interaction between selenium and titanium respectively. The waves ranging between 3500-3000 depicted the stretching vibrations of hydroxyl groups. They along with the wave at 1625 depicted the

presence of water molecules in the interlayers. A recent study that characterized the selenium and hyaluronic acid coating on titanium dental implants reported characteristic waves that were in accordance with the waves observed in our study.¹⁶

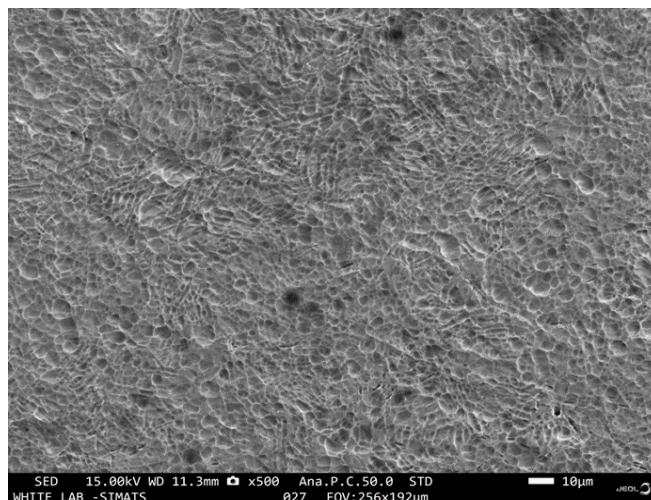


Figure 1. SEM image of the developed selenium conversion coating on titanium implant

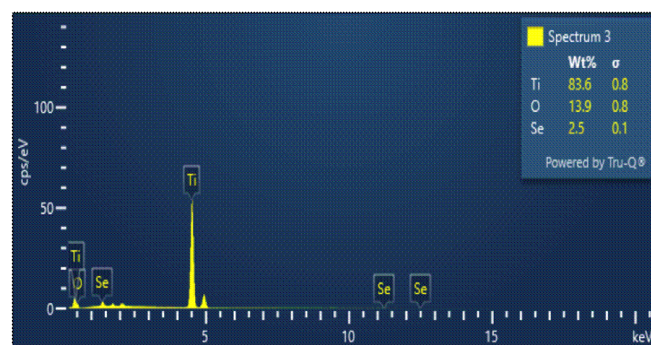


Figure 2. EDX analysis of the developed selenium conversion coating on titanium implant

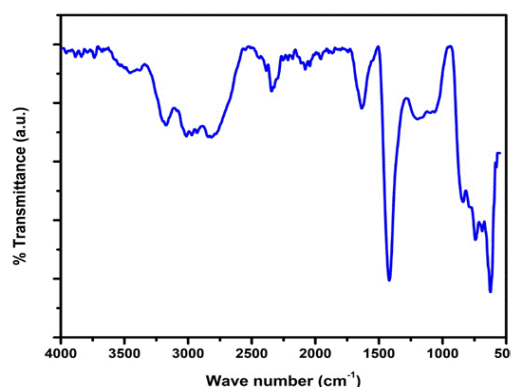


Figure 3. FT-IR analysis of the developed selenium conversion coating on titanium implant

Table 1. The absorbance peaks observed with the selenium conversion coating on titanium implant on FTIR analysis.

WAVE NUMBER (CM ⁻¹)	BOND	FUNCTIONAL GROUP	INTERPRETATION
3500-3000	Hydrogen bonded – OH	Hydroxyl group	stretching vibration of hydrogen-bonded hydroxyl groups
1625	H-O-H	-	Bending vibrations of water molecules
1419	Ti-O	Titanium oxides	interaction between Ti-O and Se
860, 800-550	Se-O	Selenite functional group	Stretching vibration of selenite ion

Biocompatibility analysis

The biocompatibility of selenium-coated titanium implants was assessed using MG-63 cells and staining techniques. The cells showed spindle-shaped morphology and well-defined organelles, with filopodial extensions and nuclear material (Figure 4). No statistically significant difference was found between groups, suggesting the selenium-coated titanium was as biocompatible as the bare titanium implant surface (Figure 5 and Table 2). A study on selenium-doped hydroxyapatite-coated titanium implants also showed excellent biocompatibility with the proliferation of pre-osteoblasts.¹⁷

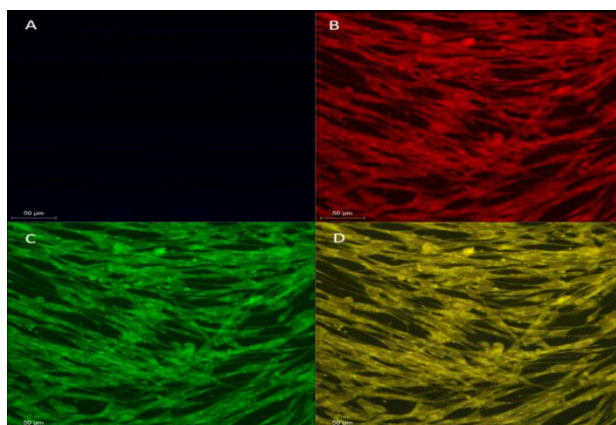


Figure 4.The biocompatibility analysis of a selenium conversion coating on a titanium implant was conducted using a confocal microscope, using various staining methods. A - blank; B - rhodamine b staining; C - Acridine orange staining; D - combination staining

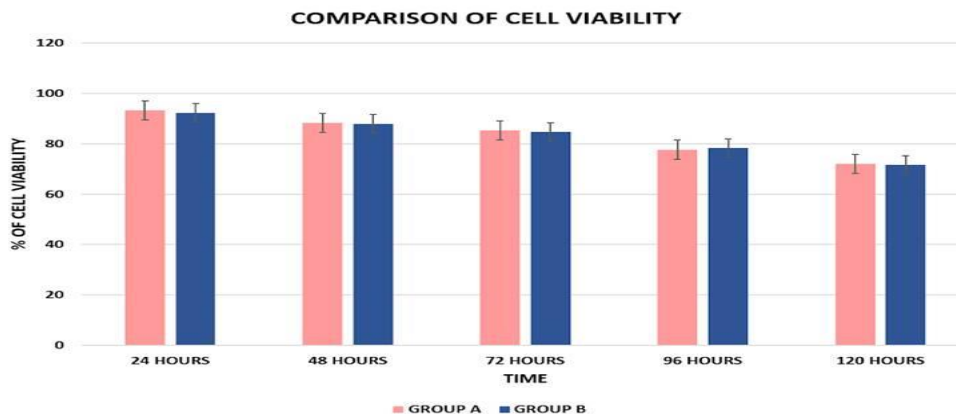


Figure 5. Comparison of biocompatibility of selenium conversion coating on titanium implants versus bare titanium implants at different time points

TABLE 2. Comparison of biocompatibility of selenium conversion coating on titanium implant (group A) and bare titanium implant (Group B) - unpaired t test

TIME	SAMPLE	N	MEAN (% OF CELL VIABILITY)	STANDARD DEVIATION	P VALUE
24 HOURS	Group A	3	93.33	1.154	0.653
	Group B	3	92.33	1.527	
48 HOURS	Group A	3	88.33	1.527	0.275
	Group B	3	88.00	2.645	
72 HOURS	Group A	3	85.33	1.643	1.000
	Group B	3	84.67	1.943	
96 HOURS	Group A	3	77.67	2.516	0.632
	Group B	3	78.32	3.511	
120 HOURS	Group A	3	72.00	2.645	0.833
	Group B	3	71.56	3.055	

Corrosion analysis: The corrosion analysis of coated and bare implant surfaces was performed and the results are depicted in Figure 6. According to potentiodynamic data, the coated surface demonstrated greater corrosion resistance by having a lower corrosion current (I_{corr}) and a higher positive corrosion potential (E_{corr}) than the bare metal. Electrochemical impedance spectroscopy, represented by the Nyquist plot, revealed a larger impedance arc for the coated surface, signifying higher resistance to current flow. Bode impedance analysis at low frequencies showed a two-fold increase in impedance for the coated surface, confirming enhanced corrosion resistance. The Bode phase angle at mid-frequency levels indicated near-ideal capacitive behavior for the coated surface. Overall, the coating demonstrated excellent electrochemical performance and corrosion resistance, which could improve the long-term in vivo stability of implants. These findings align with previous research on selenium conversion coatings for magnesium alloys, which showed minimal corrosion rates.¹⁸

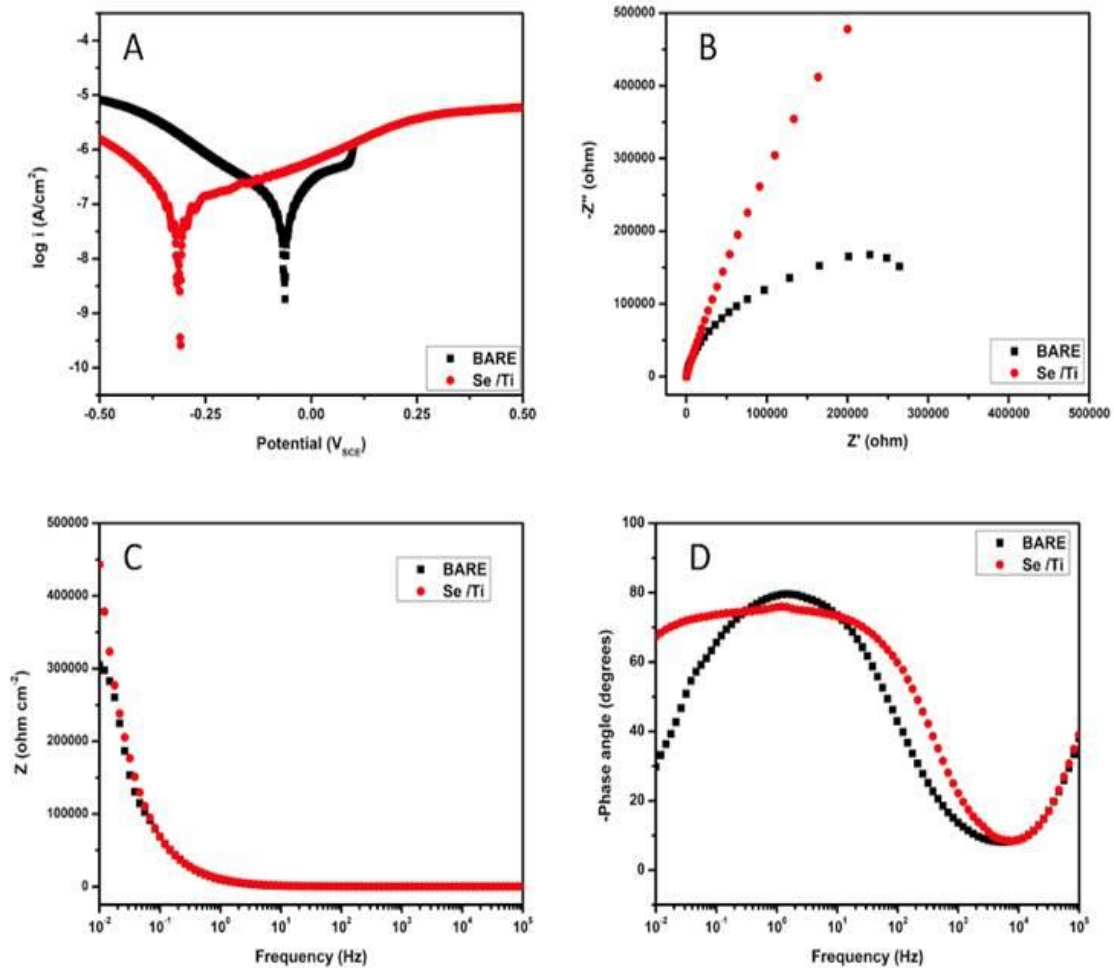


Figure 6. Corrosion analysis of the developed selenium conversion coating on titanium implant. A - potentiodynamic polarization, B - Nyquist plot, C - bode impedance, D - bode phase angle

DISCUSSION

Attaining a stable osseointegrated implant with healthy well-integrated per-implant soft tissue is one of the prerequisites for the long-term success of the implant with minimal biologic complications. The aim of this study was to develop and characterize selenium conversion coating on titanium dental implants. Conversion coatings have gained meaningful notice,

due to their advantages such as improved corrosion resistance, enhanced surface roughness, and cell adhesive properties with the added benefits of customization and biocompatibility.¹⁹ They can be broadly classified as chromate, phosphate, oxide, zinc, silicate, and ceramic coatings. Each type of conversion coating has unique properties that make it suitable for specific applications.

In this study, a selenium conversion coating was applied to titanium dental implants using a chemical process and dip coating method. SEM analysis (Figure 1) revealed a uniform layer of selenium nanoparticles in spherical aggregates, with no clumping, indicating an even distribution. The rough surface of the coating would support osteoblastic cell attachment, proliferation, and differentiation. Elemental mapping (Figure 2) confirmed a 2.5% selenium coating. FTIR analysis (Figure 3 and Table 1) identified characteristic peaks for selenite, selenium-titanium interactions, hydroxyl groups, and water molecules. These findings align with previous research showing that increased surface roughness enhances apatite formation on magnesium implants.¹⁸

Confocal analysis of selenium-coated implants cultured with MG63 cells showed similar cell viability to bare titanium implants at various time points, indicating comparable biocompatibility (Figure 5 and Table 2). Spindle-shaped viable cells arranged in stacked layers with filopodial extensions suggested intercellular communication and migration (Figure 4). The nucleus and organelles were clearly visible. These results demonstrate that the coating supports cell adhesion and proliferation. Similar findings were reported in a study on selenium coatings for orthopedic implants, showing no inhibition of osteoblast growth.²⁰ They also noted a decreased bacterial adherence of *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) which they attributed to the presence of selenium. Another study that assessed the selenium doped hydroxyapatite coated titanium implants showed excellent biocompatibility and also proved antibacterial properties over the biofilms of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *S. aureus*.¹⁷ The corrosion analysis (Figure 6) further proved that the developed coating had greater thermodynamic stability and corrosion resistance in comparison to the bare titanium implants. Congruent with our findings were the results of another study which stated that selenium and hyaluronic acid-based implant surface coating showed a decreased rate of corrosion along with an increase in the polarization resistance.¹⁶ Improved corrosion resistance properties would ultimately increase the longevity of the implants owing to the reduced wear and tear, effective barrier function leading to enhanced material integrity and improved mechanical properties. These properties would ultimately increase the biocompatibility, osseointegration and the functional longevity of the implants.

Metalloid nanoparticle implant coatings have gained favor owing to their increased surface area, bioactivity,

antimicrobial properties and wear resistance.²¹

Silicon, Boron, and strontium-based coatings have been explored as coatings for orthopedic, dental, cardiovascular and neural implants. One such metalloid nanoparticle that is essential to many biological processes in the human body as a trace element is selenium. The second highest selenium concentration remains in the bone among all human tissues.²² Selenium as a component of selenoproteins also exhibits antioxidant, antimicrobial, anti-inflammatory, and anticancer properties.²³⁻²⁵ The antimicrobial mechanism of selenium has been postulated to have a multifactorial mode. Protein degradation, loss of cell membrane integrity, photocatalytic action, prevention of bacterial adhesion and microcolony formation, interference with enzymatic functions and transport mechanisms, reactive oxygen species (ROS) production have been suggested to be the various mechanisms of antimicrobial activity.^{21, 26, 27} Studies have proven antimicrobial activity of selenium nanoparticles against common oral pathogens such as *Streptococcus mutans* (*S. mutans*), *S. aureus*, *Porphyromonas gingivalis* (*P. gingivalis*), *Escherichia coli* (*E. coli*), and *Candida albicans* (*C. albicans*) with reactive oxygen damage, cell membrane damage and protein leakage stated as the main mechanism of antimicrobial action.^{28, 29} Selenium has also been proven to exert antioxidant and anti-inflammatory properties owing to its presence in the form of selenoproteins. Various selenoproteins such as the selenoprotein P, glutathione peroxidases (GPx), thioredoxin reductases (TrxR) have shown to exert a protective effect against free radicals induced oxidative stress among cells and tissues thereby maintaining a redox homeostasis.^{30, 31} Research has shown that selenoproteins play a significant role in immunoregulation, thereby preventing the emergence of hyper-responsive phenotypes that lead to chronic inflammatory states.³²

Selenium influences osteoblast differentiation, the process by which precursor cells develop into mature bone-forming cells. According to research, selenium may encourage mesenchymal stem cells to differentiate into osteoblasts, aiding osteogenesis.^{33, 34} Selenoprotein-dependent signaling pathways in osteoblasts have been shown to enhance the synthesis of bone matrix proteins, including collagen and osteocalcin, both of which are essential for bone structure and mineralization.³⁵ Several genes related to selenoproteins and at least nine genes involved in selenoprotein biosynthesis have been identified in osteoblasts and osteoclasts.³⁴ Thioredoxin reductase 1 (TrxR1), a selenoprotein, is upregulated by vitamin D and is expressed early in the osteoblast differentiation process, illustrating part of the mechanism through which selenium supports bone

formation.^{36, 37} Glutathione peroxidase 1 (GPx1), an important antioxidant enzyme in osteoclasts, plays a role in inhibiting osteoclastogenesis when activated.³⁶

In bone marrow matrix cells cultured under low selenium conditions, there was reduced expression of GPx and TrxR, along with signs of chromosomal damage.³⁸ These issues were corrected with selenium supplementation, which restored normal selenoprotein activity. Supplementing with higher-than-normal levels of selenium has been shown to influence specific pathological changes through various mechanisms, such as the suppression of Nuclear Factor Kappa B (NF-κB) activation and its associated inflammatory responses by GPx and TrxR. Selenium has also been found to protect bone marrow stromal cells from the inhibitory effects of hydrogen peroxide on osteoblastic differentiation by reducing oxidative stress and blocking Extracellular Signal-Regulated Kinase (ERK) activation.^{39, 40}

Moreover, the noteworthy aspect of the implant coating developed in this study is that it is a conversion coating, a thin protective layer of metals formulated through a chemical conversion process, where the metal reacts with a chemical solution to create a stable, typically corrosion-resistant coating. Typically, these chemical reactions transform the metal surface into a new chemical compound, often an oxide or phosphate, which adheres strongly to the substrate. The key characteristics of these coatings that prove their superiority over other physical coatings are due to the production of a thin layer of coatings that chemically bonds to the surface materials, thereby preventing delamination. They also improve the corrosion resistance and material durability, ultimately increasing the longevity of the material. Nonetheless, this study is not without its limitations, which lie in the fact that mechanical testing, antimicrobial analysis, and cell line studies to analyze the mineralization potential of the developed coating was not performed.

Based on the scientific evidence available, we postulate that selenium conversion coating as a dental implant coating would provide an antimicrobial, antibiofilm, anti-inflammatory, anti-oxidant, and osseointegrative effect owing to the presence of selenium while also improving the corrosion resistance and material durability owing to the presence of the coating as a conversion coating. This would enhance the osseointegrative outcomes while minimizing the biologic complications. The results of this study prove the successful development of selenium conversion

coating with optimal biocompatibility and good corrosion resistance. However, preliminary results are insufficient to claim clinical superiority over other standards available currently. Further long-term in vivo and clinical studies should be performed to analyze the antimicrobial, anti-inflammatory, antioxidant, osseointegrative, mechanical, and genetic expression properties and the clinical efficacy of this coating.

CONCLUSION

In conclusion, the developed selenium conversion coating on titanium implants exhibited good surface, chemical, and corrosion resistance properties with optimal biocompatibility, presenting a promising approach to enhance the performance of titanium dental implants. By leveraging selenium's antioxidant, antimicrobial, biocompatible, and osseointegrative properties, these coatings can significantly improve osseointegration, reduce inflammation, and provide corrosion resistance, which are essential factors for the long-term success of dental implants. Overall, selenium conversion coatings offer a cost-effective and sustainable solution for enhancing the functionality and longevity of dental implants, making them a valuable advancement in dental implant technology.

DECLARATIONS

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Conflicts of interest and financial disclosures: The authors declare no conflict of interest and there was no external source of funding

Ethical approval: Approval for the conduction of the study was obtained from the Institutional Scientific Review Board (SRB/SDC/UG-1931/23/PERIO/188).

Informed Consent: Not Applicable

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