



Investigation of Hemocompatibility, Antioxidant Activity, and Functional Group Properties in Hafnium Oxide Nanoparticle-Coated Titanium Discs

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ABSTRACT:

Introduction: Despite the widespread use of titanium alloys, there are many reports indicating their disadvantages including postoperative complications, distortion of post-operative metallic screws and inflammatory reactions. Challenges related to long-term success, such as peri-implantitis and oxidative stress, continue to spur research into improving implant surfaces. The investigation of Hafnium (IV) oxide (HfO₂) layer can be utilized as a surface enhancement for the gold standard titanium alloy dental implants. Its use in biomedical applications is relatively new, but early studies suggest that HfO₂ may enhance cell proliferation and offer protection against oxidative stress. This makes it an attractive material for coating implants, where it could potentially improve outcomes by reducing inflammatory responses and promoting tissue integration.

Objectives: The objective of the research was to investigate hemocompatibility, antioxidant activity, and functional group properties in hafnium oxide nanoparticle-coated titanium discs.

Methods: The research was conducted using titanium discs 15mm diameter, 2mm thickness and gross weight 0.2kg. Two groups were designed: Test- hafnium oxide nanoparticles coated titanium discs (Hf coated Ti) and Control- uncoated titanium discs (uncoated Ti). After titanium discs were treated, 200mg hafnium oxide nanoparticle powder was used and electrolytic deposition was carried out. The hafnium oxide coated discs were subjected to hemocompatibility assay, antioxidant property testing using DPPH assay and Fourier-transform infrared spectroscopy (FTIR) . The comparative results of both groups were recorded for graphical interpretation.

Results: The results of this research indicate that hafnium oxide nanoparticles coated titanium discs had acceptable hemolytic activity (4.2%). The antioxidant activity test showed that the hafnium oxide nanoparticles coated titanium discs had a higher value of optical density at 517 nm (OD = 0.03) than the pure titanium discs which suggests comparatively better antioxidant behavior. The results of FT-IR spectroscopy showed the hafnium oxide nanoparticles coated titanium discs had lesser peak values of transmittance at varied wavelengths which signifies a good bonding of functional groups.

Conclusions: The current research concluded that titanium discs coated with hafnium oxide nanoparticles had acceptable hemolytic activity, comparatively better antioxidant behavior and lesser peak values of transmittance at varied wavelengths which signifies a good bonding of functional groups.

1. Introduction

Metallic implants have been utilized for different medical purposes including orthopedics and dentistry for short as well as long term fixations since many years (Yaszemski, 2003). Titanium alloys are characterized by good corrosion resistance and biocompatibility in contact with human body fluids and

hence seem to be irreplaceable in reconstructive surgery till date (Rajaraman, Dhanraj and Jain, 2018). Despite their widespread use, there are many reports indicating their disadvantages including postoperative complications, distortion of post-operative metallic screws and inflammatory reactions. Challenges related to long-term success, such as peri-



implantitis and oxidative stress, continue to spur research into improving implant surfaces (Wennerberg, Albrektsson and Jimbo, 2015).

Techniques for enhancing the base material's qualities have been developed in an effort to improve the therapy's therapeutic result. Among the methods, the purpose of metallic materials' enhancement is their surface alterations through the use of different methods (Zafar and Khurshid, 2020; Park, 2022). Using such methods, a thin film can be applied to a variety of surfaces, including metal, glass, and polymers. Surface modifications play a crucial role in enhancing the biological response to implants. They help in enhancing osseointegration, and minimizing adverse immune responses. By altering the surface properties, such as roughness, chemistry, or coating, implants can better promote cell attachment, reduce bacterial colonization, and improve long-term success in clinical applications (Wen, 2015; Wennerberg, Albrektsson and Jimbo, 2015).

In this context, the investigation of novel coatings, such as metal oxides, offers promising avenues to address these challenges and improve implant longevity (Biju *et al.*, 2024; Salamanca *et al.*, 2024). For that reason, we decided to investigate whether the hafnium (IV) oxide (HfO₂) layer can be utilized as a surface enhancement for the gold standard titanium alloy dental implants. Hafnium oxide (HfO₂), a metal oxide has been known for its high chemical stability, corrosion resistance, and biocompatibility. Its use in biomedical applications is relatively new, but early studies suggest that HfO₂ may enhance cell proliferation and offer protection against oxidative stress (Jayaraman *et al.*, 2014; Rajaraman, Ariga, Pandiar, *et al.*, 2024). This makes it an attractive material for coating implants, where it could potentially improve outcomes by reducing inflammatory responses and promoting tissue integration (Matsuno *et al.*, 2001).

2. Objectives

In the present paper, we analyzed physicochemical and biological properties of the layer revealing its pro-osteogenic properties. The aim of the research was to investigate hemocompatibility, antioxidant activity, and functional group properties in hafnium oxide nanoparticle-coated titanium discs. Obtained results shed a promising light for HfO₂ future application in the field of nanometric coatings for biomedical applications.

3. Methods

The research was conducted using titanium discs 15mm diameter, 2mm thickness and gross weight 0.2kg purchased from Tiaano® (Ti Anode Fabricators Pvt. Ltd. Chennai, India).

The current research is an in-vitro analysis that has been performed on the following two groups: Test Group- hafnium

oxide nanoparticles coated titanium discs (Hf coated Ti) and Control Group- uncoated titanium discs (uncoated Ti). Firstly, the titanium discs purchased were polished using a polishing machine using Silicon carbide emery sheets of various thickness (400, 600, 800, 1000). After polishing, the titanium discs were treated with 2% hafnium sol. The treated discs were then washed with deionized water using a bath sonicator. After rinsing 2-3 times the titanium discs were then dried in a hot air oven at 50 degree Celsius. 200mg hafnium oxide nanoparticle powder was taken in double distilled water, sonicated well for proper dispersion of the powder in the solvent. The sonicated hafnium oxide nanoparticles in the solvent were subjected to direct current power supply. The disc was then immersed in prepared hafnium oxide solution in a direct current power supply. In the instrument anode was titanium and cathode was platinum, which was fixed and a 20 voltage current was applied. After 2 hours in the presence of an electric field, an electron transfer from the electrolyte solution to the metal surface was obtained. This ensures the hafnium oxide nanoparticles deposition on the titanium surface. The hafnium oxide coated discs were subjected to hemocompatibility, antioxidant property testing and Fourier-transform infrared spectroscopy (FTIR). The comparative results of both groups were recorded for graphical interpretation. Blood was collected from a donor, and ethylene diamine tetraacetic acid (EDTA) was used to prevent coagulation. Red blood cells (RBCs) were centrifuged at 4°C for 10 minutes and washed three times with phosphate-buffered saline (PBS) at pH 7.4 to isolate the RBCs. The hemocompatibility assay was then performed to assess the lytic behavior of the red blood cells in the presence of hafnium coated titanium disc, along with positive and negative controls. All test samples were analyzed in triplicates. After 1 h incubation, the samples were centrifuged again, and the optical density was measured at 540 nm. The percentage of hemolysis was calculated using the following formula (equation (1)) and depicted in the bar graph.

$$\text{Hemolysis}\% = \frac{(\text{sample absorbance} - \text{negative control})}{(\text{positive control} - \text{negative control})} \times 100 \dots (1)$$

Freshly made, a 2 mM methanolic solution of DPPH (1,1-Diphenyl-2-picrylhydrazyl) was wrapped in aluminum foil to keep it out of the light and prevent photochemical degradation. A detergent-based buffer (950 mL) was mixed with 50 mL of the 2 mM DPPH stock solution to reach a final DPPH concentration of 100 μM. A plate with six wells was filled with hafnium oxide nanoparticle coated titanium and uncoated titanium discs. Subsequently, the titanium plate-containing wells were filled with DPPH solution. Absorbance measurements were taken at a wavelength of 517 nm following an hour-long incubation period. To guarantee accuracy and dependability, every measurement was carried out three times.



The antioxidant property of test and control groups were tested by using 0.3ml of each group sample prepared in different concentrations (0.5–3 mg/ml) with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 minutes in a water bath. Absorbance of all sample mixtures was measured at 517 nm wavelength. Total antioxidant activity has been expressed through a graph as the number of equivalents of ascorbic acid. The FT-IR is a significant method to analyze functional group properties of materials. The infrared spectrum of absorption, transmittance, emission and photoconductivity of solid, liquid and gas can be obtained. FT-IR was recorded at ATR (attenuated reflectance mode) using BRUKER (Alpha - II) and the graph was plotted using origin software.

4. Results

Hemocompatibility

Hemolysis is a primary tool to analyze the biocompatibility of the materials. The hemolysis of the test group was 4.2 % while that of the control group was 0.6 % (Figure 1). The desirable value of hemocompatibility assay results expressed as hemolysis percentage is less than 5%. Hafnium oxide is comparatively toxic material, and full hafnium coated titanium disc was immersed in PBS solution and estimated the hemolytic activity may be due to the higher concentration of hafnium oxide leads to higher rate of lysis.

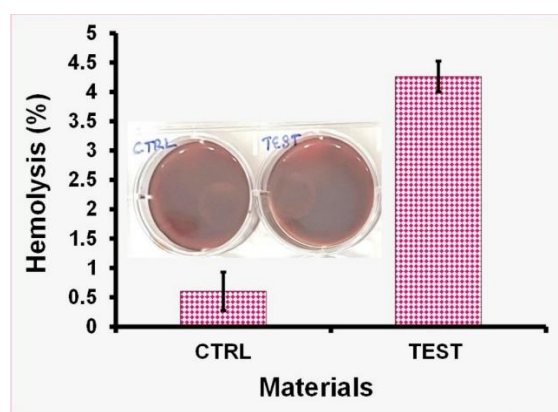


Figure 1: Figure depicting the graph of hemolysis (in %) of both the test and control group. Higher hemolysis percentage was seen in the hafnium oxide coated group (test) than the pure titanium (control) discs

Antioxidant Property

The antioxidant property was tested using DPPH assay. The optical density (OD) value at 517 nm of the control group was

very low 0.004, while for the test group it was 0.03. The antioxidant activity of Ascorbic acid was 0.044 (Figure 2). The OD values are expressed as number equivalents of ascorbic acid. Ascorbic acid is a well known material to produce radicals that indicates antioxidant behavior of the respective material. Compared to ascorbic acid, significant radical production was observed in hafnium oxide coated samples, which indicates the improved antioxidant behavior of hafnium coated titanium disc.

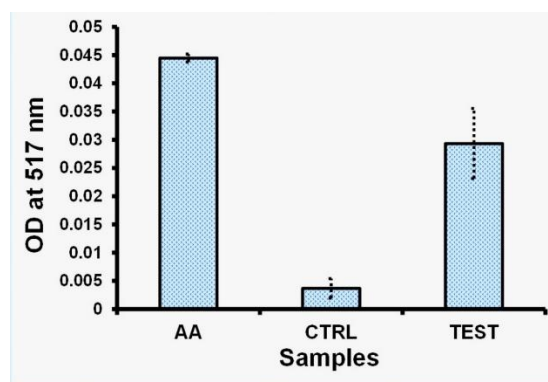


Figure 2: Figure depicting the bar graph for the optical density (OD) values at 517 nm for the test, control and ascorbic acid group. The low values for the pure titanium test group indicated there was no radical present while hafnium oxide coated discs had comparatively higher values (higher peak) which indicated the presence of the radical group.

FT- IR Spectroscopy

The results of the FT-IR study showed that peak values of transmittance for the control group (titanium oxide coatings) were at the following wavelengths- 560 cm^{-1} , 1045 cm^{-1} , 1414 cm^{-1} , 1543 cm^{-1} , 1641 cm^{-1} , all measured in cm^{-1} . From the FTIR spectrum of Ti oxide nanoparticles, Ti-O bending mode and deformative vibration of Ti-OH stretching mode is observed at 560 cm^{-1} and 11045 cm^{-1} respectively. The band at 1641 cm^{-1} can be attributed to water adsorption on the titanium oxide surface (Chougala et al, 2017). The peak values of transmittance for the test group (Hafnium oxide coating) were at 426 cm^{-1} , 526 cm^{-1} , 605 cm^{-1} , 759 cm^{-1} , 1046 cm^{-1} wavelengths (Figure 3). The peak value at 426 cm^{-1} is due to formation of Hf-O bonds. The wide peak in the range of 759 - 1046 cm^{-1} can be due to the O-H groups respectively. The peaks at 526 cm^{-1} , 605 cm^{-1} , 759 cm^{-1} indicate the presence of hafnium oxide bond. The test group hafnium oxide coated titanium discs has lesser peak values of transmittance which indicates good bonding between hafnium and functional group oxide ion (Mahendran et al, 2022).



FT-IR Spectra

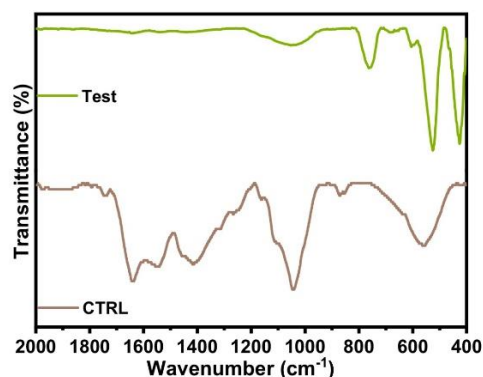


Figure 3: Figure showing graph depicting the transmittance (in %) versus wavelength (in cm^{-1}) of the test (green) and control (brown) group. The test group hafnium oxide coated titanium discs has lesser peak values of transmittance than the control group

5. Discussion

The results of this research indicate that hafnium oxide nanoparticles coated titanium discs had acceptable hemolytic activity. Although hafnium is known to be cytotoxic, in the current research the hemolysis percentage value was 4.2% which was well within the 5% threshold value. This also cements the fact that the surface coating of this metal oxide was hemocompatible. In addition to this, the antioxidant activity test showed that the hafnium oxide nanoparticles coated titanium discs had a higher value of optical density at 517 nm ($\text{OD} = 0.03$) than the pure titanium discs ($\text{OD} = 0.004$) which suggests comparatively better antioxidant behavior of the test samples which is a desirable property for dental implant surface. The results of FT-IR spectroscopy showed the hafnium oxide nanoparticles coated titanium discs had lesser peak values of transmittance at varied wavelengths which signifies a good bonding of functional groups in the test samples than the control samples. In the past, coatings like hydroxyapatite (HA) have been widely used to enhance the bioactivity of titanium implants (Stentz and Jr., 1996), silver coatings for their antimicrobial properties (Biju et al., 2024) and Diamond-like carbon (DLC) coatings offer improved wear resistance and reduced friction, making them suitable for high-stress environments (Rayannavar et al., 2024). Phosphate-based coatings, like calcium phosphate, are utilized for their ability to mimic natural bone minerals and encourage rapid osseointegration (Feng et al., 2023). Metal oxide coatings like Aluminum oxide (Al_2O_3) is known for its high hardness and biocompatibility, improving wear resistance and mechanical stability (Lavernia, 1979). Zirconium oxide (ZrO_2), often used for its aesthetic appeal due to its tooth-like color, also offers

excellent biocompatibility, corrosion resistance, and enhanced osseointegration (D'Agostino et al., 2024; Kim et al., 2024). Titanium dioxide (TiO_2) is another common coating that enhances surface roughness and bioactivity, promoting better cell adhesion and faster healing (Li et al., 2023; Wen et al., 2023).

Previously, few studies have highlighted that hafnium coatings on titanium implants demonstrated enhanced corrosion resistance and biocompatibility (Rajaraman, Ariga, Ramalingam, et al., 2024). These properties could address challenges like oxidative stress and inflammation that affect implant longevity. They are primarily used in dental and orthopedic implants to improve osseointegration, reduce bacterial colonization, and enhance the durability of titanium-based implants in clinical applications (Seweryn et al., 2020). Compared to other coatings like TiO_2 or zirconium oxide, hafnium oxide offers superior corrosion resistance and biocompatibility, with the added benefit of antioxidant properties that may further reduce oxidative stress at the implant site. Hemocompatibility is a critical factor for the success of implants as the interaction between blood components and the implant surface can trigger adverse reactions, such as thrombosis and immune responses (Chitra et al., 2020). Testing the hemocompatibility of HfO_2 -coated titanium implants is essential to ensure that the material does not induce harmful clot formation or negatively affect blood cells. By assessing hemocompatibility, researchers can determine whether HfO_2 is a safe and viable option for clinical use (Siedlecki, 2018). In addition to hemocompatibility, the antioxidant properties of HfO_2 are of particular interest, as oxidative stress plays a key role in implant failure (Lee, 2006; Shah, Rajaraman and Shanmugam, 2023). Reactive oxygen species (ROS) generated at the implant site can cause tissue damage and interfere with the healing process. Evaluating the antioxidant potential of HfO_2 coatings could provide insights into their ability to mitigate oxidative stress and enhance the overall biocompatibility of titanium implants. Finally, Fourier-transform infrared (FTIR) spectroscopy is a powerful analytical technique used to characterize surface coatings and their chemical interactions with the surrounding environment (Dumbleton et al., 1998; Rees, 2010). By using FTIR to analyze the HfO_2 coating on titanium, researchers can gain valuable information about the molecular structure of the coating, its bonding properties, and its stability over time. This data is essential for understanding how the HfO_2 coating will perform in biological environments and its potential to improve the performance of titanium implants.

In the current research, the in vitro nature of the experiments may not fully replicate the complex biological interactions occurring in vivo, limiting the direct applicability of the results to clinical scenarios. Also, a main limitation of hafnium oxide coatings is the lack of extensive long-term clinical data, which



necessitates longitudinal studies to further evaluate their performance in diverse biological environments and under mechanical stress. Additionally, the study may not account for potential variability in coating thickness or uniformity, which could impact the overall effectiveness of the surface modification. Finally, a broader range of biological and mechanical tests would be needed to confirm its safety and functionality under diverse clinical conditions.

Future research on hafnium oxide coatings could lead to the development of multifunctional implant surfaces that not only enhance osseointegration but also offer antibacterial properties and better long-term stability, ultimately improving patient outcomes. Research, including long-term in vivo studies, could validate these coatings for widespread clinical use in both dental and orthopedic implants. Additionally, exploring the antimicrobial properties of hafnium oxide coatings could open new avenues for preventing implant-associated infections. Future work could also focus on optimizing coating techniques to enhance uniformity and mechanical strength, further improving implant longevity and performance.

Conclusion

The current research concluded that titanium discs coated with hafnium oxide nanoparticles had acceptable hemolytic activity, comparatively better antioxidant behavior and lesser peak values of transmittance at varied wavelengths which signifies a good bonding of functional groups. Hafnium oxide nanoparticles can be considered as a promising option for dental implant coatings and further in vivo studies and clinical trials can be done on the same.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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