RESEARCH PAPER

Investigating the Soft Tissue Compatibility of Hafnium-Coated Titanium Implants Through Human Gingival Fibroblast Cell Lines

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Abstract

Aim: This study aims to evaluate Soft Tissue Compatibility of Hafnium-Coated Titanium Implants through Human Gingival Fibroblast Cell Lines in an in vitro environment. The focus of this research is on assessing cell morphology, cell viability, proliferation, adhesion and impact of hafnium coating on implant integration.

Materials & Methods: The study utilized titanium micro screws as the control group and hafnium oxide-coated titanium micro screws as the test group.Fibroblast cells were cultured in both groups to assess cell viability and proliferation using MTT assays, and osteoblastic differentiation was evaluated.

Results: Hafnium-coated titanium micro screws demonstrated higher levels of osteogenic markers, including BMP-2, ALP, and Runx2, compared to uncoated screws. Both groups exhibited similar biocompatibility using the MTT assay, with no cytotoxic effects on the osteoblast cells. However, the hafnium coating significantly enhanced cell proliferation and differentiation, suggesting improved osteogenic potential.

Conclusion: Hafnium oxide-coated titanium micro screws significantly improve osteoblastic activity in vitro, showing potential for enhanced osseointegration in clinical applications. The results indicate that the hafnium coating enhances osteoblast differentiation and bone formation, making it a promising material for orthopedic and dental implants.

Keywords: hafnium oxide, titanium, micro screws, nanoparticles, osteoblastic activity, HGF cell line, osseointegration, surface modification, MTT assay.

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INTRODUCTION

The success of dental and orthopedic implants is directly impacted by surface changes of titanium implants, which are essential in influencing the biological reactions at the implant-tissue interface(1–3). Surface alterations that promote titanium's integration with surrounding tissues can further boost its strength, durability, and biocompatibility, which make it a popular material(4). Surface roughness, wettability, and chemical composition have all been improved by the use of techniques including anodization, plasma spraying, and ion implantation(5). These surface characteristics are crucial for encouraging osteoblast adhesion, proliferation, and activity, all of which are necessary for stable implants and successful osseointegration(6). These changes facilitate quick and efficient integration into the bone by improving the environment for bone cells, which eventually increases the implant's effectiveness and longevity.

Soft tissue health is a critical component in implant osseointegration(7). Because it creates a biological barrier surrounding the implant, shielding the underlying bone from inflammation and bacterial invasion, it is crucial for the long-term viability of dental implants(8). In addition to improving

aesthetic results, stable and healthy soft tissue surrounding implants serves as a barrier, lowering the chance of issues like peri-implantitis and implant failure. In order to achieve soft tissue biocompatibility, fibroblasts are essential. Fibroblasts, the main cells in gingival tissue, are in charge of creating collagen and other extracellular matrix elements that support the tissue's flexibility, strength, and ability to repair(9,10). For the formation of a stable soft tissue, their capacity to adhere, multiply, and integrate with the implant surface is essential.

An implant's stability and long-term therapeutic success are improved when its surface encourages fibroblast attachment and function, which in turn facilitates soft tissue regeneration and integration(11,12). There is still much to learn about hafnium's potential as a coating material to improve soft tissue integration in dental implants. Although hafnium's corrosion resistance and biocompatibility have demonstrated encouraging results, its function in soft tissue integration surrounding implants has not yet been thoroughly examined(13) . A stable and protective seal at the implant-tissue interface depends on soft tissue compatibility, especially through fibroblast adhesion and proliferation.

Understanding how hafnium influences fibroblast behavior on implant surfaces could reveal its potential to improve soft tissue integration, minimizing the risk of inflammation and implant failure. Therefore, the aim of this study is to evaluate the adhesion and proliferation of fibroblasts on hafnium-coated titanium implants, providing insights into its suitability for soft tissue applications in dental implantology.

Materials and Methods Study Design

The reference number (SRB/SDC/UG-2040/24/PROSTHO/212) indicates that the study proposal was successfully approved by the Institutional Review Board. In order to assess the efficacy of the various therapies, a comparison group of uncoated titanium micro screws was included. On the other hand, the test group consisted of titanium implant micro screws coated with hafnium oxide nanoparticles (Haf-coated). This design made it possible to systematically assess how the hafnium coating affected the titanium micro screws' performance in comparison to their uncoated counterparts, offering important new information about the coating's possible advantages in improving.

Sample Preparation

The titanium micro screws used in this investigation were from the Republic of Korea's Jeil Medical Corporation's Lefort® System. The entire length of these micro screws was 6 mm, with a head diameter of 2 mm and an outside thread diameter of 1.5 mm. A scanning electron microscope was used to inspect the screws in order to study their surface properties (Figure 1a).

To obtain a clean and consistent surface quality, graded thickness silicon carbide emery sheets (400, 600, 800, and 1000 grit) were used to polish the screws. Deionized water in a bath sonicator was used to completely clean the titanium micro screws after the polishing process was finished in order to get rid of any remaining impurities or particles.

The screws were cleaned and then treated with a 2% hafnium sol made with powdered hafnium oxide nanoparticles from Nano Research Elements™ in Haryana, India. The titanium discs were rinsed two or three times after being treated with the hafnium sol in order to guarantee the best possible coating. Then, in order to help the hafnium coating adhere, they were dried in a hot air oven that was set to 50 °C. At the same time, 200 mg of powdered hafnium oxide nanoparticles were combined with double-distilled water and sonicated in a separate preparation to guarantee sufficient dispersion of the particles.

This step was crucial for achieving a uniform distribution of the nanoparticles. Subsequently, a direct current power source was applied to the resulting dispersion of hafnium oxide nanoparticles, facilitating the coating process on the titanium screws. The hafnium oxide nanoparticle-coated titanium screws that were obtained through this meticulous procedure were then subjected to further analysis under a scanning electron microscope (Figure 1b). This thorough methodology highlights the detailed approach taken in preparing and characterizing the titanium micro screws for the study.

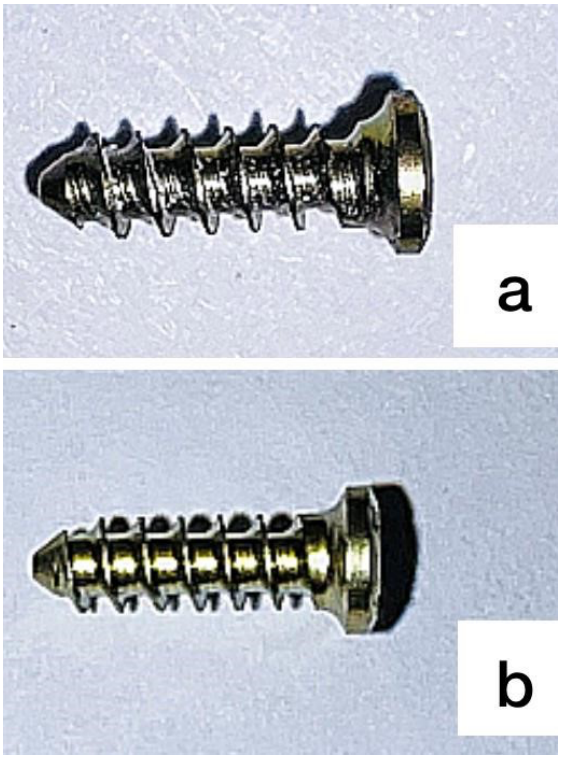

Figure 1: The figure presents scanning electron microscope images showcasing samples from both groups involved in the study: (a) illustrates the uncoated titanium micro screws, (b) displays the hafnium oxide nanoparticle-coated titanium micro screws

Establishment of Human Gingival Fibroblast cells

The Institutional Human Ethical Committee gave its clearance to the gingival tissue collection technique. During orthodontic treatment, gingival tissues were taken from the interdental papillae of adolescents in good health while their premolars were being extracted (14). Prior to tissue collection, the patients were notified and required to sign an authorized consent form. Prior to processing, tissues were weighed (20–50 mg) and stored in a sterile saline solution for one–four hours. Prior to the studies, tissue processing was carried out in a biosafety cabinet and all sterilization procedures were followed. Ten Phosphates Buffer Saline (PBS) washes were performed on the human gingival tissues in order to dilute the oral bacterial flora.

The tissues were sliced into tiny fragments of 1-2 mm^2 using a surgical blade no.11 on a sterile

Petri plate containing the culture media DMEM (Dulbecco's Modified Eagle Medium) F12 and Ham's F-12 Nutrient Mix (Thermo Fisher Scientific Inc.) after being washed in PBS. The human gingival tissue was plated onto 25 cm^2 tissue culture flasks and this was left undisturbed for 48 hours at a temperature of 37°C in a humidified incubator with 5% CO2 for 24 hours, 80% confluence. The medium was changed every 48 hours until the number of human gingival fibroblast (HGF) cells was large enough to carry out the experiment. HGF cells were seeded on hafnium-coated titanium implants at density of 5×10^4 cells/well and incubated(15).

Sample Testing

The adhesion of human gingival fibroblast cell was assessed using Calcein-AM staining and fluorescence microscopy, while gingival cell viability and proliferation was measured using the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay over 1, 3 and 5 days. This all-encompassing method enables a detailed assessment of the soft tissue biocompatibility of the test and control groups.

Results

Cell Viability

The cell viability was tested using MTT assay at Day 1, Day 3 and Day 5 for both the hafniumcoated and uncoated groups. Data indicates that cell viability remains high for both groups across all time points, with no significant differences observed. Error bars represent standard deviations (Figure 2). The MTT assay further confirmed significant proliferation of HGFs on hafnium-coated surfaces compared to non-coated controls over the tested time points.

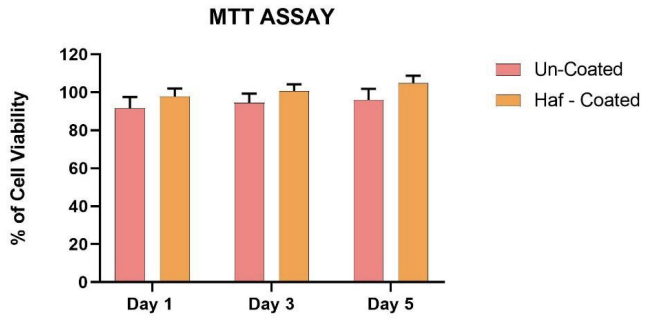

Figure 2: The bar graph represents the percentage of cell viability assessed using the MTT assay at different time points (Day 1, Day 3, and Day 5) for uncoated (pink) and hafnium-coated (orange) implants

Cell Adhesion and Proliferation

The morphological evaluation of hafnium-coated and non-coated implants was conducted using phase-contrast microscopy after incubation. Fluorescence microscopy revealed strong initial adhesion of human gingival fibroblasts (HGFs) to hafnium-coated implants. This data was checked at Day 1 and Day 3 (Figure 3) and Day 5 (Figure 4).

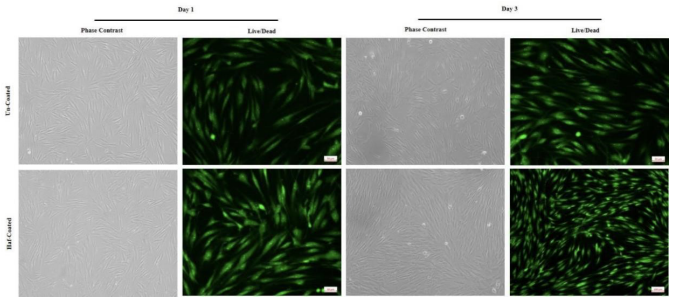

Figure 3: Phase-contrast and live/dead fluorescence microscopy images of human gingival fibroblasts (HGFs) cultured on hafnium-coated and uncoated implants at Day 1 and Day 3. The phase-contrast images (left panels) show the overall cell morphology, indicating healthy and well-spread fibroblasts in both groups. The live/dead staining (right panels) highlights viable cells in green, demonstrating strong adhesion and proliferation on both surfaces

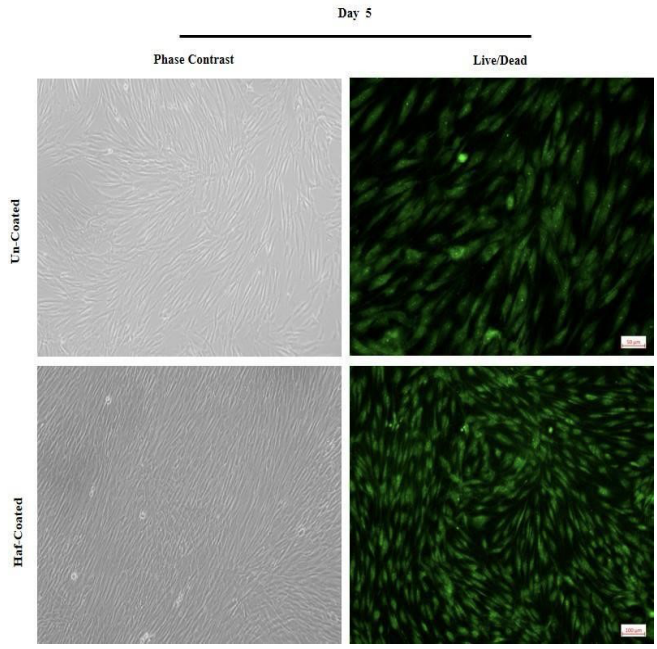

Figure 4: Phase-contrast (left) and live/dead fluorescence (right) microscopy images of human gingival fibroblasts (HGFs) cultured on hafnium-coated and non-coated implants at Day 5. The phase-contrast images reveal healthy and well-spread cells on both surfaces, with no visible morphological abnormalities. Live/dead staining shows a higher density of viable cells (green) on hafnium-coated implants, suggesting enhanced cell adhesion and proliferation

Discussion

Observations revealed that cells exhibited normal morphology on both types of implants, with no apparent alterations in shape, adherence, or structural integrity. These findings suggest that hafnium coating does not negatively impact cell morphology under the tested conditions. A higher cell density is observed on hafnium-coated implants, suggesting enhanced biocompatibility and favorable conditions for cell growth. Both groups of uncoated and hafnium oxide-coated titanium screws are biocompatible using the MTT assay and do not cause significant cytotoxicity to the human gingival fibroblast cells. The enhanced adhesion observed suggests that the hafnium coating promotes better cell attachment compared to non-coated implants. This indicates that the hafnium coating not only supports cell adhesion but also enhances cellular proliferation, suggesting its potential for improved biocompatibility and integration.

The MTT assay relies on the conversion of the reagent (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide) into formazan crystals by metabolically active cells. The amount of formazan produced is directly proportional to the number of viable cells, providing a quantitative measure of cell viability(16). When testing implant coatings, this assay helps determine the biocompatibility and potential cytotoxicity of the materials, ensuring that they promote cell growth and tissue integration without inducing harmful effects on the surrounding gingival fibroblasts, which are crucial for oral tissue repair and regeneration(16). By evaluating various coating materials with the MTT assay, researchers can identify the most promising coatings that support fibroblast health and functionality, which is vital for successful implant integration(17)(18).

Phase contrast microscopy enhances the contrast in unstained living cells by transforming variations in cellular density and refractive index into visible differences(19). This technique is useful for visualizing the morphology and behavior of gingival fibroblasts on implant surfaces without the need for staining, providing a clear image of cell contours and interactions with the coating(20). On the other hand, fluorescence microscopy utilizes fluorescent dyes or tags to label specific cellular components, providing detailed insights into cellular processes like adhesion, proliferation, and gene expression in fibroblasts on implant coatings(21). Phase contrast microscopy and fluorescence microscopy are two widely used techniques for observing human gingival fibroblasts cultured on implant coatings, each offering unique advantages for cellular analysis(22). By combining these two techniques, a comprehensive understanding of the cellular response to different implant surfaces, which is crucial for optimizing biomaterial designs for oral health applications.

Despite the promising findings, this study has certain limitations. The in vitro nature of the experiments does not fully replicate the complex biological environment of the human body, where multiple factors such as mechanical loading, immune responses, and long-term stability influence implant performance. Additionally, the study was limited to short-term observations upto 5 days and longer exposure times are necessary to evaluate the sustained effects of hafnium coatings on cell viability, proliferation, and differentiation. Another limitation is the lack of a comprehensive analysis of surface characteristics, such as roughness, wettability, and chemical composition, which play a crucial role in cell adhesion and osseointegration. A detailed physicochemical characterization of the hafnium coating would provide a deeper understanding of the factors influencing its biocompatibility and performance.

Future research should focus on in vivo studies to validate these findings in a physiological setting, assessing bone-implant

interactions over extended periods. Investigating the long-term stability of the hafnium coating, its resistance to wear and corrosion, and its impact on osseointegration will provide valuable insights into its clinical potential. Furthermore, exploring the underlying mechanisms responsible for enhanced cell adhesion and proliferation could help optimize surface modifications for improved implant performance.

Conclusion

The study concludes that hafnium-coated titanium implants exhibit excellent biocompatibility with human osteoblast. The implants support cell adhesion, proliferation, and extracellular matrix formation, which are crucial for successful integration and function of dental implants. These findings suggest that hafnium coatings can enhance the performance of titanium implants, making them a promising option for dental and orthopedic applications. Further in vivo studies are recommended to confirm these results and assess the long-term performance of hafnium-coated implants.

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