Effect of Hafnium Coating on Osseointegration of Titanium Implants: A Split Mouth Animal Study

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The behaviour of hafnium as surface coating in biological environments has not been studied. Little is known about osseointegration of hafnium-coated titanium implants. Thus, further studies of hafnium coating under biological conditions are required in order to determine the suitability of this material, as a surface coating for biomedical application. The aim of the study is to analyse the difference between hafnium-coated titanium and uncoated titanium by evaluating the osseointegration ability of hafnium metal and mechanism of which promotes better bone integration. The study was conducted with a split mouth design on 16 Wistar Albino rats of both sexes, at the age of 6-7 months, weighing \[2526 \pm 74.4\, \text{g}.\] Self-tapping titanium osteosynthesis screws (4 mm × 2 mm) (LeForte System Bone Screw®) were implanted in the mandible of rats: Group A (pure titanium screws, \(n = 12\)) and Group B (hafnium-coated screws, \(n = 12\)). The implanted screws’ stability was checked and noted with a specially customised torque apparatus during insertion and removal of implant. The tissue sections were then processed for hematoxylin and eosin and Masson's trichrome for bone and connective tissue examination, after 4 and 8 weeks of placement. Hafnium coating appears to have offered similar biocompatibility (aspartate transaminase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) enzyme assay), statistically significant improvement (independent Student’s t-test, \(p < 0.05\)) in insertion torque (25.42 ± 3.965) and removal torque (29.17 ± 2.887) than commercially pure titanium with insertion torque (22.08 ± .575) and removal torque (25.42 ± 2.575). Hafnium coating in the rat mandible showed promising osseointegration with good tissue biocompatibility. Further human trials of hafnium-coated implants are needed to understand the biological behaviour better to enhance clinical performance.

1. Introduction

Tissue engineering is a novel and well-proven approach for repair and reconstruction of bone defects. An ideal implant material should have properties that include biocompatibility, corrosion resistance, elastic modulus, and favour bone anchorage [1–14]. One of the most commonly used materials for this purpose is titanium and its alloys. In various studies conducted till date, tantalum has revealed superior properties fulfilling criteria required for an implant which include excellent chemical stability, body fluid resistance, biological inertia, and remarkable osteoconductivity. Although tantalum is shown to be promising in bone defect repair, its elastic modulus is much higher than that of human bone tissue and prone to stress shielding effect [15–25].

We wanted to evaluate alternative elements that may have the potential to offer equivalent or superior osseointegration. One such element of interest is hafnium. In the
periodic table by IUPAC, tantalum belongs to period 6 (d block) of the periodic table. Hafnium belongs to the same period and block as tantalum, in the periodic table [26–32].

Hafnium is always found in association with zirconium in mineral ores with zirconium Hf/Zr of about 2.5%. In 1984, Marcel Pourbaix proposed hafnium as a metal to be considered for surgical implants due to the passive state of the metal with properties like high ductility, strength, resistance to corrosion, and mechanical damage. Various in vitro studies were conducted on hafnium metal [33–42].

To date, the behaviour of hafnium as surface coating in biological environments has not been studied. Little is known about osseointegration of hafnium-coated titanium implants. Thus, further studies of hafnium coating under biological conditions are required in order to determine the suitability of this material, as a surface coating for biomedical applications.

This study is aimed at evaluating osseointegration of hafnium-coated titanium as compared to uncoated titanium implants. This study has two main purposes. One is to find the osseointegration ability of hafnium metal, and the second purpose is to study the mechanism of which promotes better bone integration.

2. Materials and Methods

The experimental study was conducted in accordance with the approval from the "Institutional Animal Ethical Committee," approval no. BRULAC/SDCH/SIMATS/IAEC/09-2018/015. The study was conducted on 16 Wistar Albino rats of both sexes, at the age of 6-7 months, weighing 2526.5 ± 74.4 g. Commercially pure self-tapping titanium osteosynthesis screws with a length of 4 mm and outer head diameter of 2 mm and thread diameter of 1.2 mm (LeForte System Bone Screw, Jeil Medical Corporation, Seoul®) were used for this experiment.

Two groups were utilized in these studies:

(a) Commercially pure titanium implant (control group)
(b) Commercially pure titanium implant coated with hafnium metal (test group)

2.1. Coating Procedure. Commercially pure self-tapping micro titanium osteosynthesis implant screws 4 mm long were used with 2 mm head and 1.2 mm outer thread diameter, respectively (LeForte System Bone Screw, Jeil Medical Corporation, Seoul®) (Figure 1).

The implant screws were coated with hafnium metal of 600 nm thickness using a magnetron stirrer. They were prepared by dipping the titanium screws in hafnium metal solution commercially available for industrial purposes and kept in a magnetic stirrer at 1000 rpm followed by heat exposure in a hot air oven at 70-degree Celsius for 4 days, 6 hours daily. The uncoated (Figure 2) and coated (Figure 3) implants were observed under a light microscope at 100x magnification, and the procedure was carried out till an even coating thickness was obtained.

2.2. Surgical Procedure. Surgical procedures were performed under sterile conditions in a sterile animal laboratory surgical room. Rats were anesthetized with ketamine hydrochloride intraperitoneally and xylazine intramuscularly at the dosage of 70 mg/kg body weight and 10 mg/kg body weight, respectively. The ventral part of the neck was shaved and aseptically prepared with a solution of Betadine. A 2 cm length single median vertical skin incision was made on the anterior part of the neck, exposing the fascia and muscles underneath (Figure 4).

These tissues were retracted, and the mandibular bone was exposed. A standardized, round, through-and-through osseous defect of 3 mm in diameter was created with
simultaneous driving of implant inside, similarly on the single side of the jaw, with a self-tapping screw mounted on a straight hand-piece driller controlled by the motor regulator. During the drilling process, the tissues were periodically irrigated with saline water. Care was taken during the surgery not to damage the vessels. Titanium screws were then implanted in the drilled site, in such a way that the implant should penetrate the bone slowly by the clockwise self-tapping procedure.

The implanted screws’ stability was checked and noted with a torque apparatus during insertion of implant. The torque apparatus consisted of a torque wrench and an implant hex drive; the head of which was specially customised according to the head of the implant screw (Figure 5).

The same procedure was carried in both the groups. Both the titanium and hafnium-coated screws were implanted in respective groups (Figure 6).

Then, the tissue flaps were sutured with resorbable suture threads (Vicryl 5/0, Ethicon®, Somerville, NJ, USA) and Betadine ointment was applied upon the sutured area, and then, the rats were isolated in separate cages.

2.3. Postoperative Care for the Animal. Analgesics like Fevastin 10 mg/kg body weight (intramuscularly) and diclofenac 10 mg/kg body weight (orally) were administered. The rats were examined daily for any change in body weight or signs of inflammation or infection in the surgical site.

Tissue samples were collected at the end of 4 weeks and 8 weeks by euthanizing the animals in a CO₂ chamber, and the mandibular bone alone containing the implant was dissected, photographed, and processed for histopathological examination. The excess fascial and muscular tissues adherents to the bones were removed. The mandibular bony part with the implant was fixed in 10% neutral buffered formalin. The removal torque was measured and noted in the same manner as mentioned earlier for stability using the torque apparatus, while the tissue samples were collected.

2.4. Histological Preparation. The fixed tissues were taken out and later decalcified in 20% formic acid for 7 days. Afterwards, the samples were embedded in paraffin and serial sections were cut at a thickness of 5 μm. The sections were then processed for hematoxylin and eosin staining and mounted permanently in DPX. For bone and connective tissue examination, the Masson’s trichrome staining was done. The stained samples were photographed and analysed for histopathology.

3. Results

3.1. Primary Stability (Insertion Torque) and Removal Torque. The primary stability measured using removal torque was measured for both groups studied. The mean
insertion torque/primary stability in the control group (pure titanium screws) was 22.08 ± 2.57 N/cm² and that in the test group (hafnium-coated screws) was 25.42 ± 3.96 N/cm². The mean removal torque of the control group was 25.42 ± 2.57 N/cm² and that of the test group was 29.17 ± 2.88 N/cm². The results were statistically significant (p < 0.05) when the independent Student t-test was performed (IBM SPSS Statistics 20) (Table 1). The corresponding bar graph for the primary stability and removal torque is depicted (Figures 7 and 8).

3.2. Histomorphometric Analysis. The histopathological evaluation was performed at 2 intervals, viz., 4 weeks (Figure 9) and 8 weeks (Figure 10), with two stains, namely,

### Table 1: Table showing values (mean ± SD) of independent t-test for insertion torque (primary stability) and removal torque for both the groups (p < 0.05).

<table>
<thead>
<tr>
<th>Torque values</th>
<th>Group A (pure titanium screws)</th>
<th>Group B (hafnium-coated screws)</th>
<th>Significance (<em>p value</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertion torque or primary stability (N/cm²)</td>
<td>22.08 ± 2.57</td>
<td>25.42 ± 3.96</td>
<td>p &lt; 0.05 (0.003)</td>
</tr>
<tr>
<td>Removal torque (N/cm²)</td>
<td>25.42 ± 2.57</td>
<td>29.17 ± 2.88</td>
<td>p &lt; 0.05 (0.023)</td>
</tr>
</tbody>
</table>

*Independent sample t-test.
hematoxylin and eosin stain and Masson’s trichrome stain under magnifications 4x, 20x, 40x, and 40x, respectively.

At the end of 4 weeks, histopathological evaluation demonstrated the formation and accumulation of connective tissue fibres adjacent to the implant region in the control and test groups. The presence of collagen-rich connective tissue fibres in the test group indicates the course of transformation and maturation of the endochondral ossification. The screw threads were tightly lodged in the adjacent cortical bone tissues.

At the end of 8 weeks, histopathological evaluation demonstrated the accumulation of connective tissue fibres along with the presence of thin layers of newly transformed bone (asterisk marked) which are also in connection with trabecular bone in some regions. The difference between the control and test groups is the site of formation of newly formed bone. In the control group, it is formed mostly at the inner core region of the bone away from the bone-implant contact site (*). But in the test group, the bone is formed at the implant contact site influencing the implant surfaces for better osseointegration (*). The trabecular bone tissue formation at the bone-implant contact site depicts contact osteogenesis confirmed by Masson’s trichrome stain (40x) visible as green-coloured structures.

3.3. Toxicology. The animals were sacrificed after 4 weeks and 8 weeks and sent for enzyme toxicity, viz., AST, ALT, and CK using an ELISA kit (Elabscience®) with 96 wells and an Automatic ELISA Plate Analyser (Readwell Touch, ROBONIK®). The data for the enzyme toxicity is listed (Table 2). It was found that the results are statistically insignificant ($p > 0.05$) (independent Student’s $t$-test, IBM Statistics 20).

3.4. Body Weight of Animal. The body weight of rats was measured before the surgery and after each week, and any signs of inflammation or infection were carefully monitored. The data for body weight of animals sacrificed after 8 weeks (Table 3) have been listed. The body weight of the rats studied for 4 weeks decreased from before surgery till the end of the 2nd week, after which there was an increase in the body weight. The body weight of the rats studied for 8 weeks decreased from before surgery till the end of the 4th week, after which there was an increase in the body weight.

4. Discussion

In the current study, hafnium coating appears to have offered similar biocompatibility (aspartate transaminase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) enzyme assay), statistically significant improvement (independent Student’s $t$-test, $p < 0.05$) in insertion torque ($25.42 \pm 3.96$) and removal torque ($29.17 \pm 2.88$) than commercially pure titanium with insertion torque ($22.08 \pm 0.57$) and removal torque ($25.42 \pm 2.57$). Hafnium has proved to have good tissue response and osseointegration, along with required mechanical properties [39–48]. Though these results seem to favour hafnium, it is necessary to analyse the factors that could have confounded our study.

Animal studies must have a proper protocol to be followed for care of animals used in the study as laid down by the Institutional Animal Ethical Committee (IAEC). The health of the animal was monitored throughout the study. The body weight of the animal was recorded at the start of the surgery and at the end of each week (Table 3). Signs of infection or inflammation were also checked. Proper and timely feeding of the animal was carried out every day to ensure good health of the animal. The results showed that there is an increase in the body weight towards the end of 4 weeks as well as 8 weeks, suggestive of a positive growth phase (Figures 5 and 6). Since it was a split mouth study, the health of the animal could not have affected the outcome or caused variation between the test and control groups.
The dexterity of the operator could be a confounding variable in the current study. However, the implant screws selected were a self-threading type with drill driver provided by the manufacturer of the implant screws. Hence, primary stability could not have been affected by the dexterity of the implant placement [49–52].

The implant screws of both groups were placed in the mandible of the animal. As both groups were in the same control (titanium implant screws) and test (hafnium-coated titanium implant screws) conditions, the results could be compared directly. The histopathological analysis showed no significant difference between the two groups, indicating the effectiveness of the hafnium coating in enhancing the stability of the implant screws.

**Figure 9:** Photomicrographs showing the histopathology of the control (titanium implant screws) and the test group (hafnium-coated titanium implant screws) in 4 weeks.

**Figure 10:** Photomicrographs showing the histopathology of the control (titanium implant screws) and the test group (hafnium-coated titanium implant screws) in 8 weeks.
anatomic location in the animal, this parameter could not have affected the outcome of the results. Similar studies conducted in animals also mention east variations in density of bone in the anatomically same region [53]. Magnetic stirring method was used for coating the implant screws with hafnium which ensures the same thickness of coating for all samples. Previous studies have shown the use of similar coating methods [54, 55], although the coating thickness or delamination of coating was not tested for in this study.

The accuracy of histopathology may be affected by the handling and processing of tissue samples [56, 57]. However, in this study, histopathology was carried out by an experienced senior pathologist, expert in animal tissue handling for over 15 years. Utmost care was taken to maintain consistent protocol. Effects of this would not have affected one group selectively. The limitations of the current study include the inability to measure the thickness of the coating prior to placement of the implant screws. Another limitation of this study is that the delamination of coating was not tested before the implant screws were placed in the animal model.

Future scope of the study involves studies on delamination of the coating, the scratch resistance of the coating in intraoral scenario, biofilm formation on the surface of the coating, and the side effects of leaching of the metal in the body. Researchers have emphasized that hafnium is a potential surface coating solution for titanium implants that can improve osseointegration. If research could be expanded to include hafnium as a metal for coating over dental implants or as a dental implant material to improve osseointegration, it could be used to investigate the potential of this metal in the rehabilitation of both intra- and extraoral defects, as well as in medically vulnerable patients with compromised bone quality [58]. Research could also be expanded on the possibility of newer metal alloys with hafnium for use as dental implants. Meticulous and extensive phase III and phase IV multicentre randomized control trials are required for breakthrough in this implant biomaterial.

### 5. Conclusion

Hafnium coating of endosseous implants in the current study on rat mandibles showed equivalent osseointegration and faster healing when compared to the gold standard, titanium. Hafnium is also similar to titanium in its biocompatibility with osseous tissues. Further human trials of hafnium-coated implants are needed to understand the biological behaviour better to enhance clinical performance.

### Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Authors’ Contributions

This work was carried out in collaboration among all authors. Author 1 designed the study, performed the research and the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors 2, 3, and 4 managed the literature searches and manuscript revision. All authors read and approved the final manuscript.

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### References


