Titanium Dioxide Nanotube Modified Implants: An Animal Study on Bone Formation

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This study examined the bone response to titanium dioxide nanotube modified implants. A total of 24 implants were placed in the femur of 4 beagles. Before placement, screw-shaped implants were classified into 3 groups; machined surface (group M), titanium dioxide nanotube modification of the machined surface (group MN) and titanium dioxide nanotube modification of the RBM surface (group RN). The texture morphology was observed via scanning electron microscopy. Animals were euthanized after 4 and 12 weeks of submerged healing and the histologic and histomorphometric analyses were performed. Groups MN and RN showed a significantly higher percentage of mineralized bone than that in group M ($p < 0.05$). The results of this in vivo study constitute significant evidence that the presence of the titanium dioxide nanotubes enhance osseointegration of implants.

Keywords: Titanium Dioxide Nanotubes, Osseointegrated Implant, In Vivo Bone Response.

1. INTRODUCTION

A major topic of research in dental implantology has been the development of new implant surface treatments. The surface characteristics of an implant depend on factors like topography, chemical properties, surface charge, and wettability. Among the diverse surface treatment methods for implants, “anodic oxidation” can be used to form a thin, rough, and porous oxide layer on titanium electrochemically. Titanium dioxide nanotubes on the dental implants by anodic oxidation have been reported to be advantageous for osseointegration of the implant. Kim et al. fabricated uniform titanium dioxide nanotubes (100–200 nm in diameter, 500–600 nm in length) after 10 minutes of anodic oxidation in a $1 \text{ M } \text{H}_2\text{PO}_4 + 1.5 \text{ wt}\% \text{ HF}$ electrolyte, and reported that titanium dioxide nanotubular surfaces significantly reduced water contact angles and elastic modulus compared with those prior to anodic oxidation. The implants with titanium dioxide nanotubes showed an excellent cellular response as well as good osseointegration.

This study examined the bone response to titanium dioxide nanotube modified implants in a beagle femur.

2. EXPERIMENTAL DETAILS

2.1. Implant Design and Preparation

A total of 32 screw-shaped titanium implants (ExFeel, Megagen, Daegu, Korea) were manufactured using a CNC (computer numerical control) machine. The length of titanium implants was 10 mm, and the outer diameter was 4 mm. As shown in Table I, the implants were classified into 3 groups. The RBM (resorbable blast media) implants were treated with a 40–80 mesh of hydroxyapatite and $\beta$-TCP ($\beta$-tricalcium phosphate), $\alpha$-TCP, TTCP...
Table I. Sample characteristics in this study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface characterization</th>
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<tr>
<td>M</td>
<td>Machined surface</td>
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<tr>
<td>MN</td>
<td>Nanotube modified machined surface</td>
</tr>
<tr>
<td>RN</td>
<td>Nanotube modified RBM (resorbable blast media) surface</td>
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(tetracalcium phosphate) and other calcium phosphate powders. In groups MN and RN, anodic oxidation was performed at a constant voltage of 20 V for 60 min using a direct current power supply (Fine Powder F-3005, SG EMD, Anyang, Korea) as described by Kim et al.\(^4\) The electrolyte for anodizing consisted of 1 M H\(_2\)SO\(_4\) and 1.0 wt % HF solutions with a pH of 2–3. All anodic oxidation processes were carried out at room temperature. After anodizing, the implants were washed in water for 20 min and dried for 1 hr in an oven at 200 °C. The implants were sterilized by means of gamma-ray irradiation (ISO 11137).

2.2. Scanning Electron Microscopy

A total of 32 implants were prepared. Twenty-four implants were used for surgery and 8 implants were used for surface analysis with field emission scanning electron microscopy (FE-SEM, S-4700, Hitachi, Japan). The texture morphology of 2 implants of each group and 2 RBM surface implants was observed.

2.3. Animal and Surgical Procedure

The experimental protocol was approved by the Animal Ethics Committee of Chonnam National University and all the animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Chonnam National University. The animals were kept in separate cages and fed a standard diet. Four adult beagle dogs, about 2 years old and 20 kg in weight, were used in this study. During the surgical procedures, the animals were pre-medicated with acepromacine (0.05 mg/kg intramuscularly) and morphine (0.2 mg/kg intravenously). Immediately after, they were subjected to general anesthesia by an injection of propofol (2 mg/kg intravenously). Isofluorane (1.5–2%) and O\(_2\) (100%) were used as inhalated anesthetics. The flat surface on the lateral aspect of the femur was selected for implant placement. Each animal provided eight test implant sites, with a total of 24 implants studied.

2.4. Preparation of Specimens

After 4 and 12 weeks of healing, the animals were euthanized with an overdose of sodium pentobarbital through the cephalic veins. The implants and the surrounding tissue on the lower side were removed en bloc for the purpose of histologic analysis.

The implants and the surrounding bone were fixed in formaldehyde and dehydrated in a graded series of 70%, 90%, 95% and 100% ethanol. Next, the implants with the surrounding bone were embedded in methylmethacrylate (Technovit 9100 Neu\(^®\), Heraeus Kulzer, Wehrheim, Germany). Undecalcified 100 μm thick sections were cut along the length axis of each implant using a diamond saw microsectioning system (Exakt-Apparatebau, Norderstedt, Germany). These sections were reduced to 30 μm in thickness using Donath and Breuner’s grinding techniques on a roll grinder containing sandpaper (Exakt-Apparatebau).\(^8\) The sections were stained with H&E (hematoxylin and eosin) dye.

2.5. Histologic and Histomorphometric Analysis

The histologic and histomorphometric analyses were performed using a light microscope (Olympus BX 61, Hamburg, Germany) connected to a computer. The amount of bone within the chambers was assessed by calculating the percentage of the surface area inside the chambers occupied by the bone (Fig. 1).\(^9\) The software used was Image-Pro Plus (Media Cybernetics Inc., Silver Springs, MD, USA).

2.6. Statistics

All data were analyzed using one-way analysis of variance plus post hoc Tukey’s multiple comparison test (\(p < 0.05\)) with SPSS 19 software (SPSS Inc., Chicago, IL, USA).
3. RESULTS AND DISCUSSION

The implant surfaces were examined by FE-SEM (Fig. 2). The machined surface was relatively smooth. The morphology and diameter of nanotube modified machined surface (Fig. 2(b)) and nanotube modified RBM surface (Fig. 2(c)) were similar. They consisted of highly ordered nanotubular arrays, approximately 100 nm in diameter.

After 4 weeks of healing, formation of woven bone around the machined surface implants was observed not mainly in the marrow space but in the cortical bone (Fig. 3(a)). On the other hand, titanium dioxide nanotube modified surfaces showed more new bone formation irrespective of proximity to cortical bone and osteogenesis directed from the implant surface towards the host bone (Figs. 3(b) and (c)). After 12 weeks of healing, well-developed trabecular architecture surrounding the implant was observed by a light microscope in the groups MN and RN (Figs. 3(e) and (f)) compared with the group M (Fig. 3(d)).

The percentage of mineralized bone in the group M, group MN and group RN was 42.8%, 66.7%, 65.4% ($P = 0.006$), respectively after 4 weeks and 64.7%, 79.9%, 77.4% ($P = 0.014$), respectively after 12 weeks (Fig. 4). The percentage of mineralized bone was significantly higher in the groups MN and RN than in the group M ($p < 0.05$).

The result of our study demonstrates that titanium dioxide nanotube layer significantly enhanced osteogenesis, the percentage of mineralized bone and bone deposition around both the machined and RBM surfaced implants at 4 and 12 weeks after healing. Similar results had been previously demonstrated by other experiments.$^4,10$

Although some in vitro studies of the cell behavior on nanotube surfaces have been carried out,$^{11-13}$ the mechanisms through which the properties of the nanotube layers improve osseointegration of implants have not been

Fig. 2. FE-SEM images of (a) machined surface ($\times 50,000$), (b) nanotube modified machined surface ($\times 100,000$), (c) RBM surface ($\times 50,000$), (d) nanotube modified RBM surface ($\times 100,000$).

Fig. 3. Histologic views ($\times 40$) of (a) group M, (b) group MN, (c) group RN after 4 weeks of submerged healing, and of (d) group M, (e) group MN, (f) group RN after 12 weeks of submerged healing.

Fig. 4. Percentage of the mineralized bone by histomorphometric evaluation. Group MN and RN showed significantly higher percentage of mineralized bone than in the group M (*; significant at $p < 0.05$).
defined clearly. Further studies will be needed to understand how nanotube layer enhances osseointegration of implants.

4. CONCLUSION

The effect of anodic oxidized titanium dioxide nanotube modified implants on bone formation was verified in this in vivo study. The histologic and histomorphometric analyses showed that the nanotubes induced a superior bone response and enhanced osseointegration of implants.

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References and Notes


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