MicroCT Analysis of Micro-Nano Titanium Implant Surface on the Osseointegration

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This study was to investigate the effects of micro-nano titanium implant surface on the osseointegration. A total of 36 screw-shaped implants were used. The implant surfaces were classified into 3 groups (n = 12): machined surface (M group), nanosurface which is nanotube formation on the machined surface (MA group) and nano-micro surface which is nanotube formation on the RBM surface (RA group). Anodic oxidation was performed at a 20 V for 10 min with 1 M H3PO4 and 1.5 wt% HF solutions. The implants were installed on the humerus of 6 beagles. After 4 and 12 weeks, the morphometric analysis with micro CT (skyscan 1172, SKYSCAN, Antwerpen, Belgium) was done. The data were statistically analyzed with two-way ANOVA. Bone mineral density and bone volume were significantly increased depending on time. RA group showed the highest bone mineral density and bone volume at 4 weeks and 12 weeks significantly. It indicated that nano-micro titanium implant surface showed faster and more mature osseointegration.

Keywords: Functionalized Nanotubular Titanium Implant, Bone Volume, Bone Mineral Density.

1. INTRODUCTION
Titanium (Ti) and titanium alloys are widely known as optimal materials for dental implants. Recently, anodic oxidation of titanium has attracted great deal of attention as a means of modifying its surface.1,2 As a result of anodic oxidation under high voltage, porous titanium oxide (TiO2) layer is formed. The formed TiO2 layer reduces the release of metal ion and increases resistance to corrosion. Such the surface also remarkably improved wettability, enhanced osteoblast attachment and activity, and consequently promoted osseointegration.3

In our previous study, we fabricated a functionalized nanotubular surface, which is a nanotubular TiO2 layer embedding micro-roughened surface.4,5 Kim et al. reported that the roughness of functionalized nanotubular surface was 1.57 μm and this surface was favorable for cells.3 Yun et al. reported that functionalized nanotubular surface had lower contact angle and has shown excellent results in terms of cell viability, cell differentiation and ALP activities.6 Bae et al. reported wide surface area and nanotube formation on the titanium surface delivered more bone morphogenetic protein.7 To date, functionalized nanotubular surface showed excellent results, both in vitro and in vivo. However, to date, there were no experiments in animal recipients.

The aim of this study was to evaluate osseointegration of functionalized nanotubular surface using bone mineral density and bone volume evaluation with microCT.

2. EXPERIMENTAL DETAILS
2.1. Implant Preparation
Screw-shaped implants (Exfeel™ external, Megagen Implant Co. Ltd., Gyeongsan, Korea) were used in this
Table 1. Experimental groups in this study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant surface characterization</th>
<th>N</th>
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<tbody>
<tr>
<td>M group</td>
<td>Machined surface</td>
<td>12</td>
</tr>
<tr>
<td>MA group</td>
<td>Nanotubular surface by anodic oxidation on the machined surface</td>
<td>12</td>
</tr>
<tr>
<td>RA group</td>
<td>Functionalized nanotubular surface by anodic oxidation on the RBM surface</td>
<td>12</td>
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</table>

study. The length was 8.5 mm and, the diameter was 3.75 mm. As shown in Table 1, the implants were classified into 3 groups. M group had a machined surface. MA group had nanotubular surface, i.e., the surface was anodized on the machined surface. RA group has functionalized nanotubular surface, i.e., the surface was treated by RBM, followed by anodic oxidation. The powder used for the RBM process consisted of hydroxyapatite and β-TCP (tricalcium phosphate), α-TCP, TTCP (tetracalcium phosphate) and other calcium phosphates. The anodizing procedure was carried out using 20 V direct current (DC) voltage (Fine Power F-3005, SG EMD, Korea) for 10 min in the electrolyte of 1 M H₃PO₄ solution with the addition of 1.5 wt% HF, at room temperature. After anodizing, the implants were ultrasonically washed in water for 30 min and then ultrasonically cleanse with isopropyl alcohol for 30 min followed by drying at 200 °C for 1 h. The texture morphology of the implants was observed in each group by field emission scanning electron microscopy (FE-SEM, S-4700, Hitachi, Japan).

2.2. Surgical Procedure
Six healthy male adult beagle dogs, aged 2–3 years and weighting 10–15 kg were used in this study. Animal selection management and the surgical protocol were approved by the Ethics Committee on Animal Experimentation of Chonnam National University (CNU IACUC-YB-2010-21). During the operation, anesthesia was maintained with isoflurane (Choongwae Co., Seoul, Korea) and oxygen. Using sterile surgical techniques, an incision was made in the skin to expose the proximal aspect of each humerus. The guide drill was used to drill cortical bone, and a 2 mm drill, a 3 mm pilot drill, and a 3.2 mm final drill were used sequentially. The implants were placed with ELCOMED (W&H, Bürmoos GmbH, Austria). The surgical site was closed in layers.

2.3. Morphometric Analysis with MicroCT
After 4 and 12 weeks, the beagles were euthanized with carbon dioxide inhalation. To prevent the scattering of titanium, the implants were removed. The bone volume (BV) and bone mineral density (BMD) surrounding implants were measured using a microCT system (Skyscan1172, SKYSSCAN, Antwerpen, Belgium) with 0.5 mm alumina filters and electric parameters of 60 kV and 167 μA. (Fig. 1).

Figure 1. The region of interest of microCT.

The ROI for evaluation of BV and BMD was 1 mm around the implant.

2.4. Statistics
SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A two-way analysis of variance (ANOVA) test was performed to evaluate the torque data and morphometric results. Differences between groups were examined with the Bonferroni’s adjustment, and $p < .05$ was considered statistically significant.

3. RESULTS AND DISCUSSION
The implant surfaces were examined by FE-SEM (Fig. 2). The M group showed relatively smooth surface. For the MA group, the surface showed relatively smooth surface under low magnification ($\times 5,000$). In contrast, the surface consisted of highly ordered nanotubular arrays, approximately 100 nm in diameter, under high magnification ($\times 50,000$). For the RA group, the surface showed rough surface under low magnification ($\times 5,000$). However, at higher magnification ($\times 50,000$), the surface also exhibited highly ordered nanotubular arrays of approximately 100 nm in diameter.

Quantitative analysis using microCT was reported recently, and it showed many advantages, such as non-destructive analysis, possibility of three dimensional analysis and observation in various directions. Many studies reported that micro CT analysis was accurate, compared with histomorphometric analysis, and information about the implant interface was taken by microCT without tissue injury in the dental field. Morphometric analysis using...
MicroCT had limitation because it did no implants. However, reliable results were gained not bone implant contact but bone mineral density and bone volume.

After 4 weeks, the mean bone volume of the M group, MA group and RA group was 26.6 ± 3.2%, 28.4 ± 2.9% and, 31.0 ± 2.3%, respectively. After 12 weeks, measurements increased to 28.3 ± 0.7%, 31.5 ± 1.4% and, 34.2 ± 1.0%, respectively (Fig. 3). Bone volume surrounding the implant represents the amounts of newly formed bone via osseointegration. Bone volume significantly increased with time, suggesting that bone healing occurred continuously over 12 weeks. RA group showed statistically significantly higher bone volume than M group, indicating that anodic oxidation alone didn’t accelerate osseointegration and the newly formed bone volume was affected by the micro-roughened surface, rather than nano-roughened surface.

After 4 weeks, the mean bone mineral density in the M group, MA group and RA group was 0.278 ± 0.001 g/cm³, 0.288 ± 0.017 g/cm³ and, 0.292 ± 0.010 g/cm³, respectively. After 12 weeks, the values increased to 0.281 ± 0.014 g/cm³, 0.305 ± 0.024 g/cm³ and, 0.311 ± 0.017 g/cm³, respectively. (Fig. 4) Bone mineral density represents the maturity of the newly formed bone and significantly increased with time, suggesting that bone healing occurred continuously over 12 weeks. RA group showed significantly higher bone volume than M group, indicating that anodic oxidation alone could not accelerate osseointegration and maturity of the newly formed bone was affected by micro-roughened, rather than nano-roughened surface.

To date, there is no direct animal experimental evidence in the literature to identify the reasons for the enhanced osseointegration of the nanotube implants. However, some studies of in vivo cell behavior have been carried out on nanotube surface.6,7 Although the presence of a nanotube structure may play an important role in improving the bone response, this may not be the only reason for it. The mechanisms through which the surface properties of the nanotube structured implants improve osseointegration and new bone formation have not been defined clearly. Further studies will be needed to get a better understanding of how nanotube implant surfaces may enhance osseointegration. In addition, there is a need to diversify the electrolyte, alloy and diameter of the tube, and to examine the effects.

4. CONCLUSION

Functionalized nanotubular titanium implant surface showed faster and more mature osseointegration. Further study will be needed to examine the mechanisms and properties of nanotubes and advance their potential applications.

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References and Notes


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