Comparison between bioactive fluoride modified and bioinert anodically oxidized implant surfaces in early bone response using rabbit tibia model

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ABSTRACT (218 WORDS)

Purpose: The aim of the present study was to investigate whether bioactive surfaces were more favorable to bone than bioinert surfaces by evaluating bone responses around two commercial dental implants.

Materials and methods: Bioactive fluoride-modified implants (Osseospeed™) were compared with bioinert oxidized implants (TiUnite®). A field emission scanning electron microscope, an energy dispersive spectroscope, and a confocal laser scanning microscope were used to analyze the implant surfaces. Five New Zealand white rabbits were used in the evaluation of bone response. A fluoride-modified implant was inserted into one tibia and an oxidized implant into the other. Drilling was performed bicortically. The diameter of the final drill was 3.7 mm. A cortical drill was used to create gap defects with a diameter of 5.0 mm in the upper cortex only. The rabbits were sacrificed two weeks post implant insertion. Histological specimens and light microscopy were used to measure bone-to-implant contact ratios and bone area.

Results: No significant differences were observed in surface roughness ($p > 0.05$). The gap defects were almost filled with new bone within a period of two weeks. The histomorphometry revealed no significant differences in bone-to-implant contact and bone area ($p > .05$).

Conclusions: Within the limitation of this study, the bioactive fluoride-modified surface may show no superiority to the bioinert anodized surface in early bone response.

KEYWORDS: dental implants, surface properties, fluoride modification, anodic oxidation, osseointegration

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Do bioactive implant surfaces really show a more favorable bone response than conventionally modified bioinert titanium (Ti) surfaces? Osseospeed™ (Astra Tech, Mölndal, Sweden) has a bioactive F^--modified surface. TiUnite® (Nobel Biocare AB, Gothenburg, Sweden) has a bioinert Ti surface that is modified by anodic oxidation.[1, 2] These implants are two of the most widely used oral implant systems in modern prosthetic dentistry.[3] However, no previous study has compared these implants in terms of bone response.

Osseospeed™ has surface-attached F^- ions that render this implant bioactive in bone physiology.[1] The microroughness and nanoroughness of this surface has been shown to contribute to a superior bone response [4]. The surface chemistry and topography of Osseospeed™ differ from those of its predecessor, TiOBlast™, which has a surface that is moderately roughened by blasting with titanium oxide (TiO_2) particles.[5] One study found that Osseospeed™ showed greater bone-to-implant contact (BIC) in the early phase of healing than TiOBlast™.[6]

TiUnite® has a unique oxidized Ti surface that is created through anodic oxidation.[7] The resulting TiO_2 layer has a porous structure and increased surface roughness.[3, 8] Although this TiO_2 layer is bioinert rather than bioactive, TiUnite® implants show superior osseointegration due to their enlarged surface area, which is a consequence of their increased roughness and porous structure.[3] A previous study showed that TiUnite® implants demonstrated significantly higher BIC ratios and removal torque values than turned implants 6 weeks after implant insertion.[9]

Although Osseospeed™ and TiUnite® have been reported to result in a superior bone response compared to their predecessors, they have not been compared with each other by *in vivo* studies. Furthermore, a previous study demonstrated that the calcium phosphate-coated bioactive surface showed similar bone responses to bioinert anodized and blasted surfaces.[10] The aim of the present study was to investigate whether bioactive surfaces were more favorable to bone than bioinert surfaces. Bone regeneration around two commercial dental implants was evaluated using the rabbit tibia model and a gap defect.

MATERIALS AND METHODS

Sample preparation and implant surface modification

The nine test implants had the characteristic conical seal^{TM} and microthreads^{TM} design (Astra Tech, Mölndal, Sweden). The diameter and length of the implants were 4.0 mm and 11.0 mm, respectively. Following F^- treatment, the surface of the test group implant was moderately roughened by grit blasting with TiO_2 particles (Osseospeed^{TM}, Astra Tech, Mölndal, Sweden).[11, 12] The nine control implants had the traditional external connection design (Nobel Biocare AB, Gothenburg, Sweden). The diameter and length of the control implants were 4.0 mm and 11.5 mm, respectively. The turned Ti implants were produced by using them as an anode in an electrochemical cell. During this procedure, oxidation takes place at the implant surface when a potential is applied under appropriate conditions. Oxidation provides the implants with a porous oxide surface (TiUnite®, Nobel Biocare AB, Gothenburg, Sweden).[7]

Surface characteristics

Four implants from each group were used in the surface analysis. This was performed using field emission scanning electron microscopy (FE-SEM), energy dispersive spectroscopy (EDS), and confocal laser scanning microscopy (CLSM). An image of the overall surface was provided by FE-SEM (S-4700, Hitachi, Tokyo, Japan). Analysis of the components and element content of the modified surfaces was performed using EDS (EX220, Horiba Ltd., Kyoto, Japan). The surface roughness was measured with CLSM (LSM 5-Pascal, Carl Zeiss AG, Oberkochen, Germany). Three screw-sides from each implant surface were selected at random. Two roughness parameters, S_a and S_{dr}, were measured. The area of measurement was 450 μm × 450 μm on a ×200 magnified image.

***In vivo* study**

The study was approved by the Animal Research Committee of Seoul National University Bundang Hospital (approval number: BA0909-050-036-01). Animal selection, management, preparation, and surgical protocols were performed in accordance with the Institute of Laboratory Animal Resources guidelines of Seoul National University Bundang Hospital.

Five male New Zealand White rabbits aged around 6 months and weighing 2.6~3 kg were used. The rabbits showed no signs of disease. The rabbits were anesthetized with an intramuscular injection of tiletamine/zolazepam 15 mg/kg (Zoletil 50, Virbac Korea Co. Ltd., Seoul, Korea) and xylazine 5 mg/kg (Rompun, Bayer Korea Ltd., Seoul, Korea). Prior to surgery, the shaved skin over the area of the proximal tibia was washed with betadine. A preoperative antibiotic (Cefazolin, Yuhan Co., Seoul, Korea) was administered intramuscularly. Lidocaine was injected locally into each surgical site. The skin was incised and each tibia was exposed following muscle dissection and periosteal elevation. The implant sites were prepared on the flat tibia surface using drills and sterile and profuse saline irrigation. Drilling was performed bicortically. The diameter of the final drill was 3.7 mm. A 5.0 mm-diameter cortical drill was used to create a gap defect. The cortical drill was used monocortically and created a 5.0 mm hole in the upper cortex only (Fig. 1). Each rabbit received two implants. A F^--modified implant (Osseospeed™) was inserted into one tibia and an oxidized implant (TiUnite®) was inserted into the other. The microthreads of the F^--modified implant were visible on the upper cortex. A corresponding proportion of the oxidized implant was also visible (Fig. 1). Thus, only the bone response around the macrothreads was considered. After implant insertion, the cover screws were securely fastened and the surgical sites were closed in layers. Muscle and fascia were sutured with resorbable 4-0 vicryl sutures. The outer dermis was closed with a nylon suture. Each rabbit was kept in a separate cage post-surgery.

After two weeks of bone healing, the rabbits were anesthetized and sacrificed by the administration of an intravenous overdose of potassium chloride. The tibia was exposed and the implants were surgically removed *en bloc* with an adjacent collar of bone. They were then immediately fixed in 10% neutral formaldehyde. The histomorphometry specimens were prepared as described previously [10]. General histology was evaluated by examining the specimens under a light microscope (Olympus BX, Olympus, Tokyo, Japan). Bone-to-implant contact (BIC) ratios and bone area (BA) were calculated in three consecutive threads from the bone cortex using image analysis software (Kappa PS30C Imagebase, Kappa Opto-electronics GmbH, Gleichen, Germany) connected to the light microscope.

Statistics

The Mann Whitney U-test was used to assess the statistical significance of the difference in surface roughness parameters (S_a and S_{dr}) between the test and control implants. The Wilcoxon signed-rank test was used to determine statistically significant differences between the two groups in the BIC and BA analyses. A p value of less than 0.05 was considered statistically significant.

RESULTS

The FE-SEM images of the test and the control implants are shown in Figure 2. The F^--modified surface displayed irregularities, with many depressions and small indentations, as a result of the grit blasting procedure. The oxidized surface displayed numerous open pores from which the orifices of the larger pores protruded. This porous structure is typical of the anodically oxidized titanium surface. The EDS analysis detected titanium on the surface of the test implants, and titanium and phosphorus on the control implants. Table 1 summarizes the element content of the implant surfaces.

The means and standard deviations (SDs) of S_a were 1.5 μm ± 0.1 μm for the test implants (Osseospeed™) and 1.6 μm ± 0.1 μm for the control implants (TiUnite®). The means and SDs of S_{dr} were 27.3 ± 18.3% for the test implants and 29.1 ± 6.0% for the control implants. No significant differences were observed between the groups for S_a or S_{dr} ($p > 0.05$).

Post-surgery healing was uneventful in all of the rabbits. At the time of sacrifice, all of the implants were submerged and covered by a healthy ridge of mucosa. The light microscopic findings were similar for both groups. For both types of implant, a favorable bone response was observed in the majority of the threads. The circumferential gap defect had been almost completely filled with new bone within the two week period. Good osseointegration was observed within the gap defect. Osteocytes were observed near the threads and woven bone had formed. The border of the gap defect, which distinguished the original cortical bone from the newly formed bone, was visible in both groups (Fig. 3).

The means and SDs of the BIC ratio and BA are shown in Table 2. The histomorphometric analyses revealed no statistically significant differences. Although the oxidized implant showed a numerically higher mean value, no statistically significant differences were observed between the F^--modified and oxidized surfaces in the percentage of BIC ($p > 0.05$). In addition, no significant difference was observed between the test and control implants for BA ($p > 0.05$).

DISCUSSION

In the BIC and BA analyses, no statistically significant differences were found between the bioactive F^- modified surface and the bioinert anodized Ti surface. As expected, no F^- was detected in the EDS analysis, since F^- is present on the implant surface in a trace amount that is insufficient for detection by this method of analysis. However, it is difficult to explain the effects of such a small amount of F^- on osseointegration. A previous study detected 1 atomic % of F^- on Osseospeed™, and the influence of this amount of F^- on bone response has been questioned.[3] In the present study, the bioactive F^--modified surface was not superior to the bioinert anodized surface in terms of early bone response. Further studies are required to determine whether the newly introduced bioactive implant surfaces are better in terms of osseointegration than the existing modified surfaces.

A crucial factor in the successful osseointegration of endosseous implants is a favorable interaction between implant geometry and the surface texture and tissues at the bone site.[13] The present study investigated the insertion of two implants with differing geometries (Osseospeed™ and TiUnite®) using a rabbit tibia model. To minimize the effects of implant design on bone response, the Osseospeed™ implant was inserted so that its microthreads were visible and the TiUnite® implant was inserted correspondingly. Thus, this experiment eliminated the effect of implant microstructure and focused instead on the implant surface per se. Furthermore, the implants were inserted at random, i.e., the right and left of the tibia were not assigned specifically.

Substantial bone formation was observed in the present study, and histological examination demonstrated that good osseointegration had been established around the gap defect within a two week period. This indicates that the implant surface modifications enhanced osteoconduction, as reported previously.[14] The diameter of the inserted implants was 4.0 mm, and that of the surgically created gap was 5.0 mm or larger since the cortical drill used to create the gap had a diameter of 5.0 mm. This experiment therefore excluded the effect of the primary stability of the implant, which is influenced by implant design.[15] Therefore, the results obtained for early bone formation in this experiment can be attributed to the effects of the modified surfaces.

The results of the present study show that both the F^--modified and oxidized surfaces result in good osteoconduction and high quality bone formation around circumferential gap defects during the early phase of bone healing. However, the new bone formation around the circumferential defect was assessed after a very short period of time, and so it is unclear whether contact osteogenesis had occurred. Further studies are required to investigate this.

CONCLUSIONS

The commercial oral implants Osseospeed™ and TiUnite® showed similar osteoconduction around gap defects in the early phase of healing in the present rabbit tibia model. Therefore, within the limitation of this study, the bioactive F^--modified surface may not be superior to the bioinert anodized Ti surface in terms of early bone response.

DISCLOSURE

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LEGENDS

Table 1. The element content of the implant surfaces according to EDS analysis.

	Osseospeed™	**TiUnite®**
Ti (Atomic %)	100%	87.8% ± 7.4%
P (Atomic %)	0%	12.2% ± 7.4%

Table 2. The means and SDs for BIC and BA 2 weeks post-surgery.

Groups	**BIC (%)**	**Bone Area (%)**
Osseospeed™	36.0 ± 5.4	47.4 ± 3.4
TiUnite®	42.6 ± 4.0	47.0 ± 5.4

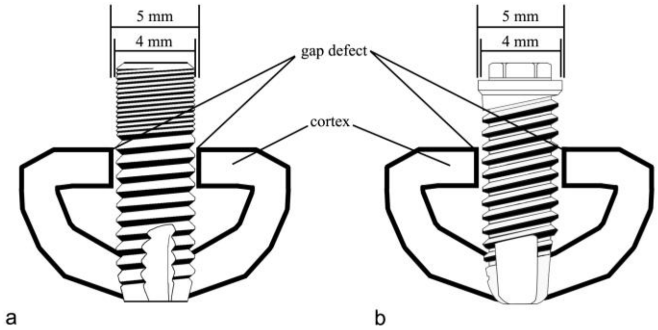

Fig. 1. A schematic diagram shows how to insert the implant into the rabbit tibia. **a**, Osseospeed™ and **b**, TiUnite® implants, both of which were 4.0 mm in diameter, were firmly engaged at the bottom of the cortex in the rabbit tibia. A hole that was 5 mm in diameter was formed at the upper cortex only. Note that the threads of the implants are not engaged at the upper cortical area.

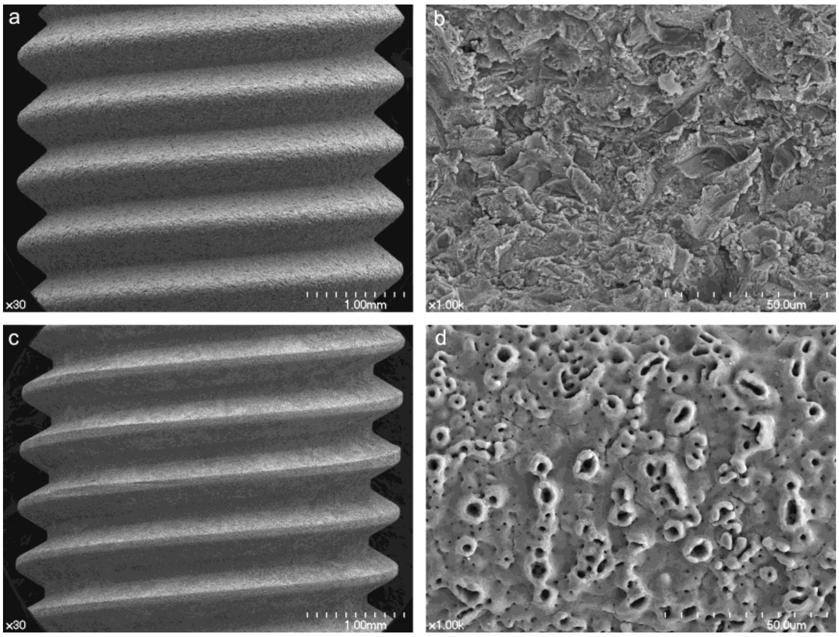

Fig. 2. SEM images of the implants. **a** and **b**, Typical indentations and irregularities on the blasted surface are shown in Osseospeed™. **c** and **d**, The porous structure, which is the result of anodic oxidation, is observed on the TiUnite® surface ((c) and (d)).

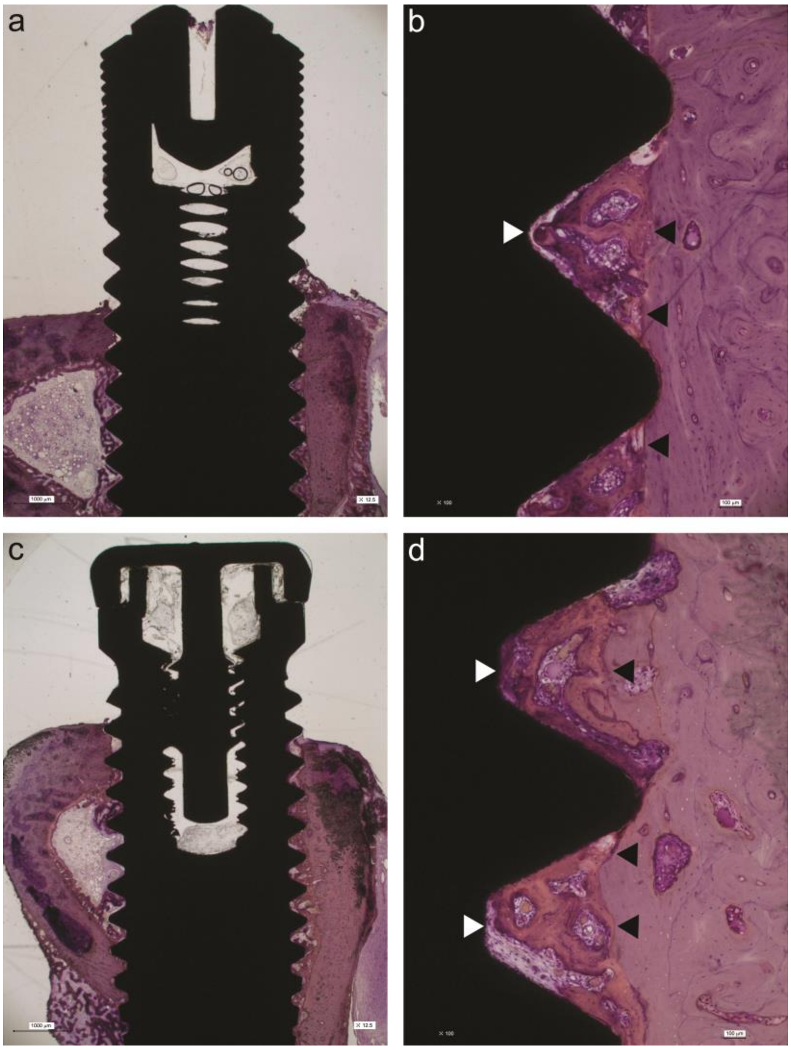

Fig. 3. a, On the light microscopic views, Osseospeed™ implant shows bone is well filled between the threads. **b**, New bone has rapidly formed for 2 weeks after implant insertion (between the black and white arrows). The margin of the gap defect remains clearly defined (black arrows). **c**, Similar findings are shown on the TiUnite® specimens. **d**, New bone has early formed enough to fill the gap defect (between the black and white arrows). The margin of the gap defect is also clearly observed (black arrows).