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Implant Science



Effects of ibuprofen-loaded TiO₂ nanotube dental implants in alloxan-induced diabetic rabbits

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ABSTRACT

Purpose: Some systemic conditions, especially diabetes mellitus (DM), adversely affect dental implant success. This study aimed to investigate the effects of ibuprofen-loaded TiO₂ nanotube (ILT) dental implants in alloxan-induced diabetic rabbits.

Methods: Twenty-six New Zealand white rabbits were treated with alloxan monohydrate to induce DM. At 2 weeks following DM induction, 3 types of implants (sandblasted, large-grit, and acid-etched [SLA], ILTN, and machined) were placed into the proximal tibia in the 10 rabbits that survived following DM induction. Each type of implant was fitted randomly in 1 of the holes (round-robin method). The animals were administered alizarin (at 3 weeks) and calcein (at 6 weeks) as fluorescent bone markers, and were sacrificed at 8 weeks for radiographic and histomorphometric analyses.

Results: TiO₂ nanotube arrays of ~70 nm in diameter and ~17 μm in thickness were obtained, and ibuprofen was loaded into the TiO₂ nanotube arrays. A total of 26 rabbits were treated with alloxan monohydrate and only 10 rabbits survived. The 10 surviving rabbits showed a blood glucose level of 300 mg/dL or higher, and the implants were placed in these diabetic rabbits. The implant stability quotient (ISQ) and bone-to-implant contact (BIC) values were significantly higher in the ILTN group (ISQ: 61.8, BIC: 41.3%) and SLA group (ISQ: 62.6, BIC: 46.3%) than in the machined group (ISQ: 53.4, BIC: 20.2%), but the difference in the BIC percentage between the SLA and ILTN groups was not statistically significant ($P=0.628$). However, the bone area percentage was significantly higher in the ILTN group (78.0%) than in the SLA group (52.1%; $P=0.000$).

Conclusions: The ILTN dental implants showed better stability (ISQ) and BIC than the machined implants; however, these values were similar to the commercially used SLA implants in the 2-week diabetic rabbit model.

Keywords: Alloxan; Dental implant; Diabetes mellitus; Ibuprofen; Nanotubes

INTRODUCTION

The high success rate of implant-supported prostheses for various edentulous defects has made this treatment intervention a reliable alternative to oral reconstruction [1]. A history of smoking, radiation therapy, hormonal therapy, and particularly diabetes mellitus (DM) are

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

systemic conditions that have been proposed to adversely affect the success of dental implants [2]. DM is a metabolic disorder characterized by chronic hyperglycemia that is caused by a decreased production or insufficient action of insulin [3]. Chronic hyperglycemia associated with diabetes is associated with changes in the host's response to periodontal healing [4].

Compared with individuals who do not have DM, those with the disorder are more likely to suffer from periodontal destruction. This could be attributed to the creation and accumulation of advanced glycation end products (AGEs) [5], which are known to activate receptors in the periodontal tissue and contribute to impaired wound healing and development of periodontal disease [6]. Santana et al. [7] confirmed that the accumulation of AGEs decreases osseous healing. In animal experiments, osseointegration was impaired under diabetic conditions [8,9]. Takeshita et al. [10] found 30% less bone-to-implant contact (BIC) with implants in diabetes-induced rats than in non-diabetic rats. Nevins et al. [11] reported significantly reduced wound healing and BIC in DM-induced animals, suggesting that osseointegration is affected by diabetes. Some studies described a correlation between diabetes and increased dental implant failure rates [2,12].

Since 1 in 10 Korean people aged 30 years or older has DM [13], dental implant surgery is performed in patients with DM in a considerable proportion of cases. Therefore, studies aimed at improving osseointegration in the diabetic environment are needed. Several methods have been proposed to improve the success rate of implants in the detrimental environment of DM, such as the use of prophylactic antibiotics, higher implant length and width, and implants loaded with bioactive material [14]. Implants loaded with bioactive material can demonstrate various effects depending on the loaded drug [14-16]. For drug application, nanotubes are fabricated through anodic oxidation on the implant surface. These nanotubes not only provide drug reservoirs, but also are capable of increasing osteoblast adhesion by increasing the surface roughness [16,17]. In a previous study [15], 2-step anodic oxidation was conducted to obtain clean-surface and open-tube structures of TiO₂ nanotube arrays; these nanotube arrays were considered suitable for drug loading.

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the conversion of arachidonic acid to the prostaglandin series of metabolites. The resultant prostaglandins themselves have been implicated in both bone resorption and bone formation [18,19]. Prostaglandin levels are elevated in inflamed tissues, and prostaglandin E₂ reportedly causes bone resorption *in vitro* [20,21]. Kornman et al. [22] reported that topically applied ibuprofen in nonhuman primates with periodontal disease significantly inhibited bone loss with no effect on polymorphonuclear leukocyte levels. Thus, ibuprofen is expected to be effective in modulating the initial bone resorption around implants.

To our knowledge, few studies have evaluated the effect of ibuprofen-loaded TiO₂ nanotube (ILT_N) dental implants in the diabetic environment. Prior to researching the improvement of osseointegration in the diabetic environment, our study group standardized the diabetic rabbit model by allowing a DM induction period of 2 weeks following the administration of alloxan monohydrate [23]. Therefore, the purpose of this study was to evaluate the effect of dental implants with surfaces containing ibuprofen-loaded TiO₂ nanotube arrays fabricated by 2-step anodic oxidation in alloxan-induced diabetic rabbits through radiographic and histological analyses.

MATERIALS AND METHODS

Experimental animals

Twenty-six New Zealand white rabbits (male, 2.6–3.75 kg) were used in this study. The animals were kept at room temperature, and water and food were provided *ad libitum*. All the animals were acclimated for 1 week before the experiments. This study was performed with approval from the Institutional Animal Care and Use Committee of Gangneung-Wonju National University (GWNVA-2016-21).

Study design

Alloxan monohydrate (Sigma-Aldrich Co., St. Louis, MO, USA) was administered to induce DM in the rabbits. At 2 weeks following DM induction, the implants were placed in the diabetic animals. Three different types of implants were prepared: sandblasted, large-grit, and acid-etched (SLA) implants (TS III™; Osstem Implant Co. Ltd., Seoul, Korea) as a positive control (SLA group); implants with ibuprofen-loaded TiO₂ nanotube arrays (ILTNA group); and machined implants as a negative control (machined group).

To evaluate differences in new bone formation on the implant surface, 2 different fluorochromes were administered intramuscularly following implant placement: alizarin red (Sigma-Aldrich Co.) 30 mg/kg, 3 weeks following implant placement; and calcein green (Sigma-Aldrich Co.) 10 mg/kg, 6 weeks following implant placement [15]. At 8 weeks following implant placement, the animals were sacrificed and the specimens were prepared for radiographic and microscopic examinations. The study design is shown in Figure 1.

Ibuprofen-loaded TiO₂ nanotube implant preparation and elution test

The implant preparation procedure for the ILTNA implants was similar to that described in a previous study [15], which is briefly explained as follows. Using a direct current power supply, machined-surface implants were anodized in an electrolyte solution containing ethylene glycol, 0.5 wt% NH₄F, and 1.0 vol% deionized water. A 2-step anodic oxidation was performed to obtain a clean-surface and open-windows for the TiO₂ nanotube arrays. Hollow TiO₂ nanotube arrays can be created on the implant surface and used as drug reservoirs.

To load ibuprofen (Sigma-Aldrich Co., St. Louis, MO, USA) onto the TiO₂ nanotube arrays, the dip-coating method was performed with 10 mg/mL ibuprofen solutions in a vacuum chamber. The implants were immersed 3 times into the ibuprofen solution for 5 s each time and then dried at room temperature [15]. The elution of ibuprofen was observed by an interferometric biosensing method, where the TiO₂ nanotube arrays formed on a flat titanium surface were used. White light from a tungsten lamp (Ocean Optics, Dunedin, FL, USA) was fed through 1 end of a bifurcated fiber-optic cable and focused through a lens onto the surface of the TiO₂ nanotube arrays at normal incidence. The reflected light was collected through the same optical setup, and the distal end of the bifurcated fiber-optic cable was connected to a charge-coupled device spectrometer (Ocean Optics S-2000, Ocean Optics) [24]. The optical thickness (OT; expressed as nL, where n is the refractive index of the porous layer and L is the thickness of the



Figure 1. Study design.
DM: diabetes mellitus.

porous layer) was determined using the Fabry-Pérot relationship ($m\lambda = 2nL$), where m is the fringe order and λ is the wavelength of maximum constructive interference for a spectral fringe of order m . The change in OT over time was monitored [15].

DM induction

Prior to investigating the improvement of osseointegration in the diabetic environment, the diabetic rabbit model was standardized by permitting a DM induction period of 2 weeks following the administration of alloxan monohydrate [23]. Alloxan monohydrate was dissolved in sterile saline, and 100 mg/kg was administered intravenously to the rabbits for 5 minutes via the marginal ear vein.

Wang et al. [25] defined DM in rabbits as being present when the morning blood sugar (BS) level is over 300 mg/dL. The severity of DM in the rabbits was categorized according to BS levels as follows: 300–400 mg/dL, mild DM; 401–500 mg/dL, moderately severe DM; and >500 mg/dL, severe DM. In the present study, the BS level was measured 2 weeks after DM induction and a rabbit was considered diabetic if the level was over 300 mg/dL.

Surgical procedure

Three types of implants (SLA, ILTN, and machined) were prepared. For the implant placement, the rabbits were first anesthetized with isoflurane (Hana Pharm Co. Ltd., Hwasung, Korea) and the surgical site was then shaved and disinfected using iodine solution. After applying local anesthesia with 2% lidocaine containing 1:100,000 epinephrine (Huons, Seoul, Korea), an incision was made along the proximal one-third of the tibia, and the bone was exposed using a blade and periosteal elevator. Under irrigation, 2 implant holes were drilled into the tibia of one leg and 1 hole was drilled into the tibia of the other leg, and each type of implant was placed randomly in 1 of the holes (round-robin method). The middle third of each implant was placed in the upper cortical bone. Following implant placement, resonance frequency analysis (RFA) was conducted, wherein the expressed implant stability quotient (ISQ) was measured using the Osstell ISQ/SmartPeg instrument (Osstell AB, Göteborg, Sweden). The surgical site was then sutured using 4-0 Vicryl sutures (Ethicon Inc., Somerville, NJ, USA). Following surgery, all the rabbits received antibiotics (0.05 mL/kg gentamicin sulfate; Samu Median Co. Ltd., Yesan, Korea) and an analgesic (0.50 mL/kg sulpyrine; Samyang Anipharm Co. Ltd., Seoul, Korea) via intramuscular injections. At 8 weeks following implant placement, the animals were sacrificed in a CO₂ chamber. The tibia was exposed and the ISQ of the implant was measured. Tibia blocks (1 cm × 1 cm) were then removed and fixed in 10% formalin.

Micro-computed tomography evaluation

To evaluate the amount of cortical bone volume around the implant, the tibia specimens were scanned using a micro-computed tomography (micro-CT) scanner (Skyscan 1173, Kontich, Belgium). The voltage, current, and exposure time of the X-rays were 130 kV, 60 μ A, and 500 ms, respectively. Micro-CT was conducted to measure the cortical bone volume around the implant. The region of interest (ROI) was set as a circle, with the long axis of the cross-section of the implant fixture as the diameter, and the 2 implant threads located in the cortical bone as the length (Figure 2). The bone tissue amount in the ROI was measured and analyzed.

Histologic evaluation

The tibia samples were fixed by immersion in a 10% neutral-buffered formalin solution (Accustain™; Sigma-Aldrich, Steinheim, Germany) for 1 day, then dehydrated in graded

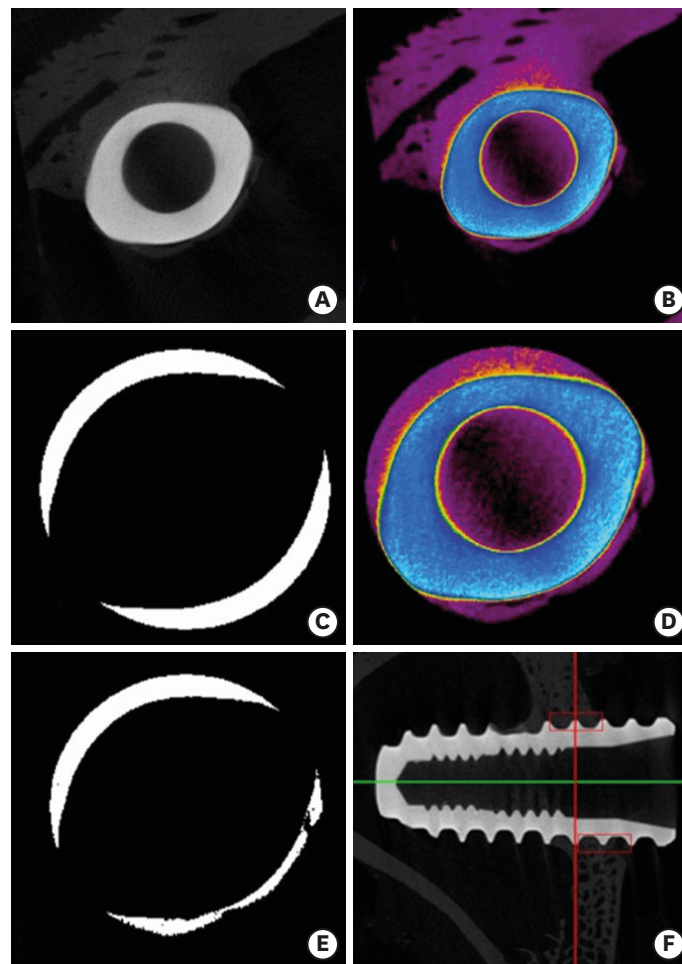


Figure 2. ROI setting for micro-computed tomography evaluation. (A) Original image, (B) color image, (C) ROI, (D) color image in the ROI, (E) bone tissue in the ROI, and (F) length of the ROI. ROI: region of interest.

series of ethanol solutions, and finally embedded in methylmethacrylate resin (Technovit 7200 VLC; Kulzer Co. GmbH, Friedrichsdorf, Germany). Following dehydration, the specimens were polymerized in a light-based polymerization unit (Exakt Apparatebau, Norderstedt, Germany). The implants were cut mid-axially in a mediolateral plane into 200- μ m thick sections using a band saw with a diamond blade (Exakt cutting-grinding system; Exakt Apparatebau). The final section was ground to no thicker than \sim 20 μ m using an Exakt microgrinder and then polished to an optical finish using the cutting-grinding technique described by Donath and Breuner [26].

All the sections were examined first using immunofluorescence microscopy (Leica Microsystems, Wetzlar, Germany), and then stained with Goldner's trichrome stain and examined using optical microscopy (BX-50; Olympus America, Melville, NY, USA). One examiner carried out the histomorphometric measurements in a blinded manner. The BIC ratio and the bone area (BA) ratio around the implant thread were measured using ImageJ software (NIH, Bethesda, MD, USA). The BIC was set as the percentage of bone contact measured along the entire surface length of 2 implant threads, and the BA was set as the percentage of total bone tissue within the area of 2 threads.

Statistical analysis

The *t*-test was used to compare the ISQ values at the time of implant placement and sacrifice. The ISQ values and the BIC ratio among the groups were assessed using 1-way analysis of variance and Scheffé *post hoc* analysis. All statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Data are presented as the mean±standard deviation (SD). *P* values less than 0.05 were considered to indicate statistical significance.

RESULTS

Mechanical features of the ibuprofen-loaded TiO₂ nanotube implant

Figure 3 shows the microscopic features of the anodic oxidized TiO₂ nanotube arrays on the dental implant surface. TiO₂ nanotubes with a pore diameter of 70 nm or less and a thickness of 17 μm or less were observed on the dental implant surface. After loading the drug in the TiO₂ nanotube arrays and drying the implant, the surface of the TiO₂ nanotubes was partially covered by the drug.

Figure 4 shows the OT change over time, demonstrating drug elution from the ibuprofen-loaded TiO₂ nanotube dental implants. The OT value of the ibuprofen-loaded TiO₂ nanotube dental implants immediately increased from ~14840.4 nm to ~14843.9 nm after placement of the implant in a deionized water bath and the value was maintained for 27 hours. The

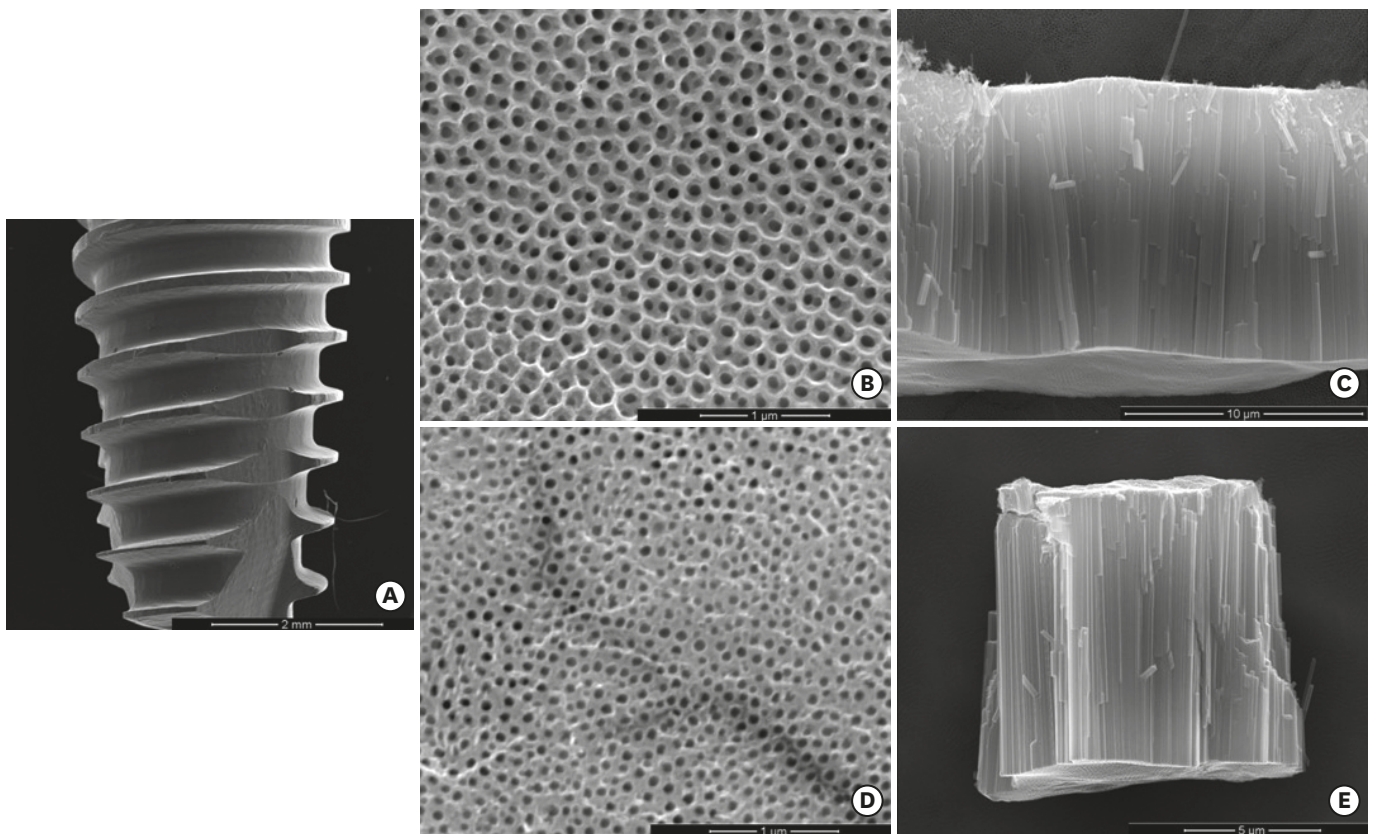


Figure 3. Field emission scanning electron microscopy images of a TiO₂ nanotube dental implant. (A) TiO₂ nanotube dental implant (low magnification) (B) top view and (C) vertical view of TiO₂ nanotube arrays on the dental implant before the ibuprofen loading process, and (D) top view and (E) vertical view of TiO₂ nanotube arrays on the dental implant after the ibuprofen loading process. After drug loading in the TiO₂ nanotube arrays and drying, the surface of the TiO₂ nanotubes was partially covered by the drugs.

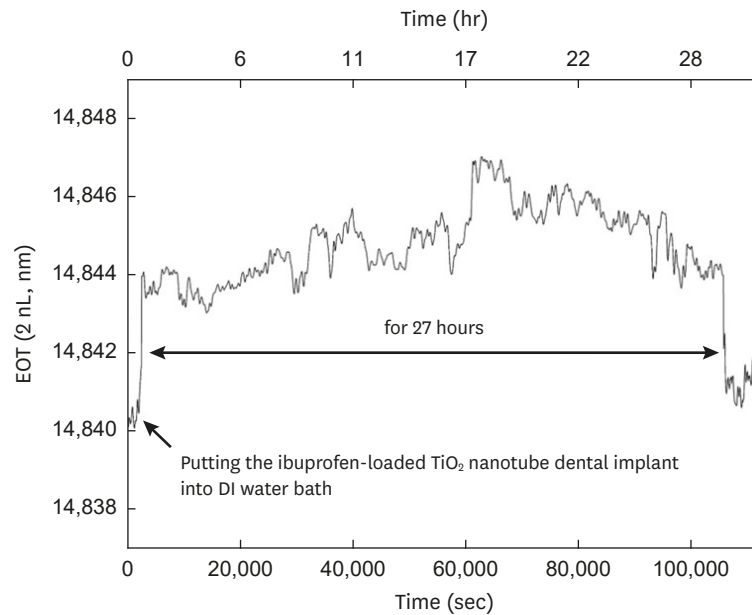


Figure 4. Changes in the optical thickness level after placement of the ibuprofen-loaded TiO₂ nanotube dental implant in a deionized water bath. EOT: effective optical thickness.

increase in OT indicates that the drug (ibuprofen) was eluted from the ibuprofen-loaded TiO₂ nanotube dental implants.

DM induction

Among the 26 rabbits injected, 14 died from complications and 2 failed to show elevated BS levels. Therefore, implants were installed in the remaining 10 rabbits that showed BS levels suggestive of diabetes. Figure 5 shows the mean BS levels at the time of DM induction (116.3±4.9 mg/dL), implant placement (2 weeks following DM induction: 423.6±68.0 mg/dL), and sacrifice (8 weeks following implant placement: 463±86.6 mg/dL). In all the experimental rabbits, the BS level was over 300 mg/dL at DM induction and before sacrifice, and the rabbits were therefore diagnosed with diabetes.

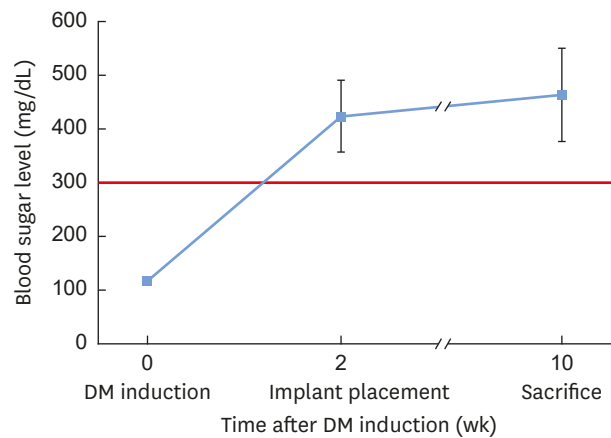


Figure 5. Changes in blood sugar levels after alloxan monohydrate administration. DM: diabetes mellitus.

Table 1. Changes in the implant stability quotient (mean±standard deviation) obtained by resonance frequency analysis

Variables	SLA group (n=10)	ILTN group (n=10)	Machined group (n=10)
At implant placement	55.2±11.6	54.2±10.9	40.8±13.1
8 wk later	62.6±7.0	61.8±3.8	53.4±8.6

SLA group: sandblasted, large-grit, and acid-etched implants, ILTN group: ibuprofen-loaded TiO₂ nanotube implants, Machined group: machined implants.

Implant stability analysis

Table 1 shows the ISQ values (implant stability). All 3 groups had increased implant stability after 8 weeks compared to the time of implant placement. The ISQ values of the SLA and ILTN groups were higher than that of the machined group at implant placement (55.2±11.6, 54.2±10.9, and 40.8±13.1, respectively). Although the ISQ values were not statistically different between the groups, the ISQ values of the SLA and ILTN groups were higher than those of the machined group at 8 weeks following implant placement (63.6±7.0, 61.8±3.8, and 53.4±8.6, respectively). The ILTN and the SLA groups had greater implant stability immediately and at 8 weeks following surgery.

Micro-computed tomography analysis

Figure 6 shows a comparison of the cortical bone volumes around the implants using micro-CT. The highest mean cortical bone volume was found in the ILTN group (0.264±0.100 mm³), followed by the machined and SLA groups (0.253±0.106 and 0.225±0.132 mm³, respectively). However, the difference in values among the groups was not statistically significant.

Histological analysis

The BIC was significantly higher in the SLA (46.3±23.8%) and ILTN (41.3±16.7%) groups than in the machined group (20.2±8.1%; *P*<0.05) (Table 2). The difference in BIC percentage between the SLA and ILTN groups was not statistically significant (*P*=0.628). However, the BA percentage was significantly higher in the ILTN group (78.0±7.9%) than in the SLA group (52.1±15.652.13%; *P*=0.000).

Figure 7 shows the histological staining and fluorescence images in the SLA, ILTN, and machined groups. In the fluorescence images, different patterns of osteogenesis were evident among the groups. Red and green fluorescence indicate bone formation at 3 and 6 weeks

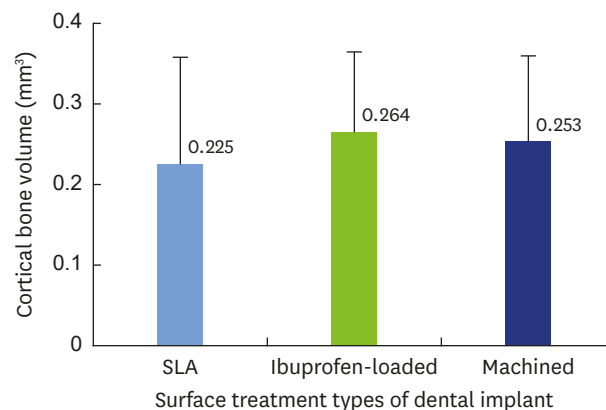


Figure 6. Cortical bone volume around the implant (mm³).

SLA group: sandblasted, large-grit, and acid-etched implants, Ibuprofen group: ibuprofen-loaded TiO₂ nanotube implants, Machined group: machined implants.

Table 2. Histomorphometric analysis

Dependent variables	Implant type	No.	Mean±SD (%)	P value
BIC (%)	SLA	10	46.3±23.8	0.000
	ILTN	10	41.3±16.7	0.001
	Machined	10	20.2±8.1	
BA (%)	SLA	10	52.1±15.6	
	ILTN	10	78.0±7.9	0.000
	Machined	10	65.2±15.7	0.066

SD: standard deviation, BIC: bone-to-implant contact, BA: bone area ratio around the implant thread, SLA: sandblasted, large-grit, and acid-etched implants, ILTN: ibuprofen-loaded TiO₂ nanotube implants, Machined: machined implants.

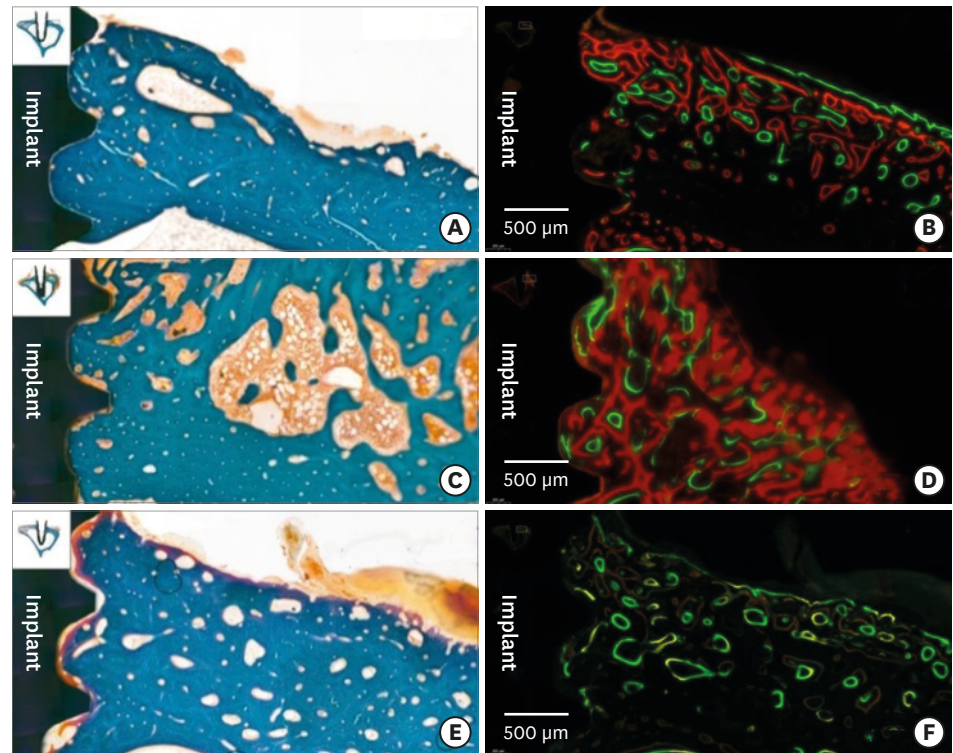


Figure 7. Histological staining and fluorescence images in the SLA group, ibuprofen group, and machined group. (A) Histological staining image from the SLA group, (B) fluorescence image from the SLA group, (C) histological staining image from the ibuprofen group, (D) fluorescence image from the ibuprofen group, (E) histological staining image from the machined group, and (F) fluorescence image from the machined group. The ILTN group appeared to be superior to the other 2 groups in terms of initial bone formation. The scale bar represents 500 μm. SLA group: sandblasted, large-grit, and acid-etched implants, Ibuprofen group: ibuprofen-loaded TiO₂ nanotube implants, Machined group: machined implants.

following implant placement, respectively. From these images, the ILTN group appeared to be superior to the machined group based on initial bone formation.

DISCUSSION

Alloxan monohydrate is a commonly used drug for experimental DM induction in animals such as rabbits, rats, mice, and dogs. A previous study [23] demonstrated that a period of more than 1 week was required for inducing DM in rabbits following the administration of 100 mg/kg of alloxan monohydrate; implants were placed following an induction period of 2 weeks. The lethality of 100 mg/kg alloxan monohydrate and the success rate of DM induction

were consistent in both studies (lethality rate: 52.6% vs. 53.8% and success rate: 36.4% vs. 38.5% in previous vs. present studies, respectively), confirming the alloxan monohydrate dose appropriate for DM induction in rabbits. Among studies using the same amount of alloxan monohydrate, one with a lower lethality rate was conspicuous [25]; in that study, to avoid hypoglycemia following DM induction, 10 mL of glucose was administered at 4, 8, and 12 hours following the induction process and added to the drinking water. Incorporating these methods would be beneficial in future studies.

The BIC values in this study were lower than those reported in previous studies [27,28]. Gehrke et al. [27] assessed bone formation around titanium surfaces incorporating calcium–magnesium deposited using sandblasting in rabbit tibia. They reported that the BIC of SLA implants in normal rabbits was 55.1% after 6 weeks, but in the present study, the SLA group was 46.3% after 8 weeks. Cordioli et al. [28] conducted a histomorphometric and biomechanical comparison of bone response to 4 different types of dental implants in rabbit tibia. They reported that the BIC of machined implants in normal rabbits was 48.6% after 5 weeks, but in the present study, the machined group had a BIC of 20.2% after 8 weeks. This difference implies a negative effect of DM on the implants.

The RFA method developed by Meredith et al. [29] evaluates the stability of implants in a non-destructive and non-invasive manner. Several studies have also reported a positive correlation between the ISQ and total BIC values [30,31]. However, in the present study, the ISQ value tended to be higher at the time of sacrifice than at implant placement, although the difference was not significant at each time point. These discrepancies could be due to differences in the cortical bone volume at various locations in the tibia. At the time of surgery, the rabbit tibia generally had a thinner cortical bone at the medial site than at the lateral site. It could be assumed that the non-significant difference in the ISQ values among the groups was because of these discrepancies in the cortical bone volume. Moreover, the lack of significant differences for cortical bone within the ROI could indicate that the randomization limited to the 2 implant threads was successful.

In the present study, the BIC ratio of the ILTN and the SLA implants was higher than that of the machined implants. However, the difference in BIC between the ILTN and SLA implants was not significant. Buser et al. [32] suggested that a tendency for higher BIC was related to increased surface roughness of the implant. Shalabi et al. [33] reported in a systematic review that the BIC is mainly affected by surface roughness. In this study, the BIC value was not significantly different between the ILTN and SLA implants because the ILTN and SLA implants have a similarly rough structure.

In the *in vitro* study, ibuprofen was eluted from the drug-loaded implant for 27 hours. Loading ibuprofen in a nanotube implant, from which it is gradually released following implant placement, can considerably improve the diabetic environment that inhibits tissue healing by controlling the inflammatory response occurring in the beginning of implant placement. In the fluorescence images, red and green fluorescence appeared as distinct thin threads in the SLA implants, whereas they appeared homogeneous in all the threads in the ibuprofen implant. These findings support that the inflammation-inhibiting function of ibuprofen promotes early osteogenesis. In orthopedic studies, NSAIDs caused certain side effects, such as decreased bone formation and inhibition of fracture healing [34,35]. In the present study, there was no significant difference in the BIC percentages between the ILTN group and the SLA positive control group.

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