The in vivo effects of hydrophilicity and fluoride surface modifications to titanium dental implants on early osseointegration

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ABSTRACT (189 words)

PURPOSE. The purpose of this study was to investigate *in vivo* histomorphometric differences in initial bone response to modified sand-blasted, large-grit, acid-etched (modSLA) and fluoride-modified (F-mod) implant surfaces in rabbit tibia models. **MATERIALS & METHODS.** Field emission scanning electron microscopy (FE-SEM), confocal laser scanning microscopy (CLSM), and X-ray photoelectron spectroscopy (XPS) were used to determine surface characteristics. Each of 3 live New Zealand White rabbits received an F-mod implant in one tibia and a modSLA implant in the other. After 1 week, the rabbits were sacrificed and the undecalcified histologic slides were prepared. Bone-to-implant contact ratio (BIC) and bone area (BA) were calculated in a defined area under a light microscope. **RESULTS.** FE-SEM, CLSM, and XPS showed that the modSLA surface was significantly rougher than the F-mod, and that the F-mod surface had a very small amount of fluoride. However, despite these surface variances, histomorphometric analyses revealed no significant differences in either BIC or BA. **CONCLUSION.** Our results suggest that the *in vivo* effects of increased hydrophilicity, when added to a titanium dental implant surface, on early bone response may be similar to the effects of surface fluoride treatment.

KEY WORDS: implant surface; surface modification; fluoride; hydrophilicity; modified SLA; rabbit tibia

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Implant surfaces influence the biologic response at the bone-implant interface, which in turn affects osseointegration.[1,2] Various surface characteristics, such as surface topography and hydrophilicity, affect bone response.[3] Surface topography has been thoroughly investigated in the literature, reaching the generally accepted conclusion that moderately rough surfaces lead to faster and stronger osseointegration than smooth surfaces.[3-5] Recently, several studies have reported evidence that changing the chemistry of implant surface by enhancing hydrophilicity or by lowering hydrocarbon contamination promotes bone healing.[6-9]

A new material has been introduced that consists of sand-blasted, large-grit, acid-etched (SLA) surface that is further chemically modified with hydrophilic properties, known as a modified SLA (modSLA) surface.[6,10] Compared with the previous SLA surface, the modSLA surface has shown more active osteogenic activities *in vitro* and stronger bone responses *in vivo*.[9,11-14] Several authors have postulated that the hydrophilicity of the modSLA surface explains the cellular activation and resulting bone healing.[6,11,13] A different modification, cathodic reduction of a titanium oxide grit-blasted titanium surface by hydrofluoric acid (HF) creates a fluoride-modified (F-mod) surface that lowers its surface hydrocarbon content.[7,15] Although the F-mod surface is hydrophobic, various reactions of the fluoride ion have been reported to promote bone formation and osseointegration both *in vitro* and *in vivo*.[7,8,15-17] It has been suggested that improved bone formation is caused by the elimination of hydrocarbon contamination as well as the presence of surface fluoride, titanium oxide, and titanium hydride.[7]

Both the modSLA and F-mod surfaces have exhibited superior bone responses to their predecessors, potentially because of their increased hydrophilicity and the chemical action of fluoride.[13,18] However, *in vivo* investigations comparing bone responses between the modSLA and F-mod surfaces are lacking, which are required to comprehensively evaluate their effects in the complex living environment.

In this study, we investigate differences in initial bone response to the modSLA and F-mod implant surfaces using a rabbit tibia model. The null hypothesis is that there is no significant difference in bone response to the different surfaces.

MATERIALS AND METHODS

Implant Preparation

We tested implants with the F-mod surface (Osseospeed, Astra Tech, Mölndal, Sweden) with a diameter of 3.5 mm and a length of 11.0 mm. We also tested implants with the hydrophilic modSLA surface (SLActive, Institute Straumann AG, Basel, Switzerland) with a diameter of 3.3 mm and a length of 10.0 mm.

Surface Characteristics

We used field emission scanning electron microscopy (FE-SEM) to study the surfaces (S-4700, Hitachi, Tokyo, Japan) and confocal laser scanning microscopy (CLSM; 5-Pascal, Carl Zeiss AG, Oberkochen, Germany) to measure the roughness of the implant surfaces. Three screw sides from each implant surface were selected at random. Two roughness parameters, S_a and S_{dr}, were measured. S_a is defined as the arithmetical mean height of the surface in 3-dimensional area surface texture parameters, and S_{dr} is defined as the developed area ratio. The area of measurement was 300 μm × 300 μm on a × 200 magnified image. X-ray photoelectron spectroscopy (XPS) detected the elements and their contents on the investigated surfaces. XPS analysis was performed using a Sigma Probe (Thermo VG Scientific, UK) at 15 kV. Three individual implants of each type were examined using FE-SEM, CLSM, and XPS.

Animal Surgery

This animal experiment was approved by the Animal Research Committee of Seoul National University (approval no. SNU-111123-1). The guidelines of the Institute of Laboratory Animal Resources of Seoul National University were followed in animal selection, management, preparation, and surgical protocol.

We used 3 male New Zealand White rabbits aged 1-2 years and weighing 2.6-3 kg. The rabbits received anesthesia with an intravenous 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). Before surgery, the shaved skin in the proximal tibia area was washed and decontaminated with Betadine. A preoperative antibiotic (cefazolin, Yuhan Co. Ltd., Seoul, Korea) was also administered intravenously.

The skin was incised and bilateral tibia were exposed after muscle dissection and periosteal elevation. The implant sites were prepared at the tibia using drills and profuse sterile saline irrigation. The flat surface on the medial aspect of the proximal tibia was first drilled with a small diameter of 1.5 mm and low rotational speed of 800 rpm. Then, the drilled hole was successively enlarged according to manufacturer guidelines. Drilling was performed bicortically. The diameter of the final drill was 3.2 mm for the F-mod implant and 3.0 mm for the modSLA implant. For the F-mod implants, a drill 3.7 mm in diameter was used monocortically to create a 3.7-mm hole in the upper cortex only (Figure 1). For the modSLA implants, a drill 3.5 mm in diameter was used monocortically (Fig. 1). Each rabbit received 1 implant in each tibia. 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 suture. The outer skin was closed with nylon suture. Each rabbit was kept in a separate cage after surgery. After 1 week of bone healing, rabbits were anesthetized and sacrificed by the administration of intravenous potassium chloride.

Histomorphometry

The tibiae were exposed and the implants were surgically removed *en bloc* with an adjacent collar of bone. The samples were then immediately fixed in 10% neutral formaldehyde. Specimen preparation for light microscopy has been described in previous studies.[19,20] Briefly, the undecalcified specimens were prepared through resin embedding and grinding by Exact® system (Exact Apparatebau, Norderstedt, Germany) according to the method described by Donath and Breuner.[21] The specimens were ground to an approximate thickness of 50 μm and stained with hematoxylin and eosin. General histology was evaluated by examining the specimens under a light microscope (Olympus BX, Olympus, Tokyo, Japan). Bone-to-implant contact ratio (BIC) and bone area (BA) were calculated in a defined area from the bone crest (Fig. 2) using image analysis software (Kappa PS30C Image-base, Kappa Opto-electronics GmbH, Gleichen, Germany) connected to the microscope.

Statistical Analysis

The Mann-Whitney U test was used to find significant differences in surface roughness parameters (S_a and S_{dr}) between implants. Wilcoxon's signed rank test was used to determine statistically significant differences in BIC and BA. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

FE-SEM images of the implant surfaces are shown in Figure 3. Both the F-mod and modSLA surfaces displayed irregularities as a result of the grit blasting procedure. The F-mod surface displayed typical features including the detection of blasted media, while the modSLA surface displayed honeycomb-shaped irregularities with sharp edges due to the acid etching procedure.

The mean and standard deviation (SD) of the S_a values for the F-mod and modSLA surfaces were 1.3 μm (0.1 μm) and 3.5 μm (0.2 μm), respectively. The modSLA surface was significantly rougher than

the F-mod surface ($p < 0.05$). The mean and SD of the measured S_{dr} values were 64.2% (4.1%) for the F-mod surface and 130.4% (9.5%) for the modSLA surface. The modSLA surface had a significantly larger area than the F-mod surface when the irregularities were smoothed out ($p < 0.05$).

XPS results are shown in Table 1. Very little fluoride was detected on the F-mod surface. Titanium and oxygen were detected on both surfaces due to the titanium oxide layer that spontaneously formed.

Histology slides are shown in Figure 4. No active bone formation was found on either surface except for minor bony spicules. The mean and SD of the BIC were 34.4% (14.8%) for the F-mod surface and 36.9% (21.1%) for the modSLA surface. Wilcoxon's signed rank test found no significant difference in BIC between surfaces ($p > 0.05$). The mean and SD of the BA were 34.8% (2.6%) for the F-mod surface and 42.6% (22.5%) for the modSLA surface. There was no significant difference in BA between surfaces ($p > 0.05$).

DISCUSSION

The F-mod and modSLA surfaces had very different surface topography based on FE-SEM images, S_a/S_{dr} values, and fluoride content by XPS analysis. In addition, the modSLA surface is known to be more hydrophilic than the F-mod surface, although hydrophilicity was not compared in this nor in previous studies.[7,10] Despite these measured differences, histomorphometric analysis found no significant difference in bone response. This suggests that the effect of fluoride on bone response *in vivo* may be similar to that of hydrophilicity.

Other studies have reported different mean S_a values of the modSLA surface, including one report of an R_a (a 2-dimensional value of S_a) of 2.6 μm as measured by optical profilometry.[3,10,22] These differences may be due to different specimens (disc and screw forms) and different measurement

equipment (confocal laser scanning microscope, optical profilometer, and atomic force microscope). Our results found no substantial effect of surface topography on bone response with an S_a of the titanium surface greater than 1.0 μm, even with additional surface modifications like fluoride treatment and increased hydrophilicity. Other studies have also compared the histomorphometric results of various implant surfaces, showing the results similar to those of this study.[19,20,23,24] The optimal surface roughness (S_a) is thought to be approximately 1.5 μm for a titanium surface blasted by aluminum oxide.[25-27] However, further studies are needed to determine the optimal topography of actively modified implant surfaces.

The modification of dental implant surfaces aims to improve the initial bone response; in fact, several studies have reported no significant difference in histomorphometry in rabbit tibia models after 4 weeks of healing.[12,19,24] Although initial bone responses are easily detected in the case of actively modified surfaces such as anodized and F-mod surfaces, significant histomorphometric differences have been difficult to find even 2 weeks after implant placement.[20,23] On the other hand, given the results of the present study, 1 week after implant insertion may be too soon to evaluate new bone formation. We hypothesize that there are critical differences in the bone response to actively modified surfaces between 1 and 2 weeks after implant placement in the rabbit tibia model.

This study has several limitations, including different implant thread designs and a small sample size. Although it would be ideal to use implants with identical macro-designs when comparing the effects of their surface modifications on bone response, implant manufacturers are generally unwilling to provide identical implants to researchers. More sophisticated experimental design is required to perform an unbiased analysis.

CONCLUSIONS

Changing the chemistry of a rough implant surface can make the surface more biocompatible. The F-mod surface adds the effects of cathodic reduction by HF to a rough blasted titanium surface, while the modSLA surface increases hydrophilicity of an SLA surface for faster osseointegration. However, both the surfaces have similar *in vivo* bone responses in a rabbit tibia model.

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DISCLOSURE

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LEGENDS

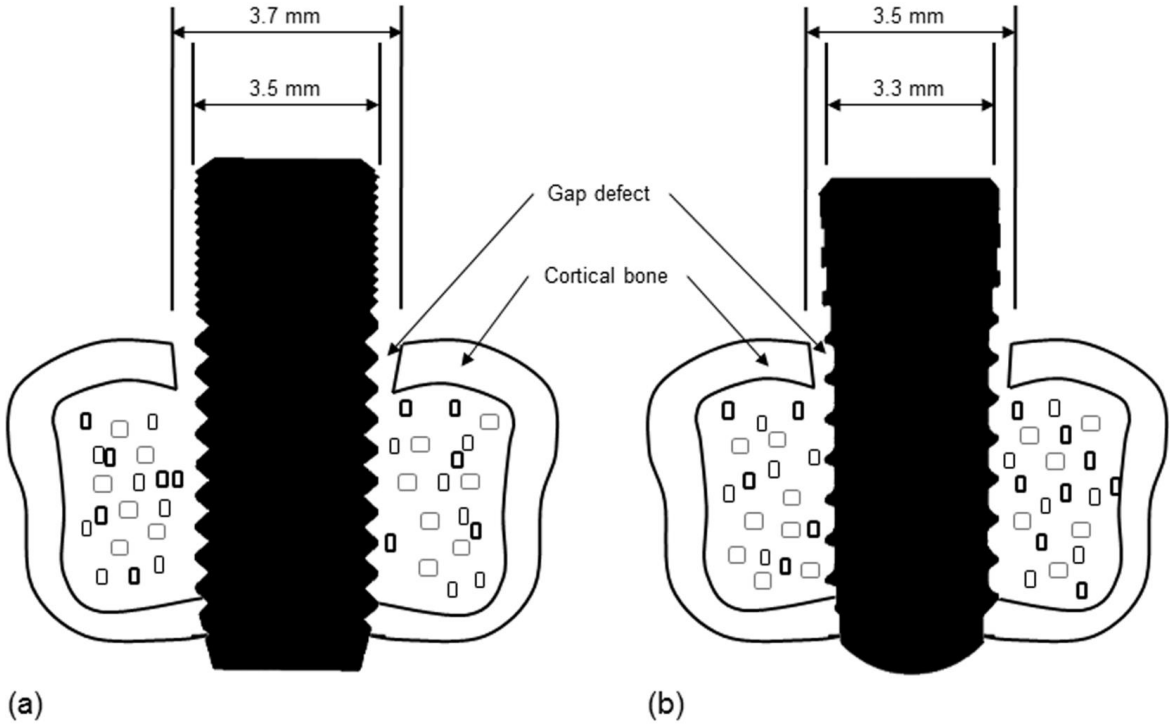


Fig. 1. A schematic diagram of implant placement in the rabbit tibia. An F-mod implant (a; 3.5 mm diameter; Osseospeed, Astra Tech, Mölndal, Sweden) and a modSLA implant (b; 3.3 mm diameter; SLActive, Institute Straumann AG, Basel, Switzerland), were firmly engaged at the bottom of the cortex in the rabbit tibia. A hole 0.2-mm larger in diameter than the implant was formed in the upper cortex. Note that the implant threads are not engaged at the upper cortical area.

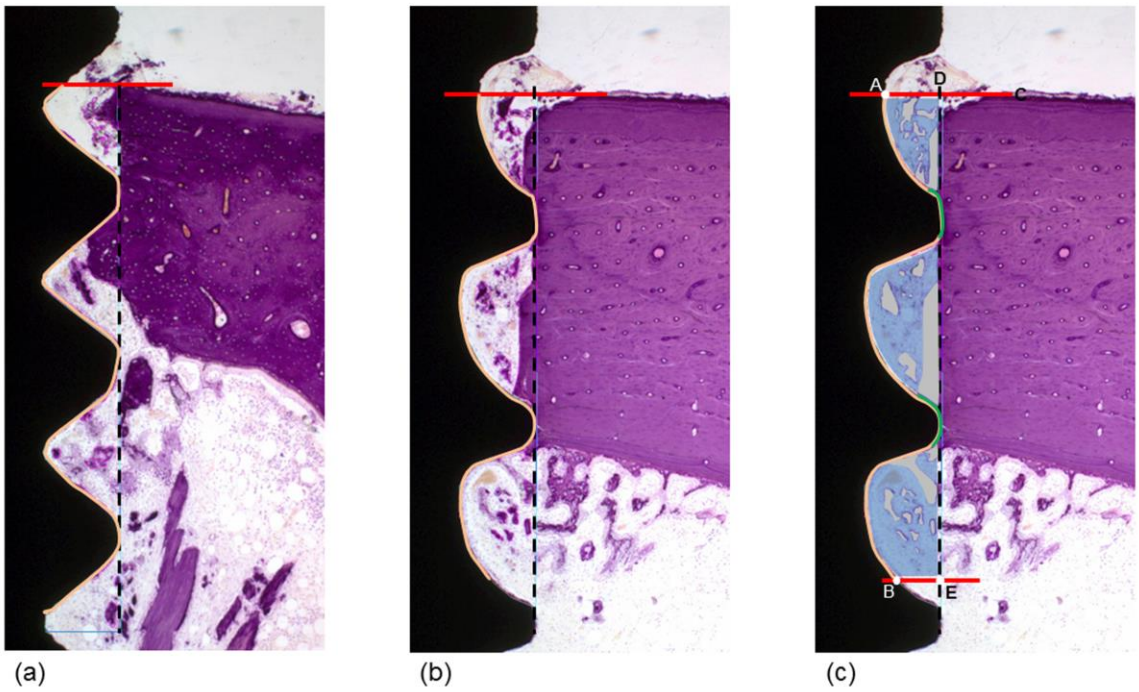


Fig. 2. Histomorphometric image analysis of light microscopy slides of (a) the F-mod surface and (b) the modSLA surface. Calculations are shown in (c). Point A is the intersection between the line at the alveolar bone crest (point C) and the implant thread contour. Point B is the end point 2.0 mm beyond the contour from point A (orange line). The green line represents the length in direct contact with the implant surface. Here, the bone-to-implant contact ratio (BIC) is defined as a ratio of the direct contact lengths (sum of the green lines) to 2.0 mm beyond the implant contour (the orange line). The grey area is filled with bone. The bone area (BA) is defined as a ratio of the sum of the grey area to the investigated total area (ABED, the blue shadowed area).

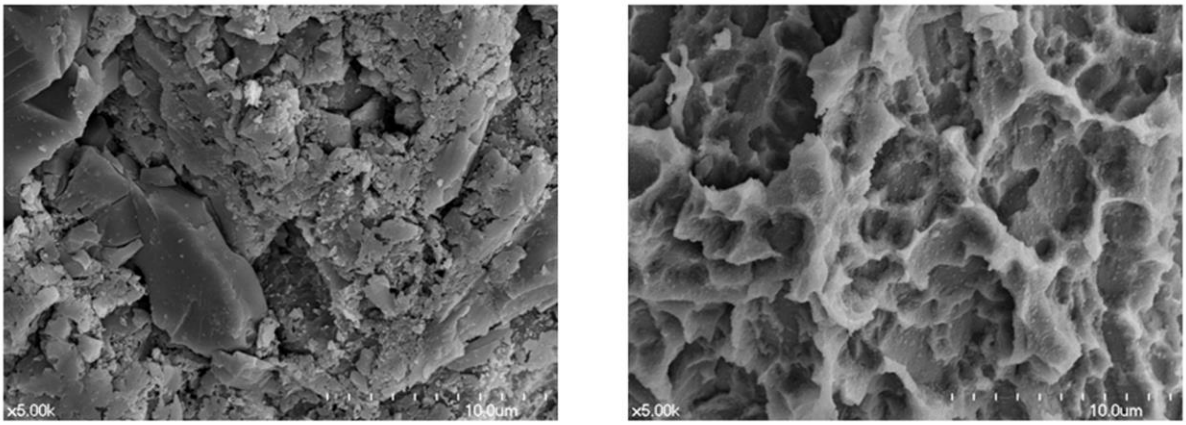
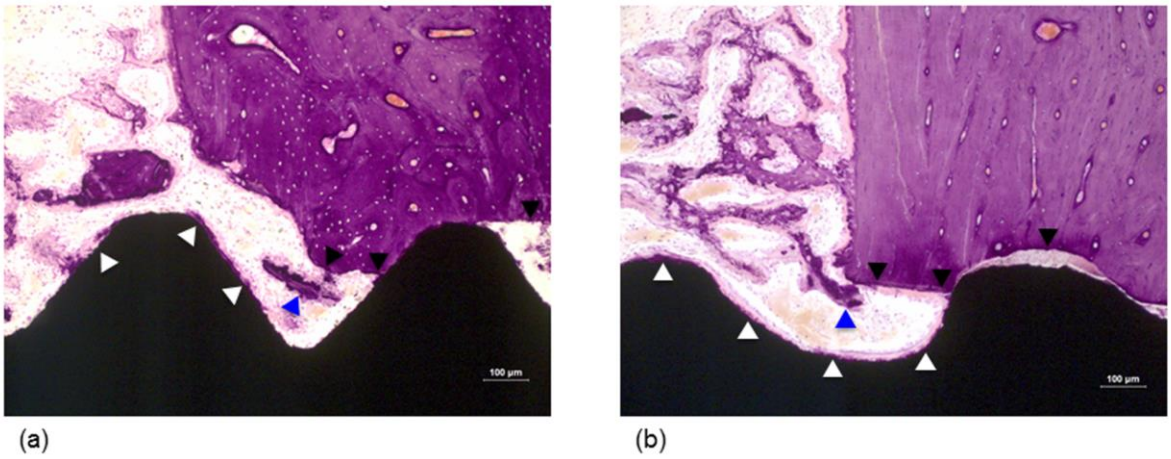


Fig. 3. FE-SEM images of the investigated implants. (a) Typical indentations and irregularities on the blasted surface are shown on the F-mod surface. (b) The honeycomb-like features, which are the results of acid etching, are observed on the modSLA surface.



(a) (b)

Fig. 4. Light microscopy views (×100 magnification) of the F-mod surface (a) and the modSLA surface (b) after 1 week of implant insertion. Little new bone formation was observed (black arrowheads) although tiny bone spicules were found between the implant threads (blue arrowheads). Notice the new bone was formed in direct contact with the surface, which is assumed to be contact osteogenesis (white arrowheads).

Tables

Table 1. The mean (standard deviation) of atomic percentages of the elements detected on the investigated surfaces by XPS.

	Ti	O	F	C
F-mod	9.9 (1.8)	35.2 (2.4)	0.2 (0.1)	53.6 (3.0)
modSLA	12.0 (0.6)	37.4 (1.1)	None	50.3 (1.3)