치의학석사학위논문

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

in vivo 상에서 친수성 물질과 불소 물질로 표면 처리한 치과용 임플란트의 초기 골유착 비교

2014년 11월

서울대학교 대학원

치의학대학원

홍 영 선

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

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이 논문을 치의학석사 학위논문으로 제출함

2014년 11월

서울대학교 대학원

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홍영선의 석사학위논문을 인준함

2015년 2월

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

학위구분 : **석사 □ · 박사 □**

학　　과 : 치의학대학원

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서울대학교총장 귀하

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

홍 영 선

Abstract

1. 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 an rabbit tibia model.

2, Materials and 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 of bone healing, the rabbits were sacrificed and histologic slides were prepared from the implant-tibial bone blocks. Bone-to-implant contact ratio (BIC) and

bone area (BA) were calculated in a defined area under a light microscope.

3. Results

FE-SEM, CLSM, and XPS showed that the modSLA surface was significantly rougher than the F-mod surface, 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 between surfaces. 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.

주요어 : implant surface, surface, modification, fluoride, hydrophilicity, modified SLA, rabbit tibia
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목 차

그림 목차

표 목차

INTRODUCTION

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, M?lndal, 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, Sa and Sdr, were measured. Sa is defined as the arithmetical mean height of the surface in 3-dimensional area surface texture parameters, and Sdr 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 (Sa and Sdr) 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 Sa values for the F-mod and modSLA surfaces were 1.3 m (0.1 m) and 3.5 m (0.2 m), respectively. The modSLA surface was significantly rougher than the F-mod surface ($p < 0.05$). The mean and SD of the measured Sdr 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, Sa/Sdr 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 Sa values of the modSLA surface, including one report of an Ra (a 2-dimensional value of Sa) of 2.6 m 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 Sa of the titanium surface greater than 1.0 m, 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 (Sa) is thought to be approximately 1.5 m 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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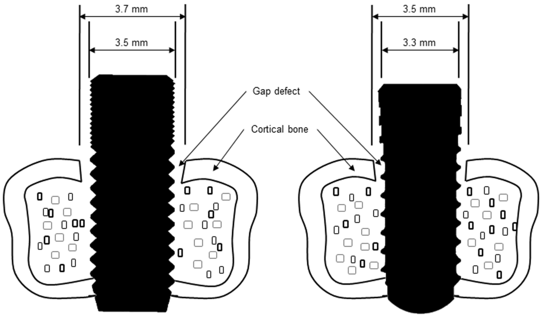
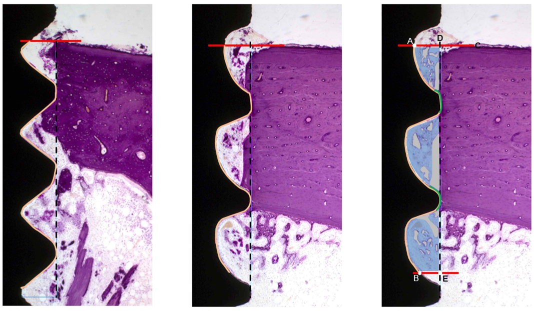


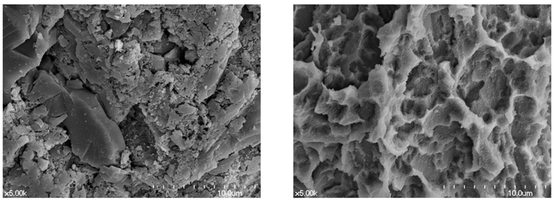
(a) (b)

Fig 1



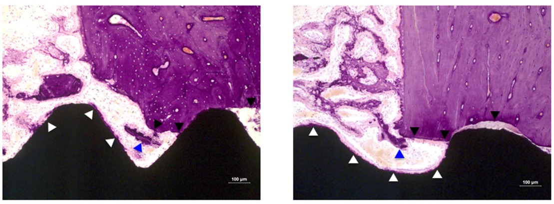
(a) (b) (c)

Fig 2



(a) (b)

Fig 3



(a) (b)

Fig 4

Figure 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, M?lndal, 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.

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)

초 록

in vivo 상에서 친수성 물질과 불소 물질로 표면 처리한 치과용 임플란트의 초기 골유착 비교

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1. 목 적

본 연구의 목적은 in vivo 상태에서 표면에 modified sand blasted, large grit, acid-etched(mod SLA)와 fluoride-modified(F-mod)를 처리한 각각의 임플란트를 토끼 경골에 식립하여 초기 골과의 반응을 조직계측학적(histomorphometric)으로 분석하고 그 차이를 조사하기 위함이다.

2. 방 법

임플란트 표면 특징을 분석하기 위해 Field emission scanning electron microscopy(FE-SEM), confocal laser scanning microscopy(CLSM), 그리고 x-ray photoelectron spectroscopy(XPS)가 사용되었다. 실험을 위하여 New Zealand white rabbit 3마리 각각의 경골에 한쪽은 불소(F-mod) 처리한 임플란트를 식립하였고 반대편에는 산(modSLA)

처리한 임플란트를 식립하였다. 1주일 후에 토끼를 희생시켰고 비탈회 조직학적 표본을 제작하였다. 광학 현미경을 이용하여 골-임플란트 접촉비율(BIC ratio)과 골-임플란트 접촉면적(BA)을 계산하였다.

3. 결 과

FE-SEM, CLSM, 과 XPS로 분석한 결과 modSLA 표면이 F-mod 표면에 비해 현저히 거칠었다. 그리고 실질적으로 F-mod 표면에는 극히 소량의 불소가 남아있었다. 이러한 표면 특징의 차이에도 불구하고, 조직계측학적으로 분석하였을 때 두 임플란트 사이에 BIC값이나 BA값은 크게 차이가 나지 않았다. 이러한 결과는 in vivo 상에서 티타늄으로 제작한 치과용 임플란트 표면에 친수성을 증가시킨 효과가 불소를 처리한 효과의 초기 골 반응이 유사하다는 것을 나타낸다.

주요어 : 임플란트 표면, 표면처리, 불소, 친수성, modified SLA, 토끼 경골

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