pISSN 1738-2696 · eISSN 2212-5469
http://dx.doi.org/10.1007/s13770-016-9126-x
ORIGINAL ARTICLE

TERM

Osseointegration of Magnesium-Incorporated Sand-Blasted Acid-Etched Implant in the Dog Mandible: Resonance Frequency Measurements and Histomorphometric Analysis

Won-Wook Song[1], Jin-Ho Heo[1], Jeong-Han Lee[1], Young-Min Park[2], Yong-Deok Kim[1,3,4]*

[1]Department of Oral and Maxillofacial Surgery, School of Dentistry, Pusan National University, Yangsan, Korea
[2]Bio Research Center, DIO Corporation, Busan, Korea
[3]Institute of Translational Dental Sciences, Pusan National University, Busan, Korea
[4]Dental Research Institute, Pusan National University Dental Hospital, Yangsan, Korea

The aim of this pilot study was to investigate the bone responses of novel magnesium (Mg)–incorporated sand-blasted and acid-etched (SLA) titanium (Ti) implant in an experimental animal model. Novel Mg-incorporated SLA Ti implant was obtained via vacuum arc source ion implantation method and Mg-ions were implanted into the SLA implant surface. Control group consisted of two commercial implants; resorbable blasting media (RBM) and SLA. Twelve implants from each group were placed into the mandibles of 6 mongrel dogs. Experimental animals were divided into 2 groups of 3 animals, with 4 weeks and 8 weeks healing time points. Resonance frequency analysis was performed at the time of fixture installation, 1, 2, 4, and 8 weeks after installation. Bone to implant contact (BIC) measurements were assessed at the 4 and 8 weeks healing time points. The overall implant survival rate was 97.2%. The Mg-incorporated SLA Ti implants showed more rapid osseointegration than control group implants at follow-up periods of 4 weeks. Histomorphometric analysis showed a tendency for BIC% values of Mg-incorporated SLA Ti implant to be higher than that of other the implant groups. The results of this study suggest that Mg-incorporated SLA Ti implant may be effective in enhancing the bone responses by rapid osseointegration in early healing periods.
Tissue Eng Regen Med 2016;13(2):191-199

Key Words: Dental implant; Animal study; Surface treatment; Bone to implant contact; Bone metabolism

INTRODUCTION

The introduction of the osseointegrated dental implants has revolutionized the rehabilitation of oral function in clinical dentistry. In an effort to enhance osseointegration of dental implants, various surface treatments have been developed, such as grit-blasting (i.e., sand-blasting), acid-etching, plasma-spray, and others [1-5]. The basic premise was to increase implant surface roughness, thereby increasing bone-to-implant contact (BIC) during the initial bone healing phase. One blasting technique uses resorbable blasting materials (RBM). Experimental studies have demonstrated a higher BIC with RBM-treated surface when compared with machine-turned surfaces [6,7]. In addition, using both sand-blast and acid etching (SLA) techniques combine the advantages of both to impart macro-roughness and micro pits to the implant surface [8]. These modifications provide for biomechanical interlocking between the implant surface and the surrounding bone. In addition, biochemical bonding also has been proposed to promote successful osseointegration [8,9].

Recently, chemical modification of dental titanium implants has been proposed to enhance osseointegration. Several studies reported surface treatment with magnesium (Mg), sulfur (S), calcium (Ca), phosphorous, or fluoride [10-12]. From these techniques, the surface chemistry of the titanium implants has been modified to incorporated specific ions in the titanium oxide layer, and these specific ions seem to reinforce the biochemical bonding of the titanium implant to bone [3,4,13,14].

Received: December 15, 2015
Revised: December 18, 2015
Accepted: December 23, 2015
***Corresponding author:** Yong-Deok Kim, Department of Oral and Maxillofacial Surgery, School of Dentistry, Pusan National University, 20 Geumo-ro, Mulgeum-eup, Yangsan 50612, Korea.
Tel: 82-55-360-5100, Fax: 82-55-360-5104, E-mail: ydkimdds@pusan.ac.kr

Bioactive, Mg-incorporated titanium implant have also been widely investigated. Sul et al. [4] reported that Mg surface chemistry stimulates faster and stronger osseointegration. In addition, several studies demonstrated positive effects of Mg-incorporated titanium implants in osteoblastic cells [8,15,16] and in animals [4,17,18]. Although these studies have shown that Mg ions enhance the cellular response of osteoblastic cells and bone regeneration *in vivo*, the effects of combined surface treatment such as SLA with Mg-incorporation have not yet been evaluated in the clinical setting. While there is evidence of possibilite enhanced osseointegration of surface treated implants at the cellular level [8], further animal and clinical studies are needed to establish the clinical use of this implant.

There are various biomechanical methods for evaluating osseointegration [19,20]. However, almost of them, including removal torque quotient, push/pull out test and tensile pull-off tests, are invasive methods and needed to sacrifice objects for evaluation, therefore, they are not suitable for evaluation of osseointegration during early healing period. The resonance frequency analysis (RFA) is a noninvasive measurement of implant stability, and it can be used to evaluate the degree of osseointegration of implant indirectly [21,22]. Furthermore, RFA is useful tool to examine the effect of different surface treatments on implant stability [23]. To our knowledge, little is known about the implant stability changes and bone responses of SLA with Mg-incorporated implants *in vivo*.

Therefore, the aims of this study were to 1) compare the early stability measurements using RFA between three different surface-treated implants: SLA, RBM, and SLA with Mg-incorporated implants and 2) to compare the bone response by histomorphometric analysis, especially BIC measurement in mandible of dogs. The hypothesis was that the Mg-incorporated SLA Ti-implant enhances clinical and histological outcomes of osseointegration more than other implants.

MATERIALS AND METHODS

Implant design and preparation

A total of 36 titanium implants (Dio Inc., Busan, Korea) sized 3.8 mm diameter and 8 mm in length were used. Twelve experimental implants had Mg-incorporated SLA surfaces (group SLA-Mg). Whereas control groups consisted of SLA implants (n=12, group SLA) and RBM implants (n=12, group RBM). The RBM surface fixtures were blasted with 180 to 425 μm sized Ca phosphate. The SLA surface was prepared with large-grit blasting followed by acid etching. For Mg-ion implantation on SLA surface, PSII processing was performed [8,24]. Ionized Mg plasma was generated when Mg ion plasma was released from an arc spot in a negatively charged surface and then injected into 90 electromagnetic filters. The ionized Mg plasma accelerated within the electric field between the substrate and sheath and then reached the surface of the substrate. The electric energy of the implantation field was 15 keV.

Surface characterization

The surface morphology of the specimens was observed by scanning electron microscopy (SEM; JSM-5800, JEOL, Tokyo, Japan). To analyze the chemistry of the implanted layer, Auger electron spectroscopy (AES; PHI650; Physical Electronics, Chanhassen, MN, USA) was used. For electron excitation in the AES analysis, a primary electron beam (3 keV and 2.6 μA) with a diameter of 40 μm was used. The samples were sputtered by two symmetrically inclined 1 keV argon ion beams at an ion incidence angle of 47° with respect to the surface normal during depth profiling. Using the relative sensitivity factor provided by the instrument producer, the atomic concentrations were calculated. For Rutherford backscattering spectroscopy (RBS) analysis, helium ions were used as an ion beam source and accelerated using a Pelltro apparatus (6SDH2, NEC) at a voltage of 2 MeV. The charge of the helium ions was 20 μC, the incident angle was 0°, and the scattered angle was 10°. The measured spectrum was fitted to a theoretical spectrum and a 16-layer sample was measured and quantified (Figs. 1 and 2).

Animals

Six mature 24 month old mongrel dogs with a mean weight of 25 kg were used in this study, which was approved by the animal ethics committee at the Pusan National University, Korea. All subjects were healthy enough to receive implantation on mandible and had no periodontal problems.

Surgical technique

Teeth extraction

Two premolars were extracted from each dog bilaterally under general anesthesia. Every subject has treated identically with the same anesthesia protocol. All, dogs were premedicated with atropine (IV, 0.01 mg/kg, Huons Co., Seoul, Korea), and received presurgical antibiotics (IV, cephazoline 1 g+gentamicin 5 mg, Shinpoong Co. Ltd., Seoul, Korea) and analgesics (IM, tramadol, 100 mg/25 kg, Shinpoong Co. Ltd., Seoul, Korea). Anesthesia was induced with IV propofol (6.6 mg/kg, Jeil Pharm Co. Ltd., Seoul, Korea). The dogs were intubated and mechanically ventilatedi using muscle relaxants (vecuronium, 0.1 mg/kg, Korea United Pharm. Inc., Seoul, Korea). Additional propofol was added as necessary to maintain anesthesia.

Local anesthesia with 2% lidocaine (Lidocaine HCL; Huons Co., Seoul, Korea) was injected prior to extraction. Full thickness mucoperiosteal flaps were elevated around the teeth to be extracted. Teeth were hemisected with using carbide fissure burs under copious saline irrigation. Using dental elevators and forceps, two premolars were extracted from each side of the mandible. Interrupted sutures using 3-0 vicryl (Ethicon Inc., Somerville, NJ, USA) achieved primary closure of the extraction socket wounds.

Implantation

4 weeks after extraction, each of the 6 dogs were treated with 3 implants bilaterally (n=36). The general anesthesia protocol was the same as that used for extraction. Local anesthesia was also used. After elevation of full thickness mucoperiosteal flaps, three implant sites were prepared in each mandibular quadrant (Fig. 3).

A uniform technique for drilling and insertion of implants was performed using a UF drilling kit (DIO Inc., Busan, Korea) with copious saline irrigation. Implantation sites were initiated using a small round bur. Then, initial drilling was performed with a 2 mm twist drill considering the implant length and direction. The pilot and 3 mm twist drills were used. Implants were installed using fixture mounts in a mechanical handpiece. The fixtures were tightened manually and healing abutments were applied. Interrupted sutures were used with 3-0 vicryl after irrigating and rinsing the operative field with saline. Three different types of implants (RBM, SLA, and SLA-Mg) were placed alternatively in the right or left mandibles of 6 dogs (n=4 per each group); thus, each of the three types of implants were evenly distributed (Table 1). The animals were divided into 2 groups of 3, group A and group B. Animals in group A were allowed a healing period of 4 weeks following implant placement, whereas animals in group B were had an 8 week healing period.

Postoperative care

Every dog received subcutaneous injection of cephazoline (1 g/25 kg), gentamicin (5 mg/25 kg) and ketoprofen (2 mg/kg) for 1 week after extraction and implantation.

Stability analysis

Every implant was tested with RFA (Osstell mentor, Integration Diagnostics Ltd., Goteborg, Sweden) at 0 (placement), 1, 2, 4, and 8 weeks after implantation. RFA is a suitable device for evaluation of the implant stability quotient (ISQ), which signifies clinical stiffness of implants without the need for invasive procedures. Under separate general anesthesia the healing abutments were removed in each dog and a transducer was applied to top of each fixture being evaluated. ISQ were

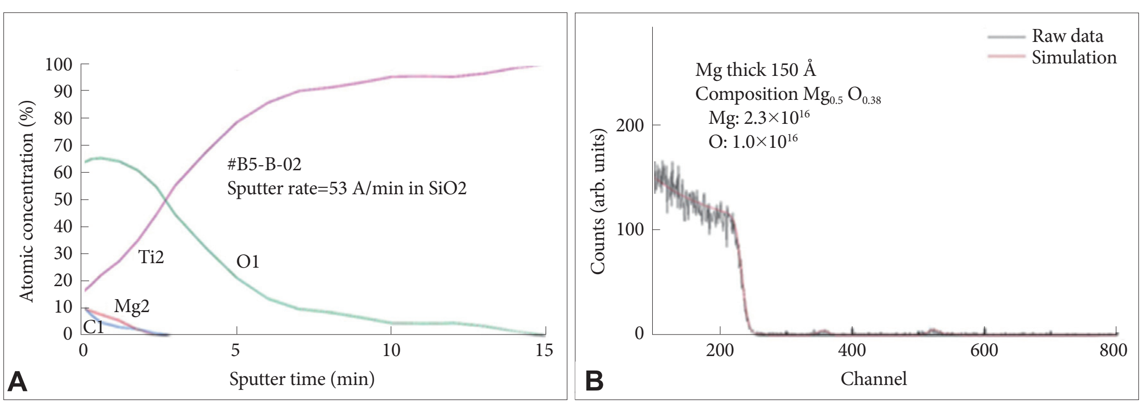

Figure 1. (A) Auger electron spectroscopy depth profiling. (B) Rutherford backscattering spectroscopy (RBS) analysis.

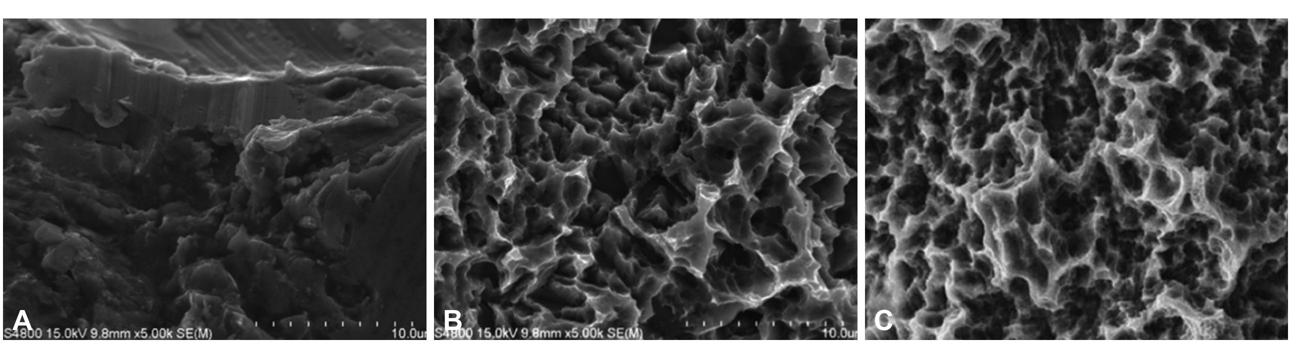

Figure 2. SEM images showing the surface morphology. (A) RBM. (B) SLA. (C) SLA-Mg. SEM: scanning electron microscopy, RBM: resorbable blasting materials, SLA: sand-blast and acid etching, Mg: magnesium.

measured by using the RFA device perpendicular to the transducer. After evaluation, the healing abutments were replaced and tightened.

Histomorphometric analysis

The slide specimens were produced using the EXAKT Cutting & Grinding system. This is a system for making specimens of teeth, bone, metal and other tissues, without disruption of interfaces. It is usually used for undecalcificated specimens. En bloc resection of implants with surrounding tissues was done after sacrificing the dogs. Each specimen was fixated in 10% neutral buffered formalin solution, (Sigma Aldrich, St. Louis, MO, USA) of each specimen for 2 weeks and dehydration procedures continued under graded series of ethanol. Then specimens were infiltrated with a mixture of ethanol and a graded series of resin solutions (Technovit 7200 VLC, Heraeus KULZER, Hanau, Germany). After infiltration, specimens were embedded for 24 hours in a Light Polymerization Unit (EXAKT 520, EXAKT Technologies, Inc., Oklahoma City, OK, USA) to cure the resin.

After processing as above the desired slices were obtained using the EXAKT cutting system (EXAKT 300 CP, EXAKT Technologies, Inc., Oklahoma City, OK, USA) and the slices were attached to slides using Precision Adhesive Press (EXAKT 402, EXAKT Technologies, Inc., Oklahoma City, OK, USA).

Further cutting was accomplished with the EXAKT cutting systema in 200±50 μm thickness and the section were ground to approximately 30±5 μm by using the EXAKT grinding system (EXAKT 400 CS, EXAKT Technologies, Inc., Oklahoma City, OK, USA). Finally, the slide specimens were stained with 1% toluidine blue.

Measurements

Histomorphometric analysis was performed with a BX51 microscope (Olympus, Nagano, Japan) attached to a CCD camera (SPOT Insight 2 Mp scientific CCD digital Camera system, Diagnostic instruments Inc., Sterling Heights, MI, USA) and adaptor (U-CMA3, Olympus, Nagano, Japan). SPOT imaging software V 4.0 (Diagnostic instruments, Inc., Sterling Heights, MI, USA) was used. Examination of specimen occured using 12.5× magnification and images were photographed under 40× magnifications for measurements. BIC was measured as the percentage of the length of mineralized bone in direct contact with the implant surface between the 3rd and 5th thread of each implants to avoid the marginal cortical bone.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). All of the data was classified according to RFA and BIC values were presented as mean±SD. The Fried-

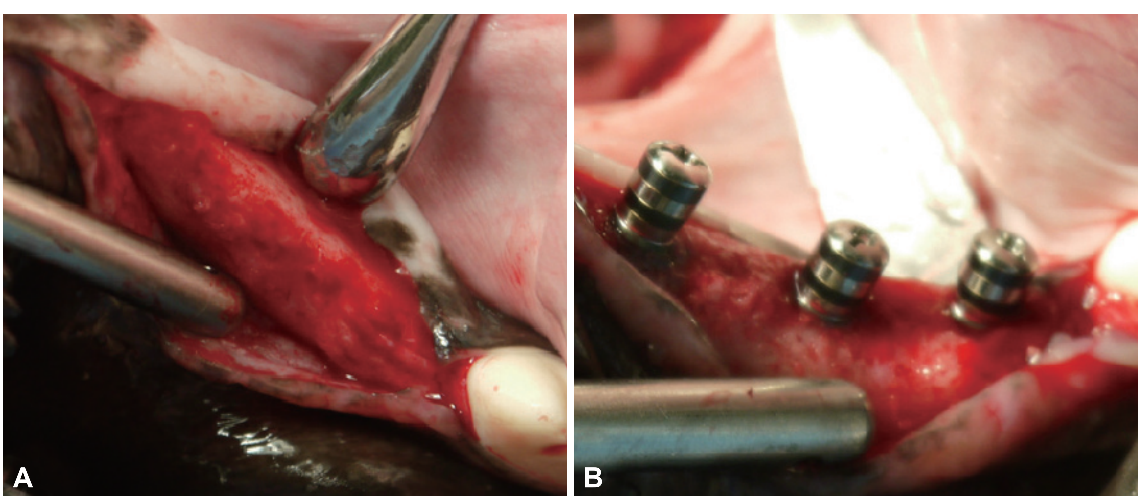

Figure 3. Implant insertion surgery in the mandible of dog. (A) Exposure of the bone surface (4 weeks after teeth extraction). (B) Installation implants and connection of healing abutment.

Table 1. Study design with implants treated on dogs

	Group A			Group B		
	1	2	3	4	5	6
Lt.	RBM	SLA-Mg	SLA	RBM	SLA	SLA-Mg
Rt.	SLA-Mg	SLA	RBM	SLA	SLA-Mg	RBM

Group A: Given a healing period of 4 weeks following implant placement, Group B: Given a healing period of 8 weeks following implant placement. Mg: magnesium, RBM: resorbable blasting materials, SLA: sand-blast and acid etching man test was used for the analysis of RFA and the Kruskal-Wallis test was used for the BIC% values. In addition, to compare ISQ values between each weeks in the same implant group, Kruskal-Wallis test with Wilcoxon signed rank test were used. $p<0.05$ was considered statistically significant.

RESULTS

Surface characteristics

SEM images of a control group implants surface and a Mg ion-incorporated implant surface are shown in Figure 2. The RBM surface showed an irregular and rough morphology, giving a surface roughness value (Ra) of 1.2- to 1.7-μm. The SLA surface demonstrated an irregular and rough morphology, and numerous small holes were observed, giving Ra of 2.0±0.5 μm. Furthermore, small micro pits and sharp edges were clearly observed. These morphological characteristics did not change after Mg ion implantation. To determine the elemental content of the outermost surface of the SLA-Mg Ti implants, AES analysis was used. The AES depth profiles are shown in Figure 3A. The concentration of Mg, oxygen, and Ti gradually varied from the outermost surface to the bulk without forming a distinct interface. The concentration of Mg ions was approximately 9.8%. The presence of oxygen in the implanted layer, which may have been derived from residual gas, indicated that Mg oxide formed during ion implantation. The roughness of SLA Ti and SLA-Mg Ti implants was also measured. A SLA Ti implant with an average Ra of 2.0±0.5 μm was used for Mg ion implantation, and its Ra value did not change after Mg ion implantation. RBS analysis was performed to measure the retained dose of implanted Mg ions in the implanted layer. Because the Ti and Mg ion peaks overlapped, a pure carbon wafer was used as substrate. Mg ions were implanted with a dose of 2.3×10^{16} ions/cm^2 in a layer with a thickness of 150 Å.

Implant survival rate

A total of 36 implants (RBM, SLA, and SLA-Mg, n=12, respectively) were inserted into the mandibles of 6 dogs. Only a single RBM implant failed to achieve osseointegration 4 weeks after implantation (Dog 6). The overall survival rate was 97.2%.

Implant stability

Before analyzing the RFA measurements, the ISQ value of the failed RBM implant was set at 0 for the 4 and 8 weeks measurements. The results from the RFA measurements during healing periods of group A and B are summarized in Table 2. In group A (4 weeks healing group), there were no significant differences between the three types of implants or between the healing periods of the same implant group.

In group B (8 weeks healing group), at implant placement, the Friedman test indicated that there was a statistically significant difference in the mean ISQ values between the three types of implants. Test scores between the three types of implants showed χ^2 (2, n=6)=10.333, p=0.006. The SLA implant group had significantly higher mean ISQ values (85.17±4.79, p=0.027) than the SLA-Mg group (77.83±2.48) and RBM group (81.17±5.15). However, there were no statistically significant differences at 1, 2, 4, and 8 weeks between three types of implants. When comparing ISQ values during healing periods in same implant group, there were no significant differences, with the exception of the SLA group. 1 week after implantation, a significant decrease in the ISQ value was noticed for the SLA group (at implantation=85.17±4.79, 1 week after implantation=77.83±2.32, p=0.027).

In order to evaluate the differences between the three types of implants during initial healing period, especially during 4 weeks, the two groups of experimental animals were integrated and statistically analyzed. The results of integrated ISQ values during 4 weeks are summarized in Table 3. In the insertion stage

Table 2. RFA measurements in the surgery and the healing stages

Group	RFA values									
	0 week		1 week		2 weeks		4 weeks		8 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4 weeks (A)										
SLA (n=6)	77.14	4.34	75.00	11.63	77.14	6.91	80.57	2.51		
SLA-Mg (n=6)	78.50	2.66	78.67	2.25	79.50	2.07	81.33	2.66		
RBM (n=6)	76.00	8.81	78.17	2.56	79.00	2.37	79.33	3.20		
8 weeks (B)										
SLA (n=6)	85.17[a]	4.79	77.83[b]	2.32	78.33	3.78	78.33	3.93	77.67[b]	3.93
SLA-Mg (n=6)	77.83[c]	2.48	77.00	2.37	79.00	1.55	80.33	2.94	79.83	0.98
RBM (n=6)	81.17[c]	5.15	79.50	1.87	71.67	12.56	64.17	31.85	65.67	32.27

Group A and Group B was assessed respectively. The different superscript letters in the same column and row indicate the values that are significantly different ($p<0.05$). RFA: resonance frequency analysis, Mg: magnesium, RBM: resorbable blasting materials, SLA: sand-blast and acid etching

(0 week), the Friedman test indicated that there was a significant difference in mean ISQ values between the three implant types (χ^2=7.478, p=0.024). All pairwise comparisons with Wilcoxon signed rank test showed that the ISQ value of SLA group was statistically high than that of RBM group (81.25±6.11 and 78.58±7.39, respectively, p=0.037). After 4 weeks of healing time, the Friedman test indicated that there was a significant difference in the mean ISQ values between three groups (χ^2=6.837, p=0.033). All pairwise comparisons with Wilcoxon signed rank test showed that the ISQ value of the SLA-Mg group was statistically higher than that of RBM group (80.83±2.72 and 71.75±22.99, respectively, p=0.011).

The changes of ISQ values in the same implant group were analyzed (Table 3). In the SLA group, from at the time of implant installation to 1 week, a statistically significant decrease in ISQ value was noticed (81.25 and 76.42, respectively, p=0.040). After 4 weeks, a statistically significant increase in ISQ value was noted (79.58, p=0.010) and reached initial value. In SLA-Mg group, there was no decrease in ISQ value between 0 and 1 week (78.17 and 77.83, respectively). However, during the next 2 (79.25, p=0.011) and 4 weeks (80.83, p=0.010), ISQ values were significantly increased. Furthermore, the ISQ value at 4 weeks healing was statistically greater than that of at implant installation (80.83 and 78.17, respectively, p=0.007). In the RBM group, although a small decrease in ISQ value occurred at 2 and 4 weeks after implant installation, no statistically significant difference was found at any observation time points.

Histomorphometric analysis

Every implant achieved bone to implant contact. Bone filled almost the entire area within the threads, although it was occasionally interrupted by soft tissue. There were no signs of inflammation in the surrounding bone (Fig. 4).

Every group showed increased BIC% at 8 weeks than 4 weeks after installation. The SLA-Mg implant group showed higher values of BIC% compared with that of SLA and RBM implant groups. However, these data did not show statistically significant differences according to type of implants at either 4 weeks or 8 weeks (Table 4).

DISCUSSION

This study examined the bone response to Mg-incorporated SLA Ti implants during various healing periods. The results of the present study suggest that Mg-incorporated SLA Ti implant improve bone response as compared with commercially available RBM or SLA Ti implants by rapid osseointegration.

Modification of implant surfaces is a modern trend in implant dentistry to enhance osseointegration with bone [1-5,25]. Modifications have included, RBM (i.e., hydroxyapatite and Ca phosphate) and SLA techniques. In addition, bioactive implants, which are expected to release specific ions, have been reported for biochemical bonding of implants [10-12]. Among the various ions, we focused on Mg ions. Although several studies reported that Mg ions implanted in RBM-treated Ti surface provided enhanced osseointegration capacity *in vitro* [26] and *in vivo* [18,27] and one study reported the improved osseointegraion capacity of SLA treated titanium disc *in vitro* [8], the effect of Mg-incorporated SLA Ti implant has not yet been fully elucidated. Therefore, in this study, a novel Mg-incorporated SLA Ti (SLA-Mg) implant was assessed to evaluate bone response compared with commercial RBM or SLA Ti implants.

RFA measurement was used in this study to analyze the differences of implant stability between three groups. As previously described, RFA is a valuable method of non-destructive and quantitative assessment of the stability of an implant and reflects differences of bone response to the different surface chemistries of implants.

There were no significant differences among the implant groups at any observation periods in the 4 week healing group. Whereas, in the 8 week healing group, baseline RFA values at implant placement indicated that the SLA group had significantly higher mean ISQ values than the SLA-Mg group and the RBM group (χ^2=10.333, p=0.006) (Table 2). Because the geometric design was same in three groups, this discrepancy might be due to differences of bone properties of experimental ani-

Table 3. Integrated ISQ values of three group implants until 4 weeks

	SLA (n=12)		SLA-Mg (n=12)		RBM (n=12)		*p* value
	Mean	SD	Mean	SD	Mean	SD	(Friedman)
0 week	81.25[a]	6.11	78.17[c]	2.48	78.58	7.39	0.024*
1 week	76.42[b]	8.86	77.83[c]	2.37	78.83	2.25	0.275
2 weeks	77.83[b]	5.72	79.25[b]	1.76	75.33	9.43	0.614
4 weeks	79.58[a]	3.45	80.83[a]	2.72	71.75	22.99	0.033*

The same superscript letters in the same column indicate the values that are not significantly different (p>0.05). *Friedman test was used for the analysis of RFA between three group implants. ISQ: implant stability quotient, Mg: magnesium, RBM: resorbable blasting materials, RFA: resonance frequency analysis, SLA: sand-blast and acid etching mals (density, volume, thickness, and etc.). However, the investigators could not find any other significant results when analyzing group A and group B respectively. Therefore, the two groups of experimental animals were integrated and statistically analyzed to assess bone response in the early healing periods.

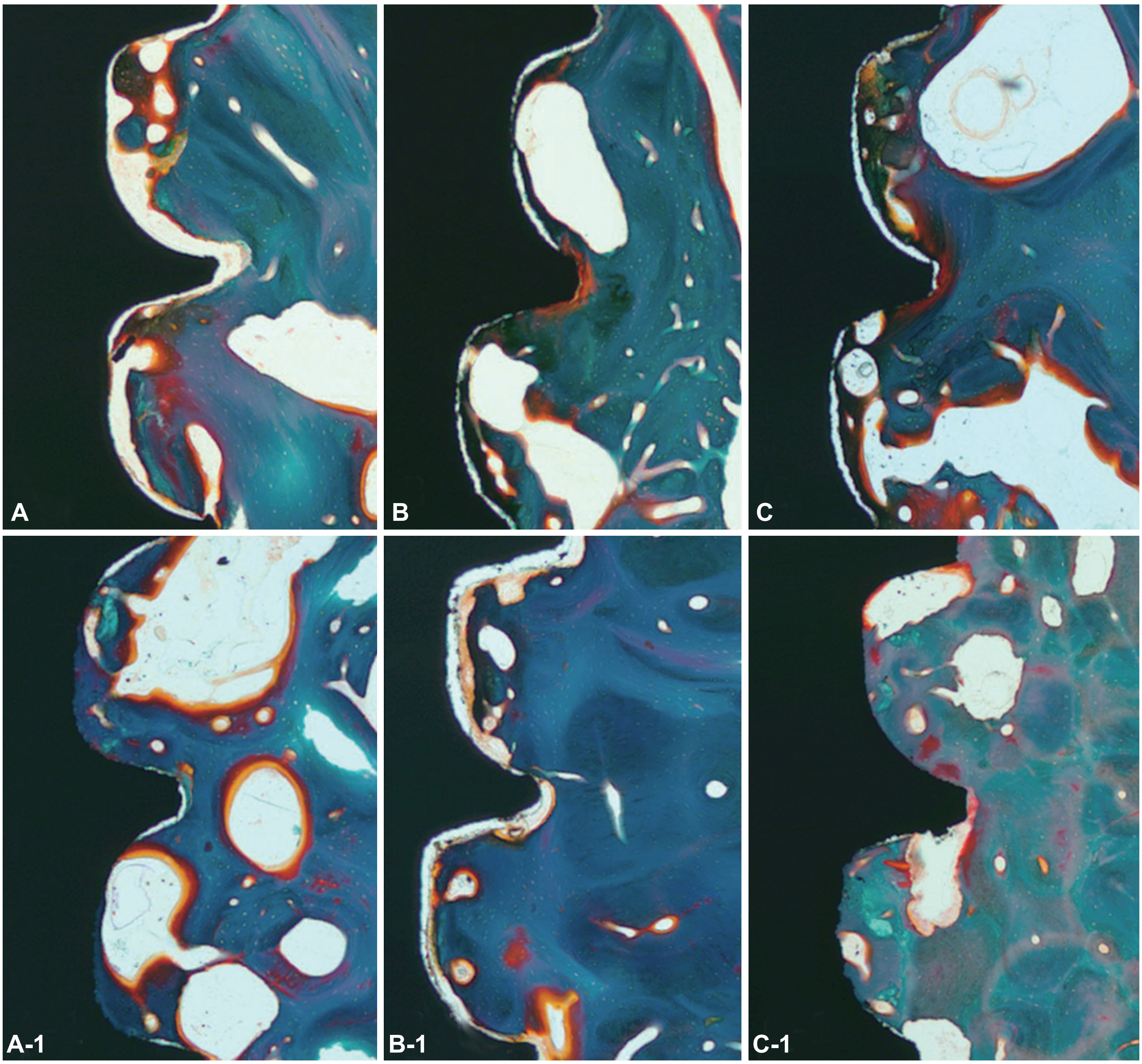

Figure 4. Histological section of the three type implants (×40 magnification). (A) RBM at 4 weeks. (A-1) RBM at 8 weeks. (B) SLA at 4 weeks. (B-1) SLA at 8 weeks. (C) SLA-Mg at 4 weeks. (C-1) SLA-Mg at 8 weeks. BIC% was increased at 8 weeks compared with 4 weeks in all group. The SLA-Mg implant showed a higher degree of BIC% in both 4 and 8 weeks. RBM: resorbable blasting materials, SLA: sand-blast and acid etching, Mg: magnesium.

Table 4. Bone to implant contact area (%)

	SLA		SLA-Mg		RBM	
	Mean	SD	Mean	SD	Mean	SD
4 weeks	49.36	13.20	56.23	10.63	50.92	13.98
8 weeks	56.05	9.34	60.23	12.85	51.89	18.55

Mg: magnesium, RBM: resorbable blasting materials, SLA: sand-blast and acid etching

Baseline RFA values at implant placement indicated that the SLA group had significantly higher mean ISQ values than the RBM group (χ^2=7.478, p=0.024), and 4 weeks after healing the SLA-Mg group had significantly higher mean ISQ values than the RBM group (χ^2=6.837, p=0.033). It seems that the osseointegration failure of one RBM implant resulted in these findings. The failing implant was ranked 61 (ISQ value) at implant placement and set to an ISQ value of 0 at 4 weeks after healing. However, the ISQ values of all the other implants at 4 and 8 weeks were greater than 70. Therefore, osseointegration had occurred in all three group implants.

There were significant differences between three group implants in terms of ISQ value changes at the 4 week healing time point. Although RBM group showed no statistical significant changes during 4 weeks experimental periods, there was a tendency for a decrease in ISQ values from 2 weeks to 4 weeks. Furthermore, in the SLA group, there was significant decrease in ISQ value 1 week after implant placement and the reduced ISQ value was maintained for 2 weeks. After 4 weeks, the ISQ value had returned to initial value. These findings are consistent with other previous clinical investigations, in which demonstrated decreased ISQ values in early healing periods comparing to that of implant placement [28-30]. Therefore, it is reasonable to assume that initial bone resorption after implantation may result in a decreased stiffness of SLA and RBM implants in the present study. However, interestingly, the SLA-Mg implant group did not show a significant decrease in ISQ values during any of the experimental periods. In contrast to control groups, the mean ISQ value of the SLA-Mg implant group started to increase from 2 weeks and significantly surpassed the initial value at both 2 and 4 weeks. Considering the almost identical surface morphologies between SLA, SLA-Mg implants, it is reasonable to assume that Mg-incorporation plays an important role in cell binding and osseointegration during the early healing periods.

It has been reported that Mg may contribute to improved osteoblast function and differentiation and result in enhanced bone healing [16,26]. Furthermore, Kim et al. [8] recently investigated the effects of Mg-incorporated SLA Ti surface on mesenchymal stem cells by comparing it with the SLA Ti surface. They reported improved cell viability, initial cell adhesion, differentiation, and Ca accumulation. They therefore concluded that the Mg-incorporated SLA Ti surface may result in earlier osseointegration. These *in vitro* findings provide some clues for interpreting the bone responses in the present study. RFA measurements are good reflections of the osseointegration processes [23]. Taken together with early increased in ISQ values of the SLA-mg implant used in the present study, we could conclude that Mg-incorporated SLA Ti implants achieve more rapid osseointegration than other implant types through earlier osteoblast differentiation in bone remodeling periods.

In general, it has been reported that BIC% increased during healing period [31-33]. In accordance with these previous studies, mean BIC% of the three types of implants at 8 weeks was higher than that at 4 weeks, although there were no statistically significant differences between two experimental periods. In addition, reports from *in vivo* studies about Mg-incorporated implants [18,27,34] indicated that the mean BIC% of Mg-treated implants was significantly higher than those of control groups. The authors suggested more predictable bone apposition around Mg-incorporated implants. The present study showed similar findings. Although a statistical difference did not exist, SLA-Mg revealed a tendency to show higher BIC% values than in the other types of implants. From these results, the osteoconductivity of a novel Mg-incorporated SLA Ti implant seems to be superior or at least similar to the other implant.

The current investigation has some limitations. The sample size was relatively small. In addition, the present study has conducted under relatively short healing periods and did not assess the implants under occlusal loading conditions. Furthermore, the precise mechanisms resulting in rapid osseointegration of Mg-incorporated SLA Ti implant remains unclear. Therefore, further studies are needed to include prolonged healing times and implant loading. The investigation regarding the role of Mg-ion implantation on osteoconductivity is also needed. However, the results of the current study suggest that a novel Mg-incorporated SLA Ti implant may result in more rapid osseointegration in the early healing periods and therefore, early loading of this type implant may be possible.

In conclusion, the present study examined the bone response to a novel Mg-incorporated SLA Ti implant. The RFA measurements and histomorphometric analysis showed that the Mg-incorporated SLA Ti implant achieved more rapid osseointegration and greater BIC in early healing periods.

Acknowledgements

This study was supported by Dental Research Institute (PNUDH-DRI 2012-02), Pusan National University Dental Hospital.

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

This study was approved by the animal ethics committee at the Pusan National University, Korea.

REFERENCES

1. Buser D, Nydegger T, Oxland T, Cochran DL, Schenk RK, Hirt HP, et al. Interface shear strength of titanium implants with a sandblasted and acid-etched surface: a biomechanical study in the maxilla of miniature pigs. J Biomed Mater Res 1999;45:75-83.
2. de Groot K, Geesink R, Klein CP, Serekian P. Plasma sprayed coatings of hydroxylapatite. J Biomed Mater Res 1987;21:1375-1381.
3. Sul YT. The significance of the surface properties of oxidized titanium to the bone response: special emphasis on potential biochemical bonding of oxidized titanium implant. Biomaterials 2003;24:3893-3907.
4. Sul YT, Johansson C, Albrektsson T. Which surface properties enhance bone response to implants? Comparison of oxidized magnesium, TiUnite, and Osseotite implant surfaces. Int J Prosthodont 2006;19:319-328.
5. Wennerberg A, Ektessabi A, Albrektsson T, Johansson C, Andersson B. A 1-year follow-up of implants of differing surface roughness placed in rabbit bone. Int J Oral Maxillofac Implants 1997;12:486-494.
6. Novaes AB Jr, Souza SL, de Oliveira PT, Souza AM. Histomorphometric analysis of the bone-implant contact obtained with 4 different implant surface treatments placed side by side in the dog mandible. Int J Oral Maxillofac Implants 2002;17:377-383.
7. Piattelli M, Scarano A, Paolantonio M, Iezzi G, Petrone G, Piattelli A. Bone response to machined and resorbable blast material titanium implants: an experimental study in rabbits. J Oral Implantol 2002;28:2-8.
8. Kim BS, Kim JS, Park YM, Choi BY, Lee J. Mg ion implantation on SLA-treated titanium surface and its effects on the behavior of mesenchymal stem cell. Mater Sci Eng C Mater Biol Appl 2013;33:1554-1560.
9. Sul YT. On the bone response to oxidized titanium implants: the role of microporous structure and chemical composition of the surface oxide in enhanced osseointegration. Gothenburg, Sweden: Thesis; 2002.
10. Sul YT, Johansson CB, Kang Y, Jeon DG, Albrektsson T. Bone reactions to oxidized titanium implants with electrochemical anion sulphuric acid and phosphoric acid incorporation. Clin Implant Dent Relat Res 2002;4:78-87.
11. Sul YT, Byon ES, Jeong Y. Biomechanical measurements of calcium-incorporated oxidized implants in rabbit bone: effect of calcium surface chemistry of a novel implant. Clin Implant Dent Relat Res 2004;6:101-110.
12. Sul YT, Johansson C, Byon E, Albrektsson T. The bone response of oxidized bioactive and non-bioactive titanium implants. Biomaterials 2005;26:6720-6730.
13. Ellingsen JE, Johansson CB, Wennerberg A, Holmén A. Improved retention and bone-tolmplant contact with fluoride-modified titanium implants. Int J Oral Maxillofac Implants 2004;19:659-666.
14. Hanawa T, Kamiura Y, Yamamoto S, Kohgo T, Amemiya A, Ukai H, et al. Early bone formation around calcium-ion-implanted titanium inserted into rat tibia. J Biomed Mater Res 1997;36:131-136.
15. Zreiqat H, Howlett CR, Zannettino A, Evans P, Schulze-Tanzil G, Knabe C, et al. Mechanisms of magnesium-stimulated adhesion of osteoblastic cells to commonly used orthopaedic implants. J Biomed Mater Res 2002;62:175-184.
16. Zreiqat H, Valenzuela SM, Nissan BB, Roest R, Knabe C, Radlanski RJ, et al. The effect of surface chemistry modification of titanium alloy on signalling pathways in human osteoblasts. Biomaterials 2005;26:7579-7586.
17. Sul YT, Johansson P, Chang BS, Byon ES, Jeong Y. Bone tissue responses to Mg-incorporated oxidized implants and machine-turned implantsin the rabbit femur. J Appl Biomater Biomech 2005;3:18-28.
18. Park JW, An CH, Jeong SH, Suh JY. Osseointegration of commercial microstructured titanium implants incorporating magnesium: a histomorphometric study in rabbit cancellous bone. Clin Oral Implants Res 2012;23:294-300.
19. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: a 1-year follow-up of removal torque of titanium implants. Int J Oral Maxillofac Implants 1987;2:69-75.
20. Thomas KA, Kay JF, Cook SD, Jarcho M. The effect of surface macrotexture and hydroxylapatite coating on the mechanical strengths and histologic profiles of titanium implant materials. J Biomed Mater Res 1987;21:1395-1414.
21. Sennerby L, Meredith N. Resonance frequency analysis: measuring implant stability and osseointegration. Compend Contin Educ Dent 1998;19:493-498, 500, 502; quiz 504.
22. Meredith N, Shagaldi F, Alleyne D, Sennerby L, Cawley P. The application of resonance frequency measurements to study the stability of titanium implants during healing in the rabbit tibia. Clin Oral Implants Res 1997;8:234-243.
23. Sul YT, Jönsson J, Yoon GS, Johansson C. Resonance frequency measurements in vivo and related surface properties of magnesium-incorporated, micropatterned and magnesium-incorporated TiUnite, Osseotite, SLA and TiOblast implants. Clin Oral Implants Res 2009;20:1146-1155.
24. Byon E, Moon S, Cho SB, Jeong CY, Jeong Y, Sul YT. Electrochemical property and apatite formation of metal ion implanted titanium for medical implants. Surf Coat Technol 2005;200:1018-1021.
25. Albrektsson T, Wennerberg A. Oral implant surfaces: part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2004;17:536-543.
26. Park JW, Kim YJ, Jang JH, Song H. Osteoblast response to magnesium ion-incorporated nanoporous titanium oxide surfaces. Clin Oral Implants Res 2010;21:1278-1287.
27. Cho LR, Kim DG, Kim JH, Byon ES, Jeong YS, Park CJ. Bone response of Mg ion-implanted clinical implants with the plasma source ion implantation method. Clin Oral Implants Res 2010;21:848-856.
28. Zhou W, Han C, Yunming L, Li D, Song Y, Zhao Y. Is the osseointegration of immediately and delayed loaded implants the same?--comparison of the implant stability during a 3-month healing period in a prospective study. Clin Oral Implants Res 2009;20:1360-1366.
29. Schätzle M, Männchen R, Balbach U, Hämmerle CH, Toutenburg H, Jung RE. Stability change of chemically modified sandblasted/acid-etched titanium palatal implants. A randomized-controlled clinical trial. Clin Oral Implants Res 2009;20:489-495.
30. Han J, Lulic M, Lang NP. Factors influencing resonance frequency analysis assessed by Osstell mentor during implant tissue integration: II. Implant surface modifications and implant diameter. Clin Oral Implants Res 2010;21:605-611.
31. Schliephake H, Sewing A, Aref A. Resonance frequency measurements of implant stability in the dog mandible: experimental comparison with histomorphometric data. Int J Oral Maxillofac Surg 2006;35:941-946.
32. Abrahamsson I, Linder E, Lang NP. Implant stability in relation to osseointegration: an experimental study in the Labrador dog. Clin Oral Implants Res 2009;20:313-318.
33. Abdel-Haq J, Karabuda CZ, Arısan V, Mutlu Z, Kürkçü M. Osseointegration and stability of a modified sand-blasted acid-etched implant: an experimental pilot study in sheep. Clin Oral Implants Res 2011;22:265-274.
34. Kim DS, Kim DG, Park CJ, Cho LR. Histomorphometry and stability analysis of early loaded implants with two different surface conditions in beagle dogs. J Adv Prosthodont 2009;1:10-18.