Effects of SLA surface on Osseointegration of a Titanium Dental Implant

Purpose: The aim of **t**his study was to evaluate the efficiency of combined surface treatments with blasting and chemical application by investigating the osseointegration level using animal study.

Materials and Methods: The samples were divided into 2 Groups according to the surface treatment method. These groups are control group (machined surface), SLA Group (Sandblasting and acid etched) each. The surface characteristics were observed with SEM. In this animal experiment, all lower premolars and 1st molars were extracted bilaterally in two beagle dogs. After a healing period of 2 months, each side of the mandible received four randomly assigned dental implants alternating between the machined surface and SLA surface. The animals were sacrificed after 8 weeks healing period. In that histometric analysis, bone-to-implant contact ratio (%, BIC) and intra thread bone density (%, ITBD) were calculated in the three upper threads. All the data were statistically analyzed with t- test (α=0.05).

Results: Surface roughness of SLA showed macro roughness under ×100 magnification level. In histometric analysis of animal test, the BIC value SLA group was more than the control groups ($P < 0.05$).

***Conclusion*:** In this in vivo study, the surface treatment combining blasting and Acid etching (SLA) was improved the degree of the early osseointegration of implant.

Albrektsson et al. (1981) recognized early on that the implant surface, including topography, chemistry, surface charge, and wettability, is one of the important factors influencing osseointegration. A great number of research were had been performed to examine altered titanium surfaces, and focused on subtractive surface techniques such as sandblasting and/or acid-etching procedure (Wennberg, A et al. 1996, 1998, Buser et al 1991, Buser et al. 1998). They demonstrated that the new titanium surface had better bone integration than machined titanium surfaces. A grater percentage of bone implant contact (BIC) were observed adjacent to microrough titanium surfaces, compared with machined titanium surface (Buser D 2001). In addition to surface roughness, the surface chemistry is another factor important for peri-implant bone apposition, because it influence surface charge and surface wettability. Surface wettability is largely dependent on surface energy, and influences the degree of contact with the physiologic environment. Increased wettability enhances interaction between the implant surface and the biologic environment (Kilpadi & lemons 1994)

The SLA surface treatment is to blast large grit particles (250-500μm) onto the implant surface and to acid-etch the titanium implant surface (Buser et al. 2004). Large grit blasting is to achieve macro-roughness of 18-23μm. Numerous reports have shown that both the early fixation and long-term mechanical stability can be improved with this roughness. In addition, the SLA surface on which micro-roughness of 2-4μm was achieved with acid etching (HCl/H_2SO_4) enhanced the osteoconductive process. The experiments showed higher bone-to-implant contact with SLA surfaces than TPS surfaces and the enhanced removal torque value. Buser et al. reported the 99% success rate of their five-year clinical study on the loading of the SLA implants at six weeks.

In this study, the surface efficiency of the combined surface treatment with the resorbable large grit medium blasting and Acid-etching was compared with that of machined surface by investigating the osseointegration level using animal experiments.

MATERIALS AND METHODS

Materials

For the animal experiments, the implants of grade4 titanium (Dynamet, Washington, PA, USA) with 3.75mm diameter and 7mm length were used. The samples were divided into 2 groups according to the surface

treatment. They are the control and the SLA groups.

The control group has machined titanium surface. For the SLA group, Biphasic calcium phosphate particles with 0.18-0.5mm diameter were blasted onto the machined titanium surface in four directions with 0.8MPa to produce macro roughness. On the surface, a mixture of HCl: H_2PO_4: H_2O was applied at 80℃ for 1 hour.

Surface Characterization

Using a sputter coater (SCD 005, BAL-TEC [Leica Microsystems GmbH], Wetzlar, Germany), each disc sample was coated with platinum. The platinum-coated samples were photographed using a field emission-scanning electron microscope (FE-SEM; Quanta 200, FEI, Hillsboro, OR, USA) at ×100 and×500 magnifications to observe their microstructures.

To investigate the surface chemical components, an energy-dispersive spectrometer (Quanta 200, FEI, Hillsboro, OR, USA) was used.

Surface Roughness Measurement

After the surface treated disc sample was coated with platinum, 3D images of the surface were taken at ×800 magnifications at 6° using a stereo scanning electron microscope (Zeiss EVO25; Zeiss, Germany) and MeX V5.1 software (Alicona, Grambach, Austria).

Using a Gaussian filter with a cutoff wavelength of kc=31μm, the Sa (the average of absolute values of the surface protrusion on the mean surface), Sq (the root mean of squared values of the protruding surface from the mean surface) and Rt (the average of the distance from the line of the maximum and minimum heights of the protruding surface) on the $340 \times 250 \mu m^2$ surface were calculated.

Experimental Animals

Two beagle dogs aged 2-3 years, each of which weighed 15kg, were used. Each beagle was reared in an individual cage wherein the temperature was maintained at 20-25°C and relative humidity, at 30-50%. A soft diet was provided with water before and after the surgery.

Preparation of the Experimental Implants

A total of 16 implants (length: 7mm and diameter: 3.75mm; Cowellmedi Co., Busan, Korea) were prepared. All the implants were screw-type and made of grade 4 commercially pure titanium. The

experimental implants were divided into two groups (8 implants per group) as *in vivo* test.

Primary Surgery

Food was withheld the night before surgery. The animals were injected with atropine sulfate (0.05mg/kg) and tiletamine/zolazepam (5mg/kg IV). During the surgery, anesthesia was maintained using isoflurane and by supplying oxygen. Lactate ringer solution (5 ml/kg/h) was injected. Ampicillin sodium (20mg/kg IV) and meloxicam (0.2mg/kg IV) were injected preoperatively. The surgical area was injected with lidocaine that contained epinephrine (1:100,000) for the local anesthesia.

All the mandibular premolar and first molar were extracted carefully to avoid damaging extraction socket. The furcation region of first molar was trimmed using a fissure bur. After verifying that no dental root remained, a suture was made with 4-0 silk.

After the extraction, Meloxicam (0.1mg/kg PO) was administered to the animals for pain relief, and amoxicillin (20mg/kg PO) was administered to them every 12 hours for 6 days.

Secondary Surgery

After checking that the alveolar bone healed well two months after the primary surgery, implant placement was performed. The general and local anesthesia procedures were the same as that of the primary surgery. Four implants were placed on each side of the mandible. An incision was made at the mid-crest of the alveolar bone.

After the full-thickness flap was dissected, the cortical bone was leveled. Then the implant site was prepared using a 3.2mm diameter drill as final drill. Eight implants were randomly placed on both sides. The distance between implants made at 4mm regular interval using a ruler.

Penicillin G procaine and penicillin G benzathine (1ml/5kg) were intramuscularly injected in the experimental animals immediately after the surgery and 48 hours after the surgery. The animal's oral cavity was rinsed twice a day with 2% chlorhexidine. A soft diet was allowed.

Sacrifice of the Experimental Animals

The experimental animals were sacrificed 8 weeks after the second surgery. They were first sedated using a zaperone and midazolam (1mg/kg, IM) before they were sacrificed via intravenous injection of 20% pentobarbital solution.

Preparation of the Specimen

The specimen that included the implant was prepared after the sacrifice of the experimental animals. The specimen was fixed for 2 weeks in a neutral buffered formalin solution, and then dehydrated by increasing the ethanol concentration to 70-100%. The dehydrated specimen was embedded in a Technovit 7200 resin. A block of the polymerized specimen was cut in the direction of the long axis of the implant at the center of the implant using an EXAKT diamond cutter. A slide with a final thickness of 30μm was made from the 400μm thick slide using an EXAKT grinding machine. The tissue was stained using hematoxylin and eosin stain.

Histometric Analysis

An observer who was blind to the experimental conditions analyzed the histological findings. The measurement was performed three times to reduce the possibility of error. The obtained images from an optic microscope were analyzed using an image analysis computer program. The center of the specimen that was cut in the direction of the buccal lingual side was used for the histological analysis. All the images of the specimens were taken under ×2.5 magnification. ×40 magnified images were used for the histological analysis, and ×100 magnified images for accurate assessment of the BIC. The following factors were analyzed (Fig. 2).

1. Bone-to-implant contact in the three upper threads except the uppermost thread: After measuring the total length of three upper threads, bone-to-implant contact ratio (%) was calculated by measuring the length of the contact with bone.
2. Intra-thread bone density in the three upper threads except the uppermost thread: Except for the top thread, the total area between three upper threads was measured. And then bone density (%) was calculated by measuring the area occupied with bone.

Statistical Analysis

Statistical analysis was performed using SPSS ver. 18.0 (SPSS, Chicago, IL, USA). To analyze measurements taken in individual test animals, groups were compared using student t-test. Values of $p<0.05$ were taken to indicate statistical significance.

RESULTS

Surface Characterization

Through SEM, the surface morphologies of each group were confirmed (Fig. 1). The SLA group showed a macro roughness at×100 magnification. The rough surface had sharp peaks with irregular heights.

Surface Roughness Measurement

Using a stereo-scanning electron microscope (Zeiss EVO25; Zeiss, Germany), 3D surface image of each sample was obtained, and their Sa, Sq and Rt were calculated. The SLA group showed the greatest degree of roughness, which was 20 times that of the control group.

Table 1. Surface roughness by stereo scanning electron microscope

Surface treatment	Sa(μm) ± SD	Sq(μm) ± SD	Rt(μm) ± SD
Control	0.08 ± 0.04	0.09 ± 0.02	1.95 ± 0.11
SLA	1.68 ± 0.22	2.15 ± 0.29	16.99 ± 3.09

Sa, the roughness per unit surface: the average of absolute values of the surface protrusionon the mean surface; **Sq,** the root mean of squared values of the protruding surface from the mean surface; **Rt** the distance from the line of the maximum and minimum heights of the protruding surface.

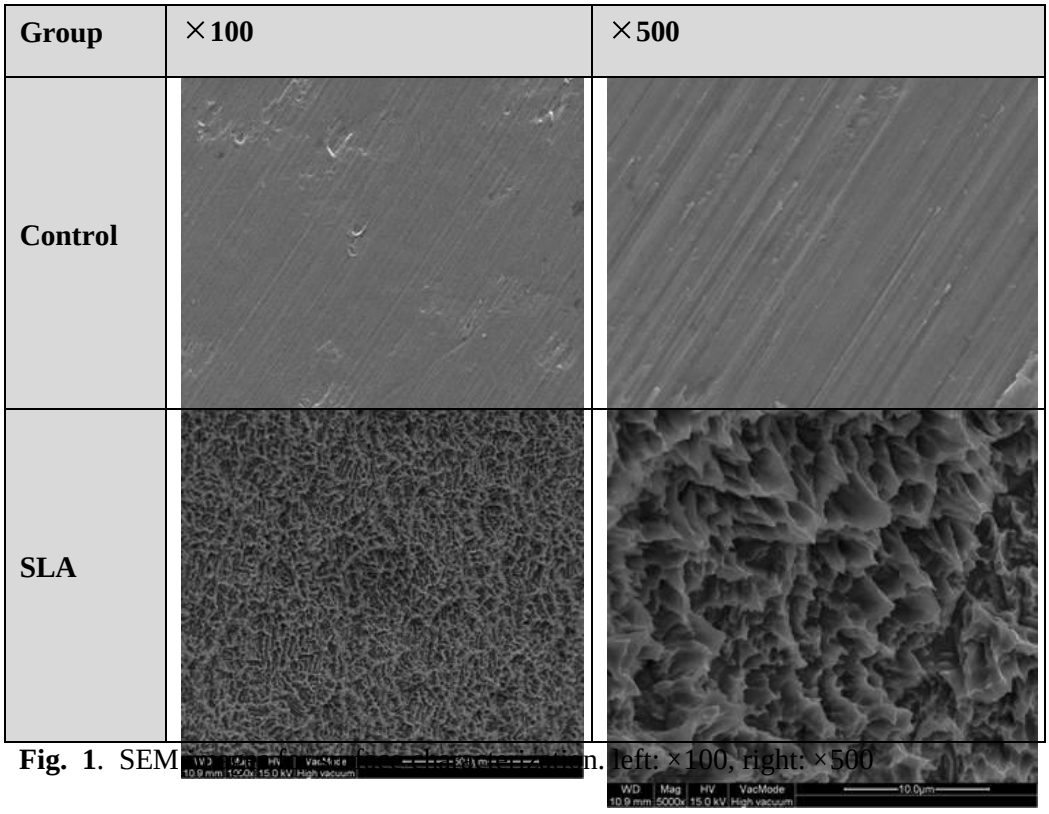

Fig. 1. SEM [illegible]n. left: ×100, right: ×500

Histological Analysis

The observation of the tissue sample treated with H&E (hematoxylin and eosin) stains showed that the osseointegration was successful with the eighth week sacrifice. In this study, the upper three threads, excluding the uppermost thread, were analyzed, and the three threads could be objectively analyzed by virtue of the sufficient existing bone. The lower area had unsatisfactory bone quality, and there were times when the intra-thread bone density (ITBD) could not be accurately measured due to the bone marrow cavity, so the area was excluded from this study. Figure 2 shows the scope of the evaluation of each sample at ×12.5 magnifications. All the groups showed satisfactory osseointegration; but in case of the control group, the BIC was partly achieved but the ITBD was unsatisfactory. On the ×100magnified images (Fig. 3), the BIC of each group is clearly shown. The SLA groups showed more mature bone formation on the implant surface than the control group. No significant inflammatory findings were observed in all the groups.

Histometric Analysis

In the three threads, excluding the uppermost thread, the BIC ratio was measured. The control group showed the BIC (%) value (mean±SD) of 33.58±8.6 and the SLA group, 58.47±12.89.

In the three threads, excluding the topmost thread, the ITBD (%) was measured. The control group showed the ITBD (%) value (mean±SD) of 34.90±7.24 and the SLA

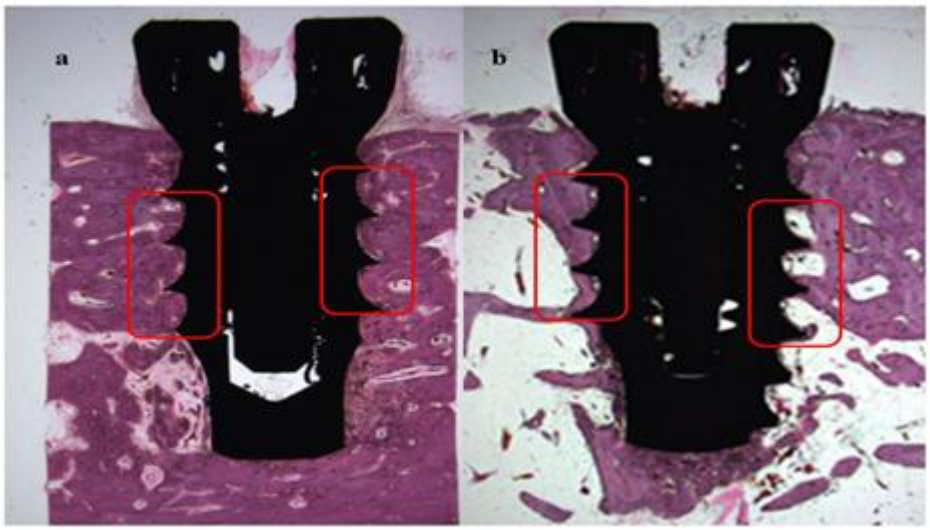

Fig. 2. The images of the specimens under ×12.5 magnification (a) SLA group, group. (b) control group

The BICs and the ITBDs values of all the groups are listed in Table 2.

Table 2. The BIC (%) and the ITBD (%) of each group

Group	N	BIC (mean±SD)	ITBD (mean±SD)
Control	8	33.58±8.63	34.90±7.24
SLA	8	58.47±12.89	53.98±13.77

BIC(%), Bone-to-implant contact ratio in the 3 threads except the uppermost thread and ITBD(%), Intra-thread bone density in the 3 threads except the uppermost thread.

DISCUSSION

A lot of animal studies have performed to examine the effects of the implant surface on bone healing and bone apposition. Microrough titanium surface regularly have a significantly greater percentage of BIC when compared with machined surface.

The SLA treated surface was achieved by blasting using biphasic calcium phosphate and acid etching. In this study, the blasting medium was biphasic calcium phosphate instead of corundum to take advantage of the typical RBM surface treatment.

Biphasic calcium phosphate is a biocompatible and resorbable blasting material.

During the osseointegration, rough titanium implant surface provides a good adherent place for osteoblasts and the initiation of new bone formation begins from the titanium surface other than the adjacent alveolar bone.

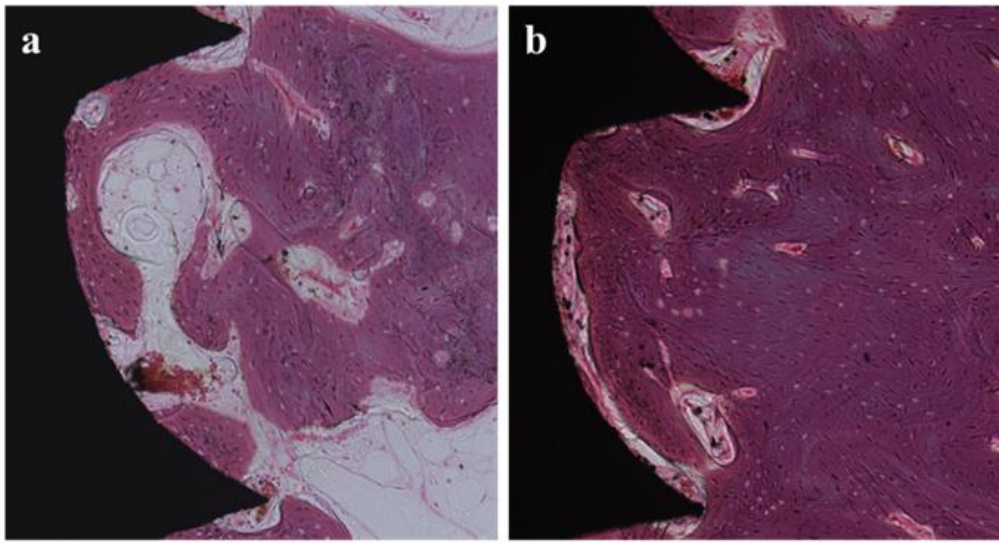

Fig. 3. The images of the specimens under ×100 magnification (**a**) control group, **(b)** SLA group.

In this situation, the contact osteogenesis can be anticipated rather than distant osteogenesis.[51] The macroroughness of the SLA group may contribute to the early osteoblasts adhesion. The macroroughness is important for an implant to enable capillary tissue and osteoblasts migration into the spaces. The roughness attempts to mimic the function of the natural extracellular matrix. The primary roles of macroroughness are: (1) to serve as an
adhesion substrate for the cell; (2) to provide temporary mechanical support to the newly grown tissue; (3) to guide the development of new tissues.

In histometric analysis, only the upper 3 threads except the uppermost thread were used for measuring the BIC and the ITBD. Because the uppermost thread usually contacted the cortical bone of the jawbone, it was excluded for the BIC and the ITBD measurement. The lower area was excluded from this study for the BIC and the ITBD measurement because it had unsatisfactory bone quality and there were times when the ITBDs could not be accurately measured due to the bone marrow cavity.

Abrahamsson et al. (2004) and Bornstein et al.(2008) studied the rate and degree of early bone formation machined and SLA surfaces. Their results showed similar characteristics of healing, with resorptive and appositional events for both SLA and machined surfaces. In the first 1-2 weeks of healing, there was a marked bony coating of the SLA surfaces with the center portion of the experimental implant device chamber less mineralized than the area adjacent to the machined implant. From week 2 onwards, the BIC percentages showed a significantly greater contact area between the newly formed bone and the titanium for implants with the SLA surface. After 6 weeks of bony healing, the differences in the bone apposition pattern and in the tissue components had disappeared, but BIC remained greater for the SLA surface. In this study, two groups were similar in the bone apposition pattern and the tissue component at 8 weeks, but BIC and ITBD represented greater for SLA group.

In this study the SLA groups showed much higher BICs to that of the control group. These observations are consistent with the previous reports regarding the BIC of surface treatment. From this observation, the SLA treatment is to be effective in the osseointegration enhancement of a dental implant.

REFERENCES

Abrahamsson, I., Berglundh, T., Linder, E., Lang, N. P. & Lindhe, J . Early bone formation adjacent to rough and turned endosseous implant surfaces: an experimental study in the dog. *Clinical Oral Implants Research* 2004; 15: 381-392

Albrektsson, T., Branemark , P.I., Hansson, H.A.& Lindstrorn, J. Osseointegrated titanium implants. Requirements for ensuring a long – lasting, direct bone-to-implant anchorage in man. *Acta Orthopaedica Scandinavica*. 1981; 52:155-170.

Bornstein M. M., Valderrama P., Jones A., Wilson T. G., Seibl R., Cochran D. l., Bone apposition around two different sandblasted and acid-etched titanium implant surface: a histomorphometric study in canine mandibles. *Clin. Oral . Impl. Res*. 2008; 19: 233-241

Bornstein MM, Schmid B, Belser UC, Lussi A, Buser D. Early loading of non-submerged titanium implants with a sandblasted and acid-etched surface: 5-year results of a prospective study in partially edentulous patients. *Clin Oral Implants Res.* 2005; 16: 631-638.

Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, Hoffmann B, Lussi A, Steinemann SG. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res.* 2004; 83: 529–533.

Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. Ahistomorphometricstudy in miniature pigs. *J Biomed Mater Res.* 1991; 25: 889-902.

Buser, D.(2001), Titanium for dental application (II): implants with roughened surfaces. In: Brunette, D.M. Tengvall, P., Textor, M., & Thomson, P., eds. Titanium in Medicine, 876-888. Berlin: Springer-Verlag.

Buser, D., Nydegger, T., Hirt, H. P. Cochran , D. L & Nolte , L. P. Removal torque values of titanium implants in the maxilla of miniature pigs. International Journal of Oral & Maxillofacial Implants 1998;13:611-619.

Kilpadi, D.V. & Lemons, J. E. , Surface energy characterization of unalloyed titanium implants. Journal of Biomedical Materials Research1994; 28: 1419-1425.

Kim BH, Kim YK, Ok JH. Development of liquid chromatographic method for the analysis of kanamycin residues in varicella vaccine using phenylisocyanate as a derivatization reagent. *J Chromatogr B Biomed Sci Appl.* 2001; 752: 173–177

Yeong WY, Chua CK, Leong KF, Chandrasekaran M. Rapid prototyping in tissue engineering: challenges and potential. *Trends Biotechnol.* 2004; 22: 643–652.