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Influence of Implant Surface Coated with pH Buffering Agent on Early Osseointegration

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Purpose: Surface treatment with pH buffering agent has been developed to achieve higher and faster osseointegration. The aim of this study was to evaluate its influence by measuring removal torque and analyzing histological characteristics.

Materials and Methods: Titanium implants with following surfaces were used in this study: sand-blasted acid-etched (SA) surface (SA group as control I group), SA surface in calcium chloride aqueous solution (CA group as control II group) and SA surface coated with pH buffering agent (pH group as test group). Removal torque test after 2 weeks and bone-to-implant contact and bone area analyses at 2 and 4 weeks were performed.

Result: The rotational torque values at 2 weeks were significantly higher in pH group (107.5±6.2 Ncm, P<0.05). The mean values of bone-to-implant contact at 2 and 4 weeks were both higher in pH group (93.0%±6.4% at 2 weeks, 88.6%±5.5% at 4 weeks) than in SA group (49.7%±9.7% at 2 weeks, 47.3%±20.1% at 4 weeks) and CA group (73.7%±12.4% at 2 weeks, 72.5%±10.9% at 4 weeks) with significances (P<0.05). The means of bone area showed significantly higher numbers in pH group (39.5%±11.3% at 2 weeks, 71.9%±10.9% at 4 weeks, P<0.05).

Conclusion: Our findings demonstrated that surface modification with pH buffering agent improved early osseointegration with superior biomechanical property.

Key Words: Dental implants; Hydrophobic and hydrophilic interactions; Osseointegration; Surface properties

Introduction

There have been extensive efforts to improve the osseointegration in implant dentistry. The modification of the implant material, design, and surface treatments has improved clinical outcomes such as early osseointegration, short loading period, stable mechanical loading, and long-term clinical

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performance[1-7].

Recently, it has been demonstrated that the surface energy of implant, which is related to hydrophilicity, is an important characteristics of implant surface to affect its biologic response[8,9]. In general, when the implant surface is positively charged, the surface turns hydrophilic. Consequently, some of the essential plasma proteins in the establishment of initial osteogenesis adsorb to hydrophilic surfaces[10]. Many studies have shown that hydrophilic surfaces tend to enhance the early stages of cell adhesion, proliferation, differentiation and bone mineralization compared to hydrophobic surface[11,12].

In another work, Schwarz et al.[13] evaluated the performance of hydrophilic sandblasted large-grit acid-etched (SLA) surface compared to non-hydrophilic implants in dogs. The surface modification seemed to increase thrombogenic responses with significances for fast bone healing, with higher bone-to-implant contacts (BICs)[13,14]. Clinical study also supports the positive effect of hydrophilicity on osseointegration by significant enhancement in bone anchorage and bone-to-implant interface[15].

Recently, Osstem Implant has released a new dental implant of which the surface is sand-blasted, acid-etched and coated with pH buffering agent to introduce hydrophilic properties.

We propose a hypothesis that this new surface promotes osseointegration during early healing period following implant placement, and accelerates the bone formation compared to conventional surfaces.

Materials and Methods

1. Study Design

Three different types of titanium implant surfaces were prepared. Conventional sand-blasted acid-etched (SA) surface (SA group as control I group), SA surface in calcium chloride aqueous solution (CA group as control II group), and SA surface coated with pH buffering solution (pH group as test group).

2. Materials

Two different dimensions of implants with the different diameter and length were used. For 2 weeks study, fixtures with lengths of 8.5 mm and diameters of 3.5 mm were prepared. For 4 weeks study, fixtures with lengths of 8.5 mm and diameters of 4.0 mm were prepared (Osstem Implant, Seoul, Korea).

A total of 30 implants were used for removal torque test, 10 implants for each SA, CA, and pH group. A total of 24 implants were used for histological evaluation, 12 implants for each 2 and 4 week, and 4 implants for each SA, CA, and pH group.

3. Animal

The protocol of this study was approved by the Animal Research Committee of Cronex Co., Ltd. (Hwaseong, Korea) (approval no. CRONEX IACUC 201710001). The guidelines of the Cronex Co., Ltd. were followed in animal selection, management, preparation, and surgical protocol.

Nine adult male miniature pigs (Cronex Co., Ltd.) aged 1 to 2 years and weighing 45 to 55 kg were used, three pigs for removal torque test and six pigs for histological evaluation.

4. Surgical Procedure

Prior to surgery, the animals were medicated with atropine (0.05 mg/kg). Anesthesia was induced with a compound of Zolazepam/Tiletamine (10 mg/kg body weight, Zoletil; Virbac Laboratories, Carros, France) and medetomidine hydrochloride (10 μg/kg body weight, Domitor; Zoetis Co., Seoul, Korea), by administering an intramuscular injection. After the induction of anesthesia, an

endotracheal tube was inserted. General anesthesia was maintained for one hour at least with 1.8% of isoflurane (Isotroy 100; Troikaa Pharmaceuticals Ltd., Gujarat, India) in oxygen at a flow rate of 100 ml/kg/min. Routine dental infiltration anesthesia (2% lidocane hydrochloride with 1/100,000 epinephrine; Huons Co., Seongnam, Korea) was used at the surgical sites. A preoperative antibiotic (cefazolin; Yuhan Co., Ltd., Seoul, Korea) was also administered intravenously. To maintain hydration, all animals received a constant rate infusion of lactated Ringer's solution while anesthetized.

The study was performed in two surgical phases. In the first phase, second, third and fourth premolars and first molars were carefully removed bilaterally in the lower jaws after reflection of full-thickness mucoperiosteal flaps and tooth separation. After the wound closure by means of mattress sutures, the sites were allowed to heal for 3 months. Prophylactic administration of clindamycine (11.0 mg/kg body weight, Cleorobes; Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 7 days. In the second phase, mid-crestal incisions were made and full-thickness mucoperiosteal flaps were reflected to expose the respective sites for implant insertion in the lower jaws.

Surgical implant sites were prepared bilaterally, at a distance of 10 mm apart, using a low-trauma surgical technique under copious irrigation with sterile 0.9% physiological saline. The flat surface on the medial aspect of the mandible was first drilled with a small diameter drill (2.0 mm) at low rotational speed of 800 rpm. The drilled hole was successively enlarged according to manufacturer guidelines. After implant insertion, cover screws were securely fastened. Primary wound closure was achieved with resorbable 5-0 Vicryl sutures and implants were left to heal in a submerged position. Following 2 and 4 weeks of healing, the pigs were anesthetized and sacrificed.

The removal torque was measured in the mandible of miniature pigs and histomorphometric analysis was conducted with histological slides containing fixtures and surrounding bony tissues.

5. Analysis Methods

1) Removal torque values measurement

A total of thirty implants were placed in three miniature pigs. Each miniature pig received five implants on each side of the mandible and the position for each implant was rotated for each animal. Thus, ten of each type of implant were placed making the total number of implants thirty. After sacrifice, the skin of the pigs was incised and the soft tissue was elevated. The cover screws were removed to measure the removal torque.

A digital torque gauge (Kanon DTDK-N5EXL; Nakamura Mfg. Co., Tokyo, Japan) was used to measure the maximum shear stress causing bone fracture of the bone and implant interface. The maximum torque limit between the fixture and fixture driver was 500 Ncm. Implants that showed higher removal torque than the threshold had a tendency to slip and it was impossible to remove them from the mandible.

2) Preparation of specimens and histologic analysis

A total of six miniature pigs were used. Four implants of each type were placed at 1 mm subcrestal level. The position of each implant was rotated for each animal. The animals were sacrificed after 2 and 4 weeks. The specimens were collected to include the implant fixture and the peripheral bone tissue. Each specimen was fixed with 10% phosphate buffered formalin, consecutively dehydrated using alcohol, and embedded with resin (Technovit 7200 VLC; Heraeus Kulzer GmbH, Wehrheim, Germany). The embedded blocks were severed in the bucco-lingual direction using a diamond band cutting system (Exakt CP; Exakt Apparatebau, Norderstedt, Germany), ground to a

30 to 40 μm thickness using a micro grinding system (Exakt 400CS; Exakt Apparatebau), and stained with H&E and Masson Trichrome for histologic observation and histomorphometric analysis.

Light microscopy (BX51; Olympus Co., Tokyo, Japan), a digital camera (DP72; Olympus Co.) and image analysis software (Image-Pro Plus; Media Cybernetics Inc., Silver Spring, MD, USA) were used in histological and histomorphometric analyses with a ×200 magnification.

3) Histometric analysis

The analysis of BIC was defined as the length fraction of mineralized bone that was in direct contact with the implant surface. Bone area (BA) was defined as the proportion of mineralized bone. The tissues located between second, third and fourth threads of the implant were included in the assessments.

4) Statistical analysis

Data are reported as mean±standard deviation. One-way analysis of variance (ANOVA) was performed for removal torque test, BIC and BA analyses. P-value of <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics 23.0 (IBM Co., Armonk, NY, USA) and Microsoft Excel 2010 (Microsoft, Redmond, WA, USA).

Result

The surgical procedures and healing following implant placement were uneventful with no indications of infection or inflammation during postoperative period in all miniature pigs.

All implants were *in situ* when animals were euthanized. There were no signs of inflammation in peri-implant tissue. Each histological ground section comprised of the implant fixture and the surrounding host bone.

1. Removal Torque Test

All the implants were stable and anchored by bone after the healing period. Fig. 1 shows the removal torques applied to different types of implants 2 weeks post-surgery. The mean resistances to removal torque for SA, CA, and pH groups are 68.5±6.4 Ncm, 86.3±7.6 Ncm, and 107.5±6.2 Ncm, respectively. There were significant differences between the pH group and SA or CA groups in removal torque values ($P<0.05$).

2. Histologic Observations

Histological results demonstrated similar bone healing surrounding all three types of implants after 2 and 4 weeks post implant placement. Inflammatory infiltrate, bone resorption and foreign body reaction were not observed at any periods and in any groups evaluated (Fig. 2, 3).

3. Histometric Observations

Results of peri-implant BIC and BA are graphically presented in Fig. 4 and 5, respectively.

The BIC% measurements for the second, third and fourth threads of the implants are presented as mean values. At 2 weeks, the BIC% values for SA was 49.7%±9.7%, for CA was 73.7%±12.4% and for pH group was 93.0%±6.4%, showing the biggest

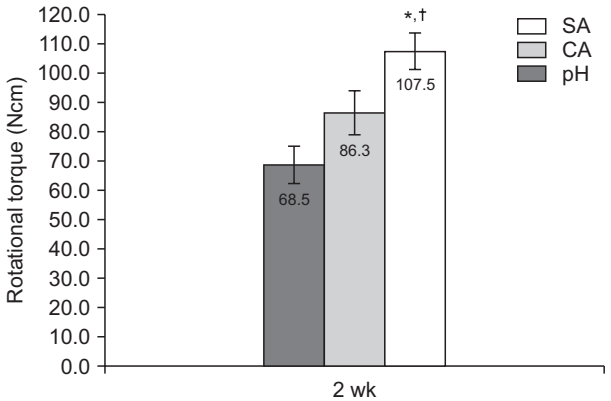

Fig. 1. Removal torque values (Ncm) after 2 weeks healing period. *Significantly different from conventional sand-blasted acid etched (SA) surface ($P<0.05$). †Significantly different from SA surface in calcium chloride aqueous solution (CA) ($P<0.05$).

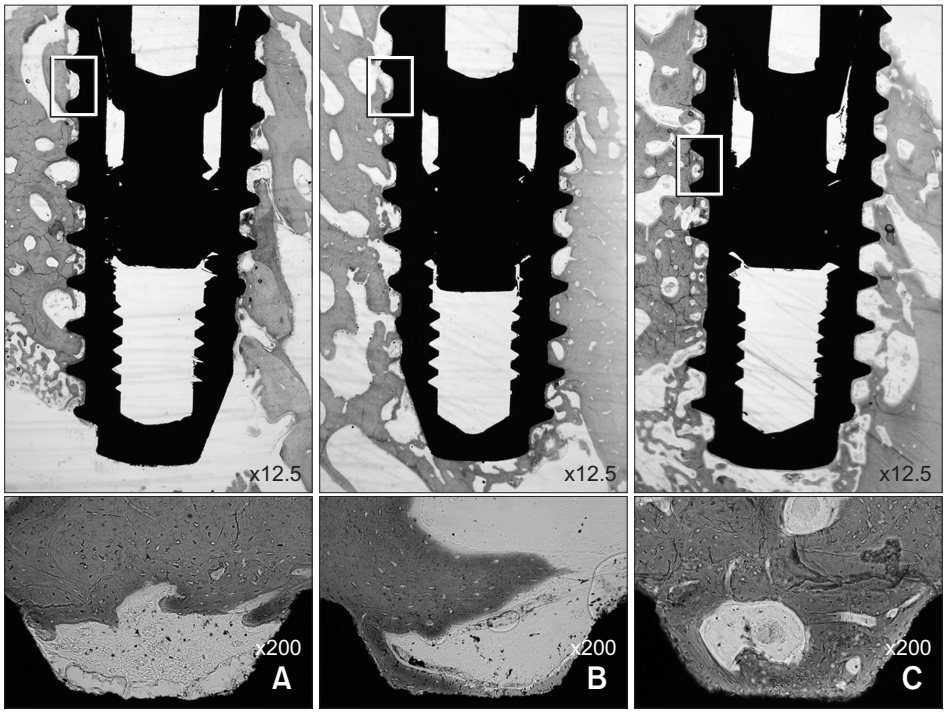

Fig. 2. Representative histological sections per group at 2 weeks healing time. The bony tissue located between second, third and fourth threads of implant was included in the assessments. (A) Conventional sand-blasted acid etched surface (SA) group, (B) SA surface in calcium chloride aqueous solution (CA) group, (C) pH group.

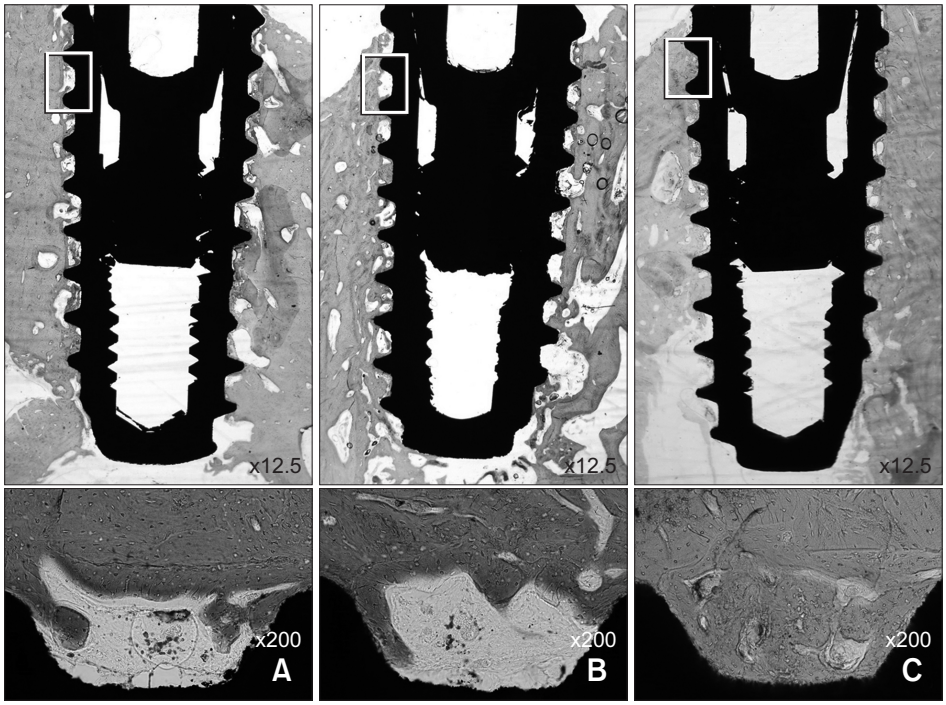

Fig. 3. Representative histological sections per group at 4 weeks healing time. The bony tissue located between second, third and fourth threads of implant was included in the assessments. (A) Conventional sand-blasted acid etched surface (SA) group, (B) SA surface in calcium chloride aqueous solution (CA) group, (C) pH group.

value in the pH group. A similar pattern was observed at 4 weeks, showing the highest BIC% in the pH group (88.6%±5.5%). The BIC% differences between groups were statistically significant ($P<0.05$).

The area of the bone tissue located between the second, third and fourth threads of three implant types were evaluated. The pH group showed 39.5%±11.3% at 2 weeks, and 71.9%±10.9% at 4 weeks, which were both higher than SA or CA

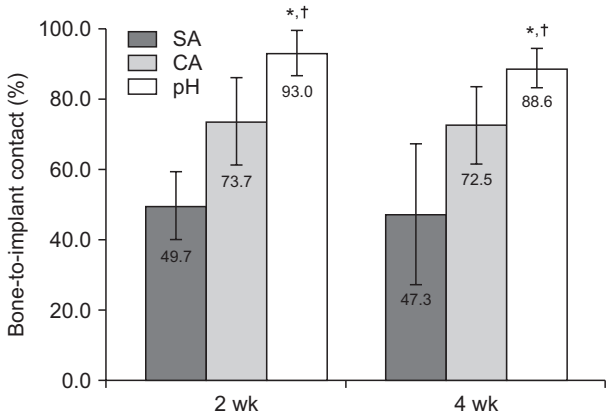

Fig. 4. Bone-to-implant contact (%) after 2 and 4 weeks of healing periods. *Significantly different from conventional sand-blasted acid etched (SA) surface (P<0.05). †Significantly different from SA surface in calcium chloride aqueous solution (CA) (P<0.05).

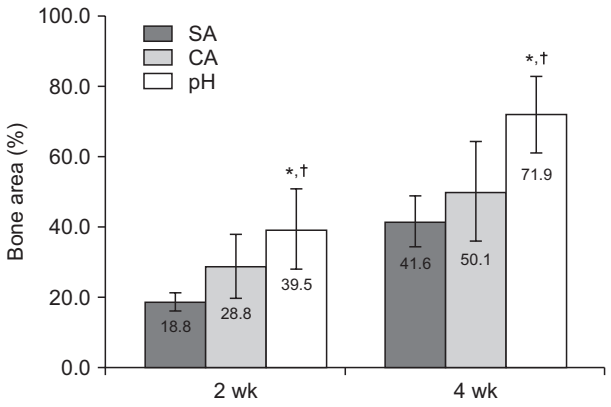

Fig. 5. Bone area (%) after 2 and 4 weeks of healing periods. *Significantly different from conventional sand-blasted acid etched (SA) surface (P<0.05). †Significantly different from SA surface in calcium chloride aqueous solution (CA) (P<0.05).

groups. SA group showed the lowest values which were 18.8%±2.6% at 2 weeks and 41.6%±7.2% at 4 weeks. The statistical analysis shows significant differences in BA% between the pH group and SA or CA group at both time periods (P<0.05).

Discussion

The aim of this study was to investigate the effect of implant surface treatment with pH buffering agent on biomechanical and histological stabilities. This was achieved by removal torque measurement and histomorphometric analyses. Our results suggest that surface treatment with pH buffering agent which promotes higher hydrophilicity enhances early osseointegration. This is demonstrated by significantly higher values in all parameters.

The mechanisms behind the improved osseointegration on the hydrophilic as opposed to the hydrophobic surfaces have not been fully understood. Many studies have shown that increased hydrophilicity induces greater BIC, osteoblast differentiation, growth factor production and osteogenic gene expression than hydrophobic surfaces[11,16-20]. A study by Donos et al.[21] also reported the evidence suggesting that the surface charge of hydrophilic surface may selectively attract proteins that affect gene regulations expressed by adjacent progenitor cells. Hong et al.[14] used *in vitro* model to compare the thrombogenic responses of hydrophilic and hydrophobic implant surfaces. Their findings showed significantly higher binding of platelets to the hydrophilic surface, accompanied by a significant increase of contact activation of coagulation cascade. The conclusions from above studies may explain our findings of higher early osseointegration from hydrophilic surface of pH group produced by pH buffering agent.

The impact of acidic environment on bone biology has long been known[22]. In acidosis, osteoclast resorptive activity is increased, and the deposition of alkaline mineral in bone by osteoblasts is reduced. However, there have been few studies investigating the effect of pH buffering properties on implant surfaces. Our study utilized pH buffering material which possesses hydrophilic nature for a coating agent on implant surfaces, and on-going investigations derived from our study could shed a light on the possible effect of pH buffering actions on osseointegration.

In the current study, removal torque values have been used as a biomechanical measure

of osseointegration in which the greater forces required to remove implants may be the indication for higher strength of osseointegration[23]. Buser et al.[24] used a miniature pig model to compare the removal torque values of two different titanium implants after 4, 8, and 12 weeks of healing. The result of sandblasted and acid-etched implants after 4 weeks was 109.6 Ncm, whereas machined and acid-etched implant showed significantly less value of 62.5 Ncm. In our study, the highest torque value was reported from the pH group (107.5 Ncm), and the lowest from SA group (68.5 Ncm) which are comparable to the aforementioned study. However, considering the shorter healing period of 2 weeks, the implants used in our study demonstrated higher performance in biomechanical stability.

In another study by Lang et al.[15], hydrophilic and hydrophobic implant surfaces were compared to evaluate the rate and degree of osseointegration. According to the study, a greater extent of BIC occurred at implants with a higher hydrophilicity at both 2 and 4 weeks post-surgery (14.8% and 48.3%, respectively). This trend corresponds that of our study, where hydrophilicity exhibited higher degree of early osseointegration. The percentages of BIC in our study, however, showed remarkably higher values compared to Lang et al.'s study[15].

The same trend in removal torque test followed that of histomorphometric analyses. At 2 weeks after the surgery, the removal torque values of test group showed significant differences compare to both SA and CA groups ($P<0.05$). Greater resistance to removal torque force may be interpreted as an increase in the BIC, although the underlying biomechanical phenomena in this type of testing are complex. This is due to the fact that removal torque tests measure the shear forces at the interface between bone and implant surface, and this does not sufficiently reflect the direct relation with bone response[25]. In spite of this shortcoming, many previous studies have used this test to evaluate the implant fixation or the degree of osseointegration[26].

Conclusion

In summary, the implant surface coated with pH buffering agent exhibits higher degree of early osseointegration than conventional surfaces. Further research is needed to investigate the potential of this surface treatment as a method to improve osseointegration.

Conflict of Interest

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

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