Histomorphometric Analysis and Removal Torque of Small Diameter Implants With Alternative Surface Treatments and Different Designs

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This study was designed to investigate the histomorphometric and biomechanical comparison of small-diameter implants with different designs. These implants can be placed surgically in narrow bone spaces, such as the lower incisor region, that have low occlusal loading. Specimens of screw-shaped pin implants were designed for the study. These specimen implants were divided into 6 groups: group 1, machined implants; group 2, resorbable blast media (RBM)-treated implants; group 3, machined implants with a long vertical groove; group 4, RBM-treated implants with a long vertical groove; group 5, RBM-treated implants with a vertical groove on the upper thread; and group 6, RBM-treated implants with a vertical groove on the lower trunk. The specimen implants were placed surgically on the medial side of the rabbit tibia. Animals were sacrificed 2, 4, and 8 weeks after surgery. The removal torque was measured and tissues were prepared for histologic and histomorphometric analysis. The bone-to-implant contact and the percentage of the bone area inside the threads were measured. RBM-treated implants with vertical groove groups showed significantly higher values of removal torque, bone-implant contact, and bone area rate than the ones of machined surface groups.

Key Words: removal torque value, resorbable blast media (RBM), vertical groove, bone-implant contact, small diameter implants

INTRODUCTION

Immediate and early loading of implants are popular procedures used in implant dentistry today. The reduced treatment times generally increase patient’s satisfaction and the efficiency of the treatment because the time to restoration is shortened. The success of immediate and early loading in terms of function and predictability has been demonstrated amply, and this technique has been suggested to be a realistic alternative to conventional loading. However immediate or early, loading is not possible in all patients, and the approach requires strict and highly effective collaboration between surgeons and restorative dentists in terms of presurgical
In the case of conventional loading, the prosthesis is attached in a second procedure after a healing period of 3 to 6 months. Therefore, a patient with a complete or partially edentulous jaw cannot avoid functional and esthetic problems until the final restoration is placed. To resolve such problems, several practitioners have recommended the use of a transitional pin implant. Transitional pin implants are placed simultaneously between the main implants, and they allow osseointegration to occur in the absence of load. Such implants support immediate fixed provisional restoration so that the patient can recover immediately with regard to esthetics and function. In most cases, the transitional pin implants are removed after the healing period. However, it has been reported that it may be very difficult to remove these implants owing to strong osseointegration; in these cases, the pin implant participates with the main implant in the final restoration. Simon and Caputo measured the removal torque of immediately loaded transitional pin implants in human subjects and reported that the pin implants are supported strongly even after immediate loading. Proussaefs performed histologic evaluation of transitional pin implants following immediate application of a load that remained for 18 months, and reported 81.3% of high bone-implant contact ratio.

Such research suggests that transitional pin implants can be used as permanent implants. To use transitional pin implants in this way, they require surface treatment, and the appropriate mechanical design and features to enhance osseointegration. Thomas and Cook insisted that bone quality is the most important factor in osseointegration and, at the same time that it is affected significantly by surface roughness. It has been reported that a titanium implant with a rough surface can increase the surface area of contact with the bone and cell adhesion. According to Buser et al, the rate of bone contact is greater when titanium implants with rough surfaces are used, as compared with titanium implants with smooth surfaces. Several methods of surface treatment are available, such as hydroxyapatite coating, titanium plasma spray, acid-etching, sand blasting, and resorbable blast media (RBM).

This study investigated the application of RBM to the surface of implants. Application of RBM is a recently developed method of surface treatment that has been used widely in clinical practice. Treatment with RBM uses calcium phosphate particles as a blast media, which are sprayed onto the surface of the titanium implant. The implant is then passivated in order to remove the excess medium. This procedure does not involve acid-etching, and therefore does not affect the fatigue strength of the implant surface. Such spray treatment not only produces a rough surface to the implant, but also removes surface contamination and increases the activity of the implant surface. This is therefore recognized as a method of surface treatment that enhances osseointegration.

This study was designed to evaluate the effect of surface treatment with RBM, together with various designs of vertical groove on machined implants for permanent use. It involved measurement of removal torque, observation of histologic findings, and histomorphometric analysis in order to study the effect of the surface treatment and mechanical design on osseointegration.

**Materials and Methods**

**Animals and implant specimens**

Twenty adult male New Zealand white rabbits, 3–4 kg in weight, were used in this study. The small diameter implant that was used in this study was made of titanium grade 5, and was screw-shaped, 2 mm in diameter, and 6 mm in length (Slimplant, YK-pros, Seoul, Korea). Two types of surface were prepared: machined and RBM treated. Vertical
groove was applied at an angle of 45° and at a depth of 0.5 mm from the center of 4 experimental implants to provide resistance to mechanical loosening (Figure 1). The implant specimens were divided into 6 groups: group 1, machined implants; group 2, RBM-treated implants; group 3, machined implants with a long vertical groove; group 4, RBM-treated implants with a long vertical groove; group 5, RBM-treated implants with a vertical groove on the upper thread; and group 6, RBM-treated implants with a vertical groove on the lower trunk. Each group contained 20 specimens which gave a total of 120 specimens.

**Surgical procedure**

Six implants were placed in the rabbit tibias, with a distance of 2.5 mm between implants. The animals were preanesthetized by intramuscular injection of a mixture of 25 mg/kg ketamine hydrochloride (Ketara; Yuhan, Seoul, Korea) and 5 mg/kg xylazine (Rompun; Bayer Korea, Seoul, Korea). Then, 0.5 mL of 2% lidocaine (1:100 000 epinephrine; Yuhan) was applied to the surgical site to provide local anesthesia.

The small diameter implant was placed on that site using a hand driver to penetrate only 1 cortical bone after 2.0-mm pilot drilling. To prevent overheating of the implant, the site was irrigated with saline solution throughout the surgical procedure.

Postoperatively, it administered 0.15 mL gentamicin (5 mg/kg; Daeseong Microbial Research Center, Seoul, Korea) as an antibiotic and 0.2 mL ketoprofen (7 mg/kg; Unibiotech, Seoul, Korea) as an anti-inflammatory drug on muscle for 1 week from the day of operation. At the end of the experimental period, the animals were sacrificed by intravenous administration of a high dose of ketamine hydrochloride (Yuhan) 2, 4, and 8 weeks after surgery. Tissue sections, which included samples from the surgical site, were obtained immediately after sacrifice. Half of each specimen was used to measure the removal torque, and the other half was fixed in 10% formalin solution (diluted with 1× PBS buffer), dehydrated using an ascending series of alcohols and embedded in glycomethacrylate resin (Technovit 7200 VLC, Kulzer, Germany) to produce undecalcified sections. Undecalcified cut and ground sections that contained the

**Figure 1.** Diagram of a specimen of the titanium small diameter implant. It is screw-shaped, 2 mm in diameter, 6 mm in length and made of grade 5 titanium (Slimplant, YK-pros). It was prepared with 2 types of surface: machined (1, 3) and resorbable blast media (RBM)-treated (2, 4, 5, 6). Vertical groove was applied at an angle of 45° and at a depth of 0.5 mm from the center of 4 experimental implants to provide resistance to mechanical loosening. The implant specimens were divided into 6 groups: group 1, machined implants; group 2, RBM-treated implants; group 3, machined implants with a long vertical groove; group 4, RBM-treated implants with a long vertical groove; group 5, RBM-treated implants with a vertical groove on the upper thread; and group 6, RBM-treated implants with a vertical groove on the lower trunk.
central part of each implant and had a final thickness of 15 μm were produced using a macro cutting and grinding system (EXAKT Co, Hamburg, Germany). The sections were stained with hematoxylin-eosin, and histomorphometric analysis was carried out. The study protocol was approved by the Ethics Committee of Kyung Hee University, Seoul, Korea.

**Removal torque test**

At 2, 4, and 8 weeks after surgery, the peak torque force required for removal of the implant was measured using a torque gauge (1.5 BTG/6 BTG, Tohnichi Mfg Co, Tokyo, Japan). This device measures the removal torque of the implants using 1.5 BTG (0.2–1.5 kgf cm) and 6 BTG (0.6–6 kgf cm), and is widely used to measure the torque required to tighten and loosen implants. The removal torque was measured to evaluate the stability of the implant in the bone.

**Histomorphometric analysis**

Specimens that had been prepared for the histologic analysis of the tissue that surrounded the implant were examined using a light microscope (Axioplan 2; Carl Zeiss, Oberkochen, Germany). The specimens were classified into 2 groups: machined surface and RBM treated group to examine new bone formation and adhesion status with the bone-implant interface on week 2, 4, and 8.

After digitizing the phase of each specimen under light microscope, the percentage of bone to implant contact (BIC%) in the first 2 threads, and the percentage of bone area inside the same threads were measured using the Kappa ImageBase program (Olympus Co, Tokyo, Japan). BIC% was calculated as the percentage of the total length of bone that was in direct contact with the implant surface. We evaluated the BIC% and bone area in the first 2 threads because the lower parts of the implants were surrounded by the marrow space, in which bony structures are almost absent.

**Statistical analysis**

SPSS version 11.0 (SPSS Inc, Chicago, Ill) was used for the statistical analysis. The data were analyzed using the multiple range tests together with a post hoc test of the least significant difference. *P* values of less than .05 were considered to be statistically significant.

**Results**

**Analysis of removal torque**

The mean and standard deviation of the removal torque for 6 groups of implant at 2, 4, and 8 weeks. Group 1, machined implants; group 2, resorbable blast media (RBM)-treated implants; group 3, machined implants with a long vertical groove; group 4, RBM-treated implants with a long vertical groove; group 5, RBM-treated implants with a vertical groove on the upper thread; and group 6, RBM-treated implants with a vertial groove on the lower trunk. *Significant difference, *P* .05.

**Figure 2.** Mean and standard deviation of removal torque for 6 groups of implant at 2, 4, and 8 weeks. Group 1, machined implants; group 2, resorbable blast media (RBM)-treated implants; group 3, machined implants with a long vertical groove; group 4, RBM-treated implants with a long vertical groove; group 5, RBM-treated implants with a vertical groove on the upper thread; and group 6, RBM-treated implants with a vertial groove on the lower trunk. *Significant difference, *P* .05.
**Histologic observations**

With both the machined and RBM-treated implants at 2 weeks, new bone was formed at the bone-implant interface on the cortical bone site of the upper implant. No infiltration of inflammatory cells or proliferation of fibrotic tissue in the interface between the implant surface and the bone had occurred, and adhesion between the new bone and the implant was observed. Small trabeculae were observed in the medullary cavity directly below the cortical bone. The new bone and trabeculae were mostly formed in the shape of the bone with large lacunae, and there was no bone resorption or remodeling of bone (Figure 3a). There was no remarkable difference between machined surface and RBM-treated implants; however, new small bones were observed to have formed independently on the implant surface in the RBM group (Figure 3b).

Increased infiltration of bone from the cortical bone to the spongiosa occurred in animals that received implants with a machined surface at 4 weeks. The thin trabeculae that were observed at 2 weeks had become thickened or had coalesced to form lamellar bone. Most of the new bone that was observed at 2 weeks had been removed, but there were small areas of lacunae and matured bone, and some bone remodeling had occurred. No infiltration by surrounding inflammatory cells or bone resorption was observed. In this group, there was some evidence that new bone that had previously adhered to the implant had been resorbed during the process of bone remodeling (Figure 3c). Histologic findings for the RBM group were similar to those for the machined surface group. However, compared with the machined surface group, there was no obvious resorption of the osseointegrated implant by bone remodeling, and compact bone was maintained at the interface (Figure 3d).

In the machined surface group at 8 weeks, the mass of bone was similar to that observed at 4 weeks. However, remarkable bone remodeling had occurred so that bone marrow was observed at the interface. Fatty and hematopoietic bone marrow was seen within the bone marrow cavity, and there was no histologic difference from preexisting bone. Areas of new and existing bone were distinguished by a boundary, but this was not clear in most cases. New bone that had been formed after reabsorption was seen in some cases (Figure 3e). The overall features of the RBM group were similar to those of the machined surface group. However, at many sites the new bone could not be distinguished from the adjacent preexisting bone, due to the almost complete formation of compact bone. In general, the connectivity of the compact bone was greater in this group than in the machined surface group (Figure 3f).

**Histomorphometric analysis**

The mean and standard deviations of the BIC% of the 2 groups at weeks 2, 4, and 8 are presented in Figure 4. At weeks 2, 4, and 8, the RBM group showed significantly higher values of BIC% than the machined group, and the ratio increased when the healing period was lengthened ($P < .05$). The bone surface area was greater in the RBM group than in the machined group at all time points (weeks 2, 4, and 8; $P < .05$). In both groups, the area increased from week 2 to week 4, but it had decreased slightly by week 8. However, the decrease from week 4 to week 8 was not statistically significant (Figure 5).

**Discussion**

It has been reported that successful osseointegration of an implant in vivo is affected by many factors, such as the surface morphology and geometric shape of the implant, the
Figure 3. Histologic sections of machined and resorbable blast media (RBM)-treated implant 2, 4, and 8 weeks after implantation in rabbit tibiae (original magnification ×40). (a) Machined implant at 2 weeks. (b) RBM-treated implant at 2 weeks. (c) Machined implant at 4 weeks. (d) RBM-treated implant at 4 weeks. (e) Machined implant at 8 weeks. (f) RBM-treated implant at 8 weeks. I indicates implant fixture; B, bone marrow. New bone formation can be observed (blue arrow). The overall features of the RBM-treated implant were similar to those of the machined implant. However, at many sites the new
bone quality and mass around the implant body, and the size and direction of the load during functional occlusion. Recent research on dental implants has focused on various surface treatments, with the aim of developing a safe implant surface that stimulates osseointegration to increase the proportion of successful surgery in practice.\textsuperscript{13–15} Therefore, studies have been performed with the aim of improving the osseointegration of implants by increasing the roughness of the surface of the implant,\textsuperscript{16–18} using various coatings\textsuperscript{19,20} or chemical treatment.\textsuperscript{21,22} It has been reported that the surface features of an implant, including surface roughness and ultrastructural morphology, have a remarkable influence on osseointegration.\textsuperscript{22}

It has also been reported that implants with a rough surface have improved stability in bone\textsuperscript{9,20} and that the features of the implant surface affect the local tissue response.\textsuperscript{15} One simple way to modify the surface of an implant is to alter the roughness of the surface. According to Goldberg et al,\textsuperscript{23} the shear stress is increased if the surface of an implant is roughened.

Wennerberg et al\textsuperscript{24} reported that the use of an implant with a rough surface leads to strong fixation in bone, and they showed a statistically significant increase in BIC\% and removal torque. However, excess roughness in the implant, may limit the healing capacity of the bone and thus reduce the contact ratio. They analyzed the surface roughness of various implants using Topscan 3D, and reported that implants with an excessively smooth surface are not appropriate for cell adhesion. Conversely, implants with an excessively rough surface caused ionic leakage and prevented the response of the bone. An implant with an optimal degree of roughness stimulates cell adhesion, and thus results in osseointegration.

To improve the mechanical resistance of an implant, it is possible to use a core or vent, to form a T-chamber on the lower part of the implant, or to produce a tap beside the implant. It is thought that such designs stimulate the proliferation of bone tissue at the implant site, which results in strong fixation.\textsuperscript{25}

The effect on fixation is not related directly to osseointegration at the interface, but is caused by physical fixation that results from bone proliferation. According to Sullivan et al,\textsuperscript{26} the removal torque of a screw-
shaped implant with a T-chamber that was 3.74 mm in diameter was more than 10 Ncm, despite a lack of osseointegration.

There are 2 general methods with which to measure the safety and osseointegration of an implant: biomechanical examination and histomorphometric analysis. A typical method of biomechanical examination is measurement of the removal torque. This involves the application of rotatory force to the implant in a counterclockwise direction. The load is measured at the point at which bonding at the bone-implant interface is destroyed in order to measure the quality of the interface of a screw-shaped implant. Such measurement is valuable for evaluation of the mechanical connectivity at the bone-implant interface. Although the method does not evaluate the degree of osseointegration precisely and directly, it is a straightforward and repeatable method, and it has been applied in both experimental animals and clinical trials. The removal torque is influenced by the surface structure and morphologic features of the implant, and by the tissue structure at the implant-bone interface. It provides an objective standard by which to evaluate the healing status at the bone-implant interface and to estimate the degree of osseointegration.

Simon and Caputo studied the removal torque in human patients with temporary pin implants to which a load had been applied immediately. The authors reported a removal torque of 16.1 ± 4.8 Ncm and 24.0 ± 7.3 Ncm in the upper and lower jaw, respectively. They also found that the value was greater for the mandible than for the maxilla, as the time in function was longer. However, in a group that had retained the implant for more than 11 months, there was no further increase in removal torque with time. During removal, several temporary pin implants were not removed, but rather became fractured. In such cases, the torque applied was 27–35 Ncm. In the current study, relatively low values of removal torque were found as compared with the results of Simon and Caputo. In the latter study, the implants were long and the subjects were human, which resulted in increased removal torque. In our study the RBM-treated implants that had a vertical groove presented a maximum removal torque that was 4 folds higher than that of the experimental group.

Therefore, if the length of the specimen were increased and both surface treatment and mechanical resistance were applied, it may be possible to use such implants permanently in certain sites in the human body that have better bone quality than the rabbit tibia.

Histomorphometric analysis was used for quantitative analysis that is difficult to explain only with findings under a light microscope. There are 2 indicators of osseointegration: the contact ratio between bone and implant, which measures the degree of contact between bone and implant in the tissue specimen, and the bone surface area, which measures the amount of new bone formation quantitatively within the conchoids of the implant. These methods have been used to measure, compare, and analyze the responses of the surrounding bone, and the amount of new bone that was stimulated by various types of implant, surgical method, site of placement, healing period, and conditions to produce extensive data that are clinically applicable.

The BIC% is the ratio of direct contact to the total length of the implant in the interface between the implant and bone. In this context, bone contact does not refer to osseointegration, but it is a useful indicator of the bonding status at the bone-implant interface. In general, the BIC% is analyzed using a standard of "the ratio of bone-implant contact against total length of implant" or "the ratio of length against top three sequential conchoids." However, the...
value measured may be influenced greatly by the quality of the bone, the ratio of cortical bone to spongy bone, and the length of the implant. Therefore, the standard to be used must be defined in advance.

The BIC% in this study increased significantly with increasing time post surgery (weeks 2, 4, and 8) in both groups of implants. The BIC% and bone area were greater in the RBM group than in the machined group. These findings indicate that RBM treatment of small diameter implants enhances osseointegration, is helpful to new bone formation, and is associated with a reduction in the healing period.

Surface treatment using RBM influenced the stability and osseointegration of the small diameter implant, and a vertical groove also led to a significant increase in these parameters. However, it was determined that the influence of the design of the vertical groove was slight. It was thought that the effect of the design of the vertical groove was small because in the RBM group, the sharp-edged angle was destroyed during the surface treatment procedure, whereas the groove could be incised precisely in the machined implants. Additional studies of different mechanical morphologies and surface treatment methods are required in order to design a small diameter implant for permanent use.

**ABBREVIATIONS**

BIC: bone to implant contact

RBM: resorbable blast media

**REFERENCES**


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