AN EXPERIMENTAL STUDY OF NEWLY DESIGNED IMPLANT WITH RBM SURFACE IN THE RABBIT TIBIA : RESONANCE FREQUENCY ANALYSIS AND REMOVAL TORQUE STUDY

Mi-Kyoung Won, D.D.S., Chan-Jin Park, D.D.S., M.S.D., Ph.D., Kyoung-Soo Chang, D.D.S., M.S.D., Ph.D., Chang-Whe Kim, D.D.S., M.S.D., Ph.D., Yung-Soo Kim, D.D.S., M.S.D., Ph.D., Zakiahbt Mohd Isa, D.D.S., M.S.D., Ph.D.*, Yusnidar Tajul Ariffin, D.D.S., M.S.D., Ph.D.*

Department of Prosthodontics, College of Dentistry, Seoul National University, Univ. of Malaya*

Statement of problem. The importance of fixture design and surface treatment.

Purpose. The clinical success of dental implants is affected by many factors such like as degree of osseointegration, the effective load dispersion for the prostheses, and a lot of attempts have been made to overcome the difficulties. In this study, efforts were made to find the possibility of clinical acceptance of the dental implants of newly designed surface and resorbable blast media surcace.

Materials and methods. In this study, two groups of custom-made, screw-shaped implants were prepared. Tthe first with the consisting of Brånemark clone design and the other with the new design. These implants were divided into four groups according to the kinds of surface treatment. Four implants(AVANA®, Osstem, Busan, Korea)of each group were installed in twenty rabbits. Group A was consisted of Brånemark clone implant left as machined, Group B with Brånemark clone implants with RBM(Resorbable blast media) surface, Group C with newly designed implants left as machined and Group D with newly designed implants with RBM surface. One of the twenty rabbits died from inflammation and the observation was made for six weeks. Specimens from four groups were observed using scanning electron microscopy with 40, 100, 1000 magnification power and microsurface structures were measured by white-light scanning interferometry for three dimensional surface roughness measurements(Accura 2000®, Intek-Plus, Korea.). Removal torque was measured in 17 rabbits using digital torque gauge(MGT 12R, Mark-10 corp., NY, U.S.A.) immediately after the sacrifice and two rabbits were used for the histologic preparation(EXAKT 310®, Heraeus Kulzer, Wehrheim, Germany) of specimens and observed under light microscope. Resonance frequency measurement(Osstell®) was taken with the 19 rabbits at the beginning of the implant fixation and immediately after the sacrifice.

Results. Following results were taken from the experiment.

1. The surface of the RBM implants as seen with SEM had rough and irregular pattern with reticular formation compared to that of turned specimens showing different surface topographies.
2. The newly designed implant with RBM surface had high removal torque value among four groups with no statistical significance. The average removal torque was 49.95±6.70Ncm in Group A, 51.15±4.40Ncm in Group B, 50.78±9.37Ncm in Group C, 51.09±4.69Ncm in Group D.
3. The RFA values were 70.8±4.3Hz in Group A, 71.8±3.1Hz in Group B, 70.9±2.5Hz, 72.7 ±2.5Hz in Group D. Higher values were noted in the groups which had surface treatment compared to the untreated groups with no statistical significance.
4. The results from the histomorphometric evaluation showed a mean percentage of bone-to-implant contact of 45±0.5% in Group A, 55±3% in Group B, 49.5±0.5% in Group C, and 55±3% in Group D. Quite amount of newly formed bone were observed at the surface RBM-treated implants in bone marrow space.

Key Words

Dental implant, Resonance frequency analysis, Removal torque, Digital torque gauge, Resorbable blast media

For the past 30 years the use of osseointegrated implants has become a scientifically accepted and well-documented treatment modality for the rehabilitation of completely and partially edentulous patients. Osseointegration is a treatment concept based on stability[1] and the rigid fixation seems to be a prerequisite for a favorable long-term clinical outcome.[2] The term osseointegration has been used to define a direct structural and functional connection between ordered living bone and the surface of a load carrying implant, and it has been mechanically defined as continuity between implant and adjacent hard tissue.[3-5] Successful long-term stability of osseointegrated dental implants has been reported in a large number of clinical studies using commercially pure titanium implants.[6-8]

Although the implants have high survival rates,[6,9] other clinical studies,[9-11] however, reported increased failure rates in area with poor bone quality such as low bone density or insufficient bone volume and height, mainly in the posterior maxilla, especially for screw-type implants with a turned surface. Failures related to these situations are caused by bone loss as a result of lack of primary and secondary implant stability.

The density and quantity of the bone, the surgical technique, and the design of implant determine primary stability. Secondary stability is the stability of implant after primary healing and can be increased by bone formation and remodeling at the implant-bone interface.

Successful osseointegration of endosseous implants results from a favorable interaction between the implant geometry, surface texture and the tissues at the bone site.[12] Implant material, the macro-design and surface structure are among the crucial factors influencing the clinical outcome of an implant.[13] Many attempts have been made over the past decade to improve bone anchorage of dental implants. Especially, surface macrostructure and microstructure have developed to establish a stable fixation between the implant and the bony tissue and to improve load transfer and to evoke favorable bone and cell response.

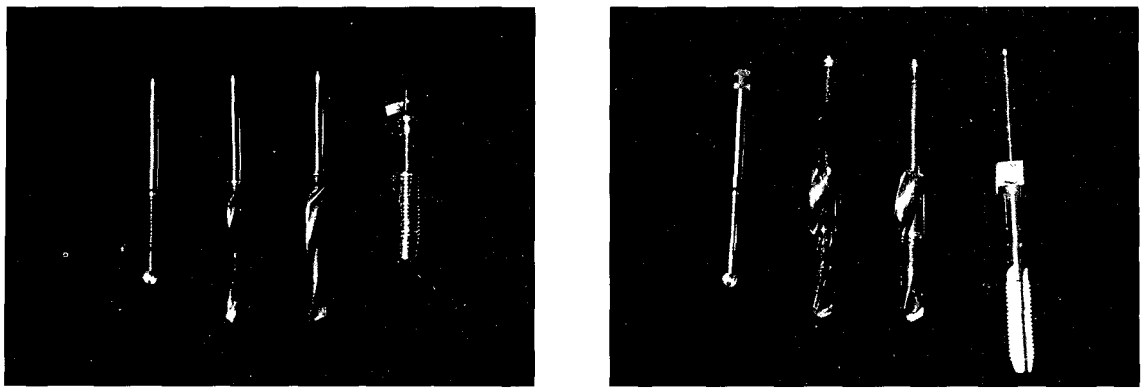
The current paper will focus on the in vivo investigations, especially those that were evaluated with biomechanical tests, i.e. resonance frequency analysis(RFA) and removal torque. From a mechanical standpoint, the removal torque technique measures the strength of the bone-implant interface in terms of shear, while the RFA is considered to measure the stability during bending.[14]

The purpose of the present study was to evaluate bone tissue reaction around a newly design threaded implants (AVANA®, Osstem, Busan, Korea) with new surface treatment called resorbable blast media (RBM) placed in the rabbit tibia using mainly resonance frequency analysis and removal torque measurement. The resonance frequency measurement and anchorage of newly design implants with RBM surface was compared with those of conventional Brånemark clone with turned surface, Brånemark clone with RBM surface, and the new design with turned surface screws.

MATERIAL AND METHODS

1. Animals and Surgical technique

Twenty adult New Zealand white rabbits of both sexes weighing 3.5 to 4 kg were included in this study. Prior to surgery, animals were acclimated to the vivarium for a period of observation to insure that they were healthy and stable. The animals were anesthetized using intramuscular injections of keta (8.8mg/kg) for surgical procedure. Prior to surgery, the shaved skin was carefully washed with a mixture of iodine and 70% ethanol. 1.8mL of lidocaine 2% were injected locally into the surgical sites. The tibial metaphysis was exposed by incisions through the skin, fascia, and periosteum. By intermittent drilling using low rotary speed (not exceeding 2000r.p.m) with copious saline irrigation, 2 holes were



(A) (B)

Fig. 1. A : drills for Brånemark clone implants. B : drills for new-design implants.

drilled 7mm apart in the central portion of each tibia and sequentially enlarged to 3mm. After tapping and slightly countersinking the sites, the implants were gently screwed into place, until the implant shoulder was leveling with the bone surface. All implants penetrated the first cortical layer only, never engaging the opposite cortical side. Each rabbit received four implants, from each of the different groups (group A, B, C, D) which were randomly assigned to their implantation sites. Then, the skin and fascia layers were closed separately using resorbable sutures. After the operation, the animals received Cefazolin IM injection per day for 7 days. The animals were kept in separate cages and immediately after surgery they were allowed to bear full weight. The follow-up time was six weeks. One week after surgery, one animal died due to an unknown inflammation. The other nineteen animals were sacrificed using an overdose of carbon dioxide.

2. Implant preparation: design and surface treatment

A total of 80 custom-made, screw-shaped implants were used. 40 implants were Brånemark clone designed, and other 40 implants had a new design(AVANA®, Osstem, Busan, Korea). These implants were divided into 4 groups according to surface treatment.) Group A and B Brånemark clone implants have 0.6mm pitch, and group C, D newly design implants have 0.8mm pitch.

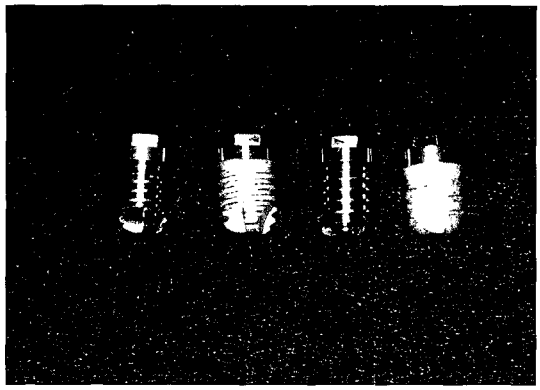


Fig. 2. Implants from each group. Group A, B, C, D

- Group A: 20 Brånemark clone implants left as machined.
- Group B: 20 Brånemark clone implants with RBM surface.
- Group C: 20 newly designed implants left as machined.
- Group D: 20 newly designed implants with RBM surface.

3. Scanning electron microscopy and scanning interferometry

The electron microscopy used to observe the characteristics of the microstructure of the RBM implants and to compare it with the surface of the machined implants was performed on a scanning electron microscopy. (× 40, 100, 1000, JSM-840A, JEOL, Japan)

Microsurface structure(of 2 implants of each material from each group) was measured by white-light scanning interferometry for three dimensional surface roughness measurements: Acurra 2000(INTER PLUS, Korea). In 2 samples from each of the 4 groups, 3 threads were selected randomly and scanned along their circumference in 4 different area, yielding 12 measurements for each surface topography. Three height parameters, R_a, R_q, and R_z, were used for quantitative characterization of the surface roughness. R_a describes the arithmetic mean of departures of the roughness profile from the midline., R_q is the root mean square parameter corresponding to R_a, and R_z measures the average height difference between the 5 highest peaks and the 5 lowest valleys.

4. Resonance frequency measurement

Resonance frequency analysis (RFA), a novel technique for the clinical measurement of implant stability and osseointegration, was presented by Meredith et al.[15] This method is non-invasive technique that measures the implant stability in terms of interfacial stiffness (Hz). The frequency response of the system was measured by attaching the transducer, i.e. an L-shaped cantilever beam, to a screw implant. The excitation signal was a sine wave varying in frequency from 5kHz to 15kHz with peak amplitude of 1 volt. The first flexural (bending) resonance frequency of the resulting system was measured.[16,17] The resonance frequency was measured immediately after implant placement (20 rabbits) and at sacrifice six weeks later (19 rabbits).

5. Removal torque

To test the implant stability, specifically the strictly interfacial shear strength, removal torque was measured at the time of animal sacrifice, i.e. six weeks after implantation. According to Roberts et al, in rabbits it takes 6 weeks for the woven bone to be replaced by lamellar bone with adequate strength for load bearing.[18]

The small diameter hex top was 0.7mm and too short to measure. Using resin cement (Panavia 21, Kuralay, Japan), small diameter fixture mount was attached (Fig. 3) and the force needed to unscrew the implants (n=72, 18 rabbits) was measured using a digital torque gauge (Model MGT12, Mark-10 Corp., 458 West John Street Hicksville, NY 11801 USA) It has the measuring torque range of 135Ncm and the accuracy of 0.5% of full scale of 1 digit, i.e. 0.3ozin (about 0.2Ncm) The implants were subjected to slowly increasing torque until loosening was detected, and the peak torque value was measured.

6. Histologic preparation of specimens

The remaining two animals were sacrificed without subjecting the implants to removal torque, and the specimens (including implants; n=4) and surrounding tissues were washed in saline solution and fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.15mol/L cacodylate buffer at 4℃ and pH 7.4. The specimens were further dehydrated in ascending concentrations of alcohol rinses and infiltrated with glycolmethacrylate resin (Technovit 7200 VLC, Kulzer & Co, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally at about 100㎛ and ground to a final thickenss of about 25㎛ (EXAKT 310, GMBH & Co, Germany) as described by donath[19]

One section was obtained for each implant and stained with hematoxylin and eosin. The histomorphometric analysis was performed using a light microscope connected to a personal computer. The percentage of bone to implant contact around threads, which engaged only in cortical plate was observed with 40 magnification power of light microscope. No measurement was taken from the cancellous bone area in the rabbits, because not much of significance was there to measure the percentage of contact in hollow marrow space.

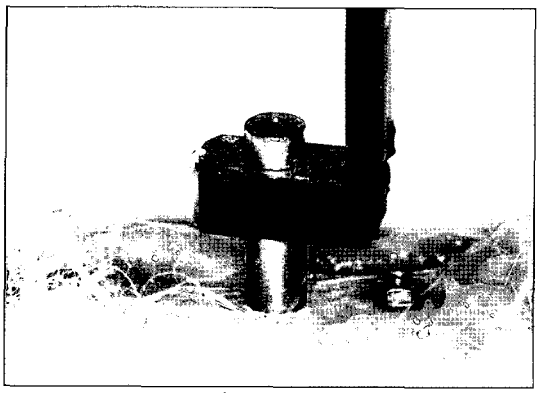


Fig. 3. Transducer attached to implant which placed into rabbit tibia.

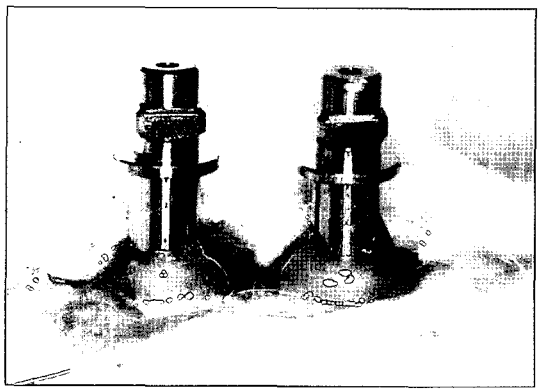


Fig. 4. Small diameter fixture mount was attached to the implant. Without this procedure, the hex top of implant would be worn-out.

7. Statistical analysis

Mean value of resonance frequency analysis were calculated and subjected to a repeated measure ANOVA to test for significant differenced between 4 investigated groups. Scheffe's test between all groups was perfomed for removal torque evaluation. Statistical testing was carried out at the 5% significance level.

RESULTS

1. Topographic Evaluation

The scanning electron microscopy analysis and surface roughness measurements demonstrate the different surface topographies between the surface of the RBM implants and the turned implant. The surface of the RBM implants as seen with SEM had a rough and irregular pattern, while the turned specimens showed relatively smooth surface. (Fig. 6) At higher magnification, the surface appears reticulated, with undermining deformation of the metal remaining after impaction of the resorbable hydroxyapatite material blasted under pressure on the

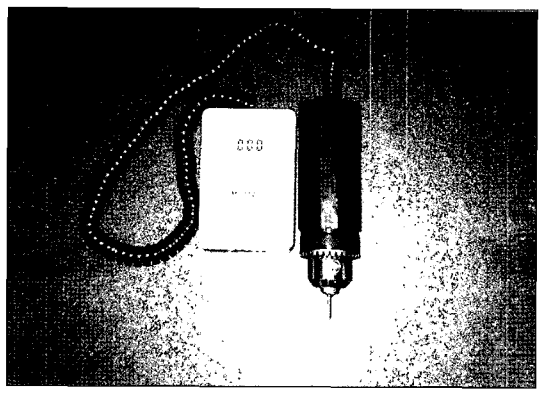


Fig. 5. Digital torque gauge for removal torque measurement.

surface of the implant (Fig. 6, B3, ×1000)

The Brånemark clone implant with turned surface showed an average surface roughness 0.26μm; corresponding values for newly designed implant with RBM surface were 1.12μm.

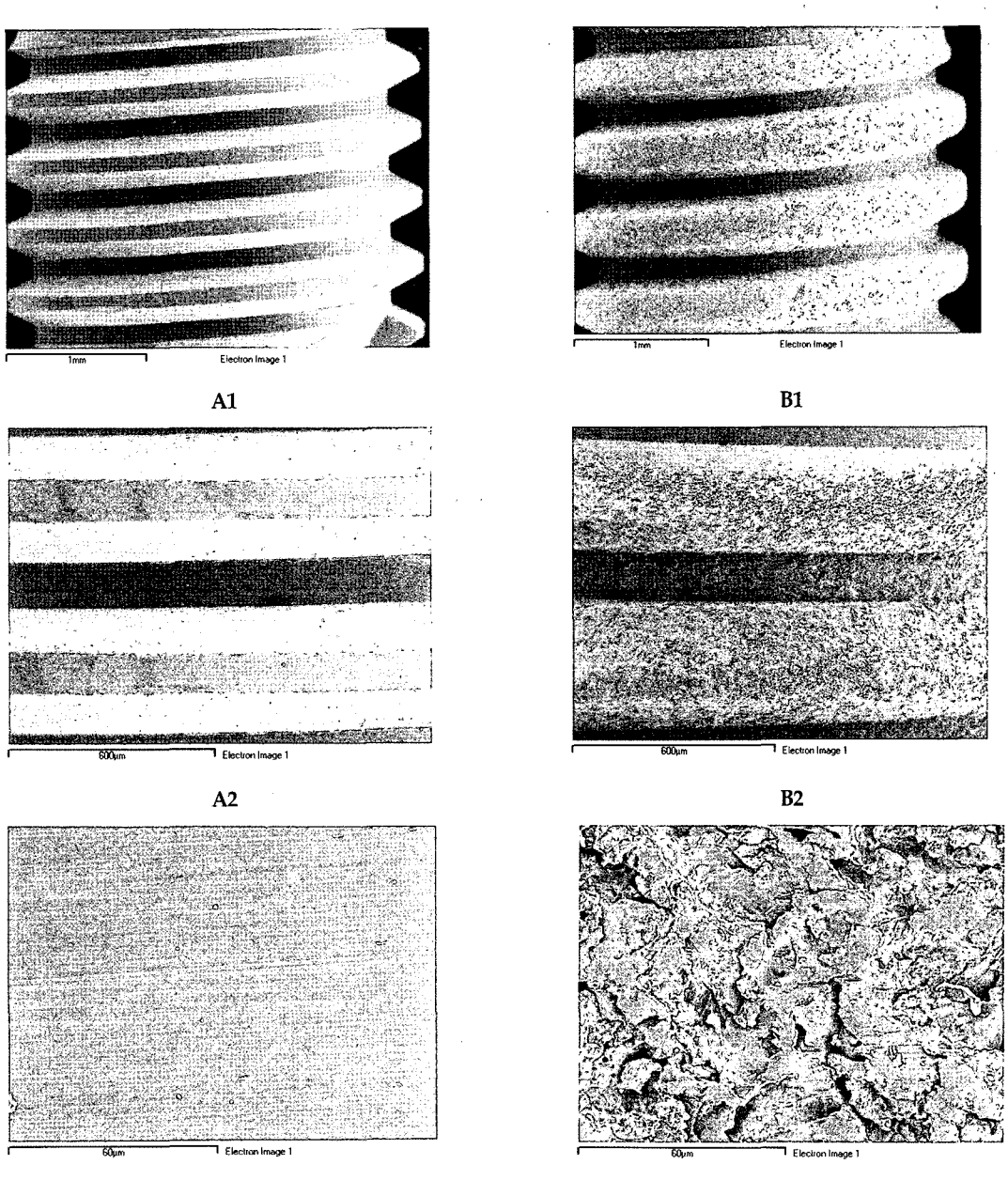


Fig. 6. Scanning electron microscopy of custom-made implants. (A1) Implant with machined surface ×40 mag. (A2) Grooved pattern on machined surface ×100 mag. (A3) smooth turned surface ×1000 mag. (B1) Implant with RBM surface. It has coarse roughness ×40 mag. (B2) ×100 mag. (B3) At higher magnification, the metal presents a uniform reticulation (×1000)

2. Resonance frequency analysis (RFA)

After initial implants installation, all implants of 4 groups were measured by connecting transducer to implant and orienting it parallel to long axis of implant. The mean RFA values were 68.9 for group A implants, 66.2 for group B implants, 70.2 for group C implants, 65.9 for group D implants. The mean RFA values six weeks after implant insertion were 70.8±4.3 for group A implants, 71.8±3.1 for group B implants, 70.9±2.5 for group C implants, and 72.7±2.5 for group D implants. There were no statistical significant differences between groups.

3. Removal torque measurement

Six weeks after implant placement, the average removal torque was 49.99±6.70Ncm for group A implants, 51.15±4.40Ncm for group B implants, 50.78±9.37Ncm for group C implants, and 51.09±4.69Ncm for group D implants. The removal torque results are summarized in Table II. The torque

Table I. Mean surface roughness(in μm)

Group	R_a(SD)	R_q(SD)	Rz(SD)
Group A (Brånemark clone, turned)	0.26	0.31	1.13
Group B (Brånemark clone, RBM)	1.02	1.18	4.53
Group C (New design, turned)	0.29	0.34	1.24
Group D (New design, RBM)	1.12	1.26	4.36

Table II. Mean values of RFA after six weeks of healing time.

Group	Group A	Group B	Group C	Group D
Mean RFA	70.8	71.8	70.9	72.7
SD	4.3	3.1	2.5	2.5

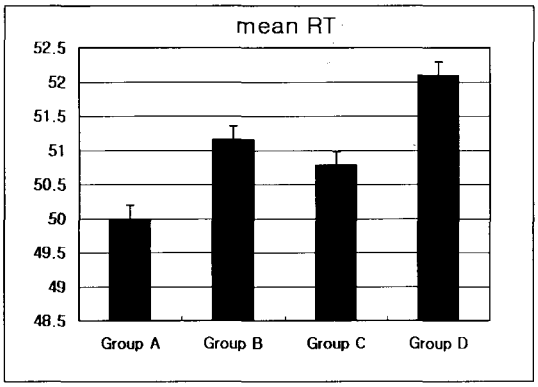


Fig. 8. Torque values for implants inserted in the tibia of rabbits 6 weeks (n=17)

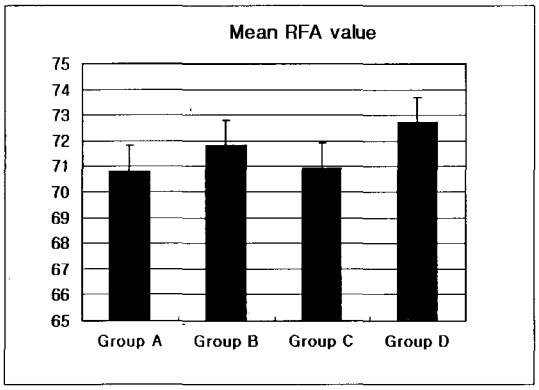


Fig. 7. Mean values of the resonance frequency measurements (Hz) after six weeks of healing time. (n=19) There were no statistically significant differences between the groups.

Table III. Torque values after 6 weeks (n=17)

Group	Group A	Group B	Group C	Group D
Mean RT	49.99	51.15	50.78	52.09
SD	6.70	4.40	9.37	4.69

measurements yielded no statistical significance. The highest removal torque corresponded to the newly designed implant with RBM surface, while the lowest was demonstrated by the turned, Brånemark clone implants. Higher torque was needed to unscrew RBM surface implants compared to the torque needed for the turned, Brånemark clone implants.

4. Histomorphometric evaluation

A typical cross-section showed the triangular-shaped tibia and a central bone marrow cavity with the implant inserted through the superior cortex. On the microscope, all 8 implants were well integrated into bone and a mature cortical bone surrounded the implants. The implants were in contact with cortical bone layer exclusively along the upper threads in the cortical region, while the threads in the bone marrow were in contact with much of mar-

Table IV. Percentage of bone-to-implant contact 6 weeks after Implant Placement

Rabbit No.	Group A	Group B	Group C	Group D
1	45	52	49	55
2	46	58	50	60
Mean	45.5	55	49.5	57.5
SD	0.5	3	0.5	2.5

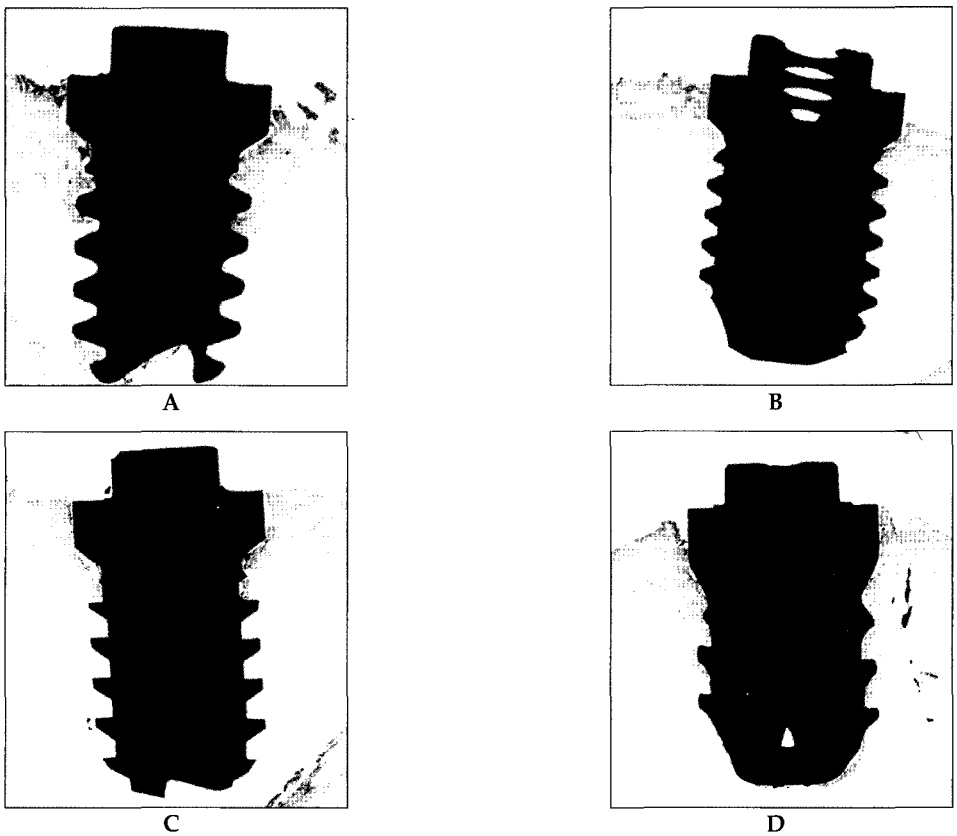


Fig. 9. 25micron ground sections of 4 implants from each group. All implants were well integrated into bone. (A) Turned Brånemark clone implant as Group A, (B) Brånemark clone implant with RBM surface as Group B, (C) Newly designed implant with turned surface as Group C, (D) Newly designed implant with RBM surface as Group D. Note newly formed bone in the marrow space. Note the bone formed at the implants body.

row tissue. Some new bone formation was observed only newly designed with RBM surface in contact with marrow space. Qualitative histologic differences among the remaining 3 groups were not seen.

Results showed a mean percentage of bone-to-implant contact was 45±0.5% for the turned Brånemark clone implants, 55±3% for the Brånemark clone implants with RBM surface, 49.5±0.5% for the turned newly designed implants, and 57.5±2.5% for the newly designed implants with RBM surface. The histomorphometric analyses were summarized in Table IV.

DISCUSSION

The quality of the implant surface is one of the six factors described by Albrektsson[13] et al which influence the healing at the implantation site and subsequently affect the osseointegration. The roughness of the implant surfaces favors distribution of stress, retention of implants in the bone, and bone response with bone trabeculae growing in a perpendicular direction to implant surface.[20,21] Studies have reported that adequate growth of bone in the interior of the pores or cavities left by the surface treatment requires that these must be approximately 100μm in size. The growth of bone tissue into cavities of this size allows a mechanical interlocking of the implants with bone.[22] Wong et al.[20] have reported that bone matrix may be deposited in pore sizes of only 1-2μm, which resulted in an increase in push-out force. Analysis of RBM implants using scanning electron microscopy revealed rough surface and pore size of (2.5-4 μm). This allowed bone growth into these pores so that mechanical interlocking of the implant with bone was possible and resulted in an improved bone -implant interface as described by other articles.[23-27]

Implants with RBM surface had higher value than control on RFA measurement and histomorphometric evaluation. These implants also achieved higher degree of bone-to-implant contact compared to turned implants. And newly designed implant with RBM surface had new bone formation around bone marrow space contrary to implants of other groups. This result is thought to arise from the novel design and the RBM surface. In vitro studies have also shown that the superficial roughness of the materials can influence cell function, matrix deposition, and mineralization.[28-30] In a series of studies, Wennerberg et al, systematically investigated the effect of surface roughness of implants and the response in rabbit bone.[31-34] And the authors concoluded that implants with a surface roughness of Ra 1 to 1.5μm seemed to be at an optimal roughness with regard to retention in bone as well as bone-to-implant contact as measured by histomorphometry. And this obervation is also in accordance with observations by von Recum & van Kooten[35] that reported excellent tissue attachment without signs of inflammation when implanting filter membranes with pore size of 1-3μm. Based on these results, it seems that the benefit of increasing roughness on a micrometer scalereaches a maximum level between 1.0 to 1.5μm. The Ra value of RBM treated implants was within this limits seemed to have resulted in favorable bone response.

Irrespective of whether resonance frequency analysis (RFA) or removal torque (RT) was used, the biomechanical data showed the similar results. Newly designed implant with RBM surface implants had the highest value on both experiments but not much statistically significant difference. Both new design and surface characteristics of the implant enabled it. The newly designed of dental implant with half-rounded shape at the apex, flatness at the top of the threaded surface, and 45 degrees of reverse bevel at the bottom surface set the stress to the apex of the dental implant during lateral or oblique load. Forcing the stress to the top part rather than the apex part of the dental implant is not highly recommended, because there is much accumulation of stress at the top part of the dental implant. The cortical bone which integrate with the top part of the dental implant is unable to disperse the attained stress

causing the concentration of stresses at the referred area. The dental implants, which took part in the experiment, are much capable of dispersing the stress especially to the direction of apex compared with general threaded types or other models of dental implant.[30]

RT and RFA are well-documented biomechanical techniques that evaluate the stability and stiffness of the osseointegrated implant interface. Removal torque evaluated the biomechanical bond between implant and the bone. Several studies have reported that RT techniques offer reliable test-to-control comparisons.[12,36] RFA is a recently introduced non-invasive test method and this technique has been utilized in a number of experimental tests to record a change in implant stiffness.[17,31,37-40] RT and RFA are quantitative method for assessment of implant stability and osseointegration and also enable us to compare different implant system, such as changes in implant geometry or surface modifications.

CONCLUSION

1. The surface of RBM implants as seen with SEM had a rough, irregular pattern than that of the turned implants that seemed to have a smoother surface. At higher magnification, the RBM surface showed reticular pattern.
2. Ra values for machined implants were 0.2-0.3μm; corresponding values for newly designed implants with RBM surface were 1.0-1.2μm.
3. The average removal torque was 44.99$\pm$6.70Ncm for group A implants, 51.15$\pm$4.69Ncm for group B implants, 50.78$\pm$9.37Ncm for group C implants, 51.15$\pm$4.40Ncm for group D implants. There was no statistically significant difference.
4. The mean RFA values were 70.8 for group A implants, 71.8 for group B implants, 70.9 for group C implants, 72.7 for group D implants. There was no statistically significant difference.
5. A mean percentage of bone-to-implant contact was 45$\pm$0.5% for group A implants, 55$\pm$3% for group B implants, 49.5$\pm$0.5% for group C implants, 57.5$\pm$2.5% for group D implants. The light microscopic picture demonstrated implants with RBM surface had higher values with respect to bone-to-implant contact and group D implants showed new bone formation around implant body.

Within the limits of this 6-week experimental study, it may be stated that the overall pattern of newly designed implants with RBM surface resulted in significantly higher percentage of bone-to-implant contact and comparable removal torque and resonance frequency measurement when compared to machined, Brånemark clone implants. And the present investigation revealed the fact that RBM surface treatment has some advantage over turned surface in bone-to-implant contact, removal torque and resonance frequency measurement.

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Reprint request to:
DR. YUNG-SOO KIM, D.D.S., M.S.D., Ph.D.
DEPT. OF PROSTHODONTICS, COLLEGE OF DENTISITY,
SEOUL NATIONAL UNIV.
28-1 YEONGUN-DONG, CHONGNO-GU, 110-749, SEOUL KOREA
yungskim@snu.ac.kr