BONE RESPONSE OF THREE DIFFERENT SURFACE IMPLANTS: HISTOMORPHOMETRIC AND RESONANCE FREQUENCY ANALYSIS IN DOGS

Woo-Seok Song, D.D.S., Yung-Soo Kim, D.D.S., M.S.D., Ph.D., M.Sc(O.S.U.), Chang-Whe Kim, D.D.S., M.S.D., Ph.D., Kyung-Soo Jang, D.D.S., M.S.D., Ph.D., Young-Jun Lim, D.D.S., M.S.D., Ph.D.

Department of Prosthodontics, Graduate School, Seoul National University

Statement of problem. Reducing treatment time in implant dentistry is a matter of main concern. There are so many factors affecting the success rate of immediate or early loaded implant for the initial bone response. The especially microscopic properties of implant surfaces play a major role in the osseous healing of dental implant.

Purpose. The aims of this study were to perform a histologic and histomorphometric comparison of the healing characteristics anodically roughened surface, HA coated surface and RBM surface implant, and to compare of ISQ values measured by Osstell™ for resonance frequency analysis in dogs mandible during 2 weeks.

Material and method. Bone blocks from 2 dogs were caught after covered healing for 0 day(2 h); Group I, 1 week; Group II and 2 weeks; Group III. One longitudinal section was obtained for each implant and stained with hematoxylin and eosin. Histomorphometric analysis was done with Kappa Imagebase system to calculate bone-to-implant contact and bone volumes inside the threads. ISQ values were measured in every time of surgery schedule.

Conclusion.

The experiment revealed that:

- 1. The percentages of bone-to-implant contact on the fixture in each group were not significantly different (P > 0.05).
- 2. The percentages of bone area inside the threads on the fixture in each group were not significantly different (P > 0.05).
- 3. The ISQ level showed clinical stability of each fixture during 2 weeks(all ISQ level \geq 71).

Key Words

Surface, Histomorphometry, ISQ, RFA, Anodic oxidation, RBM, HA

Dental implant based on the original work by Brånemark et al¹⁴ has been documented widely and proven to be a very reliable treatment choice in the

restoration of edentulous jaws.

Reducing treatment period in implant dentistry is a matter of main concerns. There are so many factors affecting the success rate of immediate or early loaded implants. And the macroscopic and microscopic features of implant surfaces have been described as one of the major factors of osseointegration.⁴ The osseous healing characteristics of various surface structures have been investigated in several experimental and clinical studies.⁵⁻⁷

The implant surfaces and types most frequently described in the literature and made may be subdivided into implants with roughened surfaces by coating[e.g. resorbable blast media (RBM), hydroxyappatite coated (HA)], implants with roughened surfaces with electrochemical modification (anodic oxidation) of commercially pure titanium implants and implants with roughened surfaces without coating [e.g. sand-blasted or acid-etched]. These surfaces were known to promote initial healing capacity by rougheness.⁸⁻¹⁰

The evaluation of the clinical outcome of implant therapy has mainly been made through clinical and radiographic examinations, which have been aimed at locating failed implants and ongoing periimplant bone resorption. However, with resonance frequency analysis(RFA), it is now possible to measure the degree of implant stability at any time during the course of implant treatment and loading.11 The technique is now commercially available as Osstell™ (Integration Diagnostics, Savedalen, Sweden) and a new unit to describe implant stability quotient(ISQ). ISQ is recorded as a number between 1 and 100, 100 is representing the highest degree of stability. Each transducer is calibrated by the manufacturer, which makes all measurements directly comparable.

The aims of this study were to perform a histologic and histomorphometric comparison of the healing characteristics of anodically roughened surface (TiUnite, Nobel Biocare, Göteborg, Sweden), HA coated surface (Replace select, Nobel Biocare, Göteborg, Śweden) and RBM surface (Kimplant, Avana®, Osstem, Busan, Korea), and to compare of ISQ values measured by Osstell™ for reso-

nance frequency analysis in dogs mandible during 2 weeks.

MATERIALS AND METHODS

Fixtures (Fig.1)

- RBM surface (Kimplant* , Avana®, Osstem, Busan, Korea)
 Diameter 4.0mm × 10mm straight form (* : project name)
- 2) Anodically roughened surface (TiUnite**, Nobel Biocare, Goteborg, Sweden) Diameter 4.0mm × 10mm MK III
- 3) HA coated surface (Replace Select**, Nobel Biocare, Goteborg, Sweden)

 Diameter 4.3mm × 10mm straight form

 (**: commercially available)

Experimental animals and Anesthesia

Two adult healthy beagle dogs weighing 10Kg were used. One is adult female(dog1) and another is adult male(dog2).

The dogs were anesthetized with combination of ketamine10mg/kg (Yu-han, Gunpo, South Korea) and Rompun 0.15mL/kg (Bayer Korea, Ansan, South Korea) intramuscularly. The partially extracted mandibles were prepared for the sites of fixtures and washed and decontaminated with Betadine (Sung Kwang, Buchun, South Korea) and prior to surgical draping. One milli-

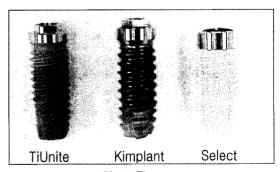


Fig. 1. Fixtures.

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	Day 0	1 Week	2 Weeks
Dog1	Installation		
Group I	ISQ		
Both	Biopsy		
Dog2	Installation		
Group II	ISQ	ISQ	
Right		Biopsy	
Dog2	Installation		
Group III	ISQ	ISQ	ISQ
Left			Biopsy

liter of 2% lidocaine (Yu-han; 1:100,000) was administered local to the implant sites.

Surgical procedures and implant placement

A controlled surgical technique was used to place 9 implants (Dog1; 6 fixtures/ Dog2; 3 fixtures). Using sterile surgical techniques, an incision was made in the mucosa to expose alveolar bone, and the mucoperial steal flap was elevated. The implant site was drilled in usual manner, by using drills of each own system with increasing diameters under constant irrigation with sterile saline.

The order of installation was TiUnite-Kimplant-Select (from the anterior site).

Group I : Fixtures submerged during 2 hours (0 day)

Group ■ : Fixtures submerged during 1 week Group ■ : Fixtures submerged during 2 weeks

ISQ measurements

ISQ measuring by Osstell™ (Integration Diagnostics, Sävedalen, Sweden) was done on each schedules of experiment.(Table. I) Each transducer is calibrated by the manufacturer, which makes all measurements directly comparable. In this study, transducer "Type F13 L5;100132" for Replace select and "Type F1 L 8.5; 10050" for external hexagon(Kimplant and TiUnite) were used.

Preparation of specimens and histomorphometric analysis

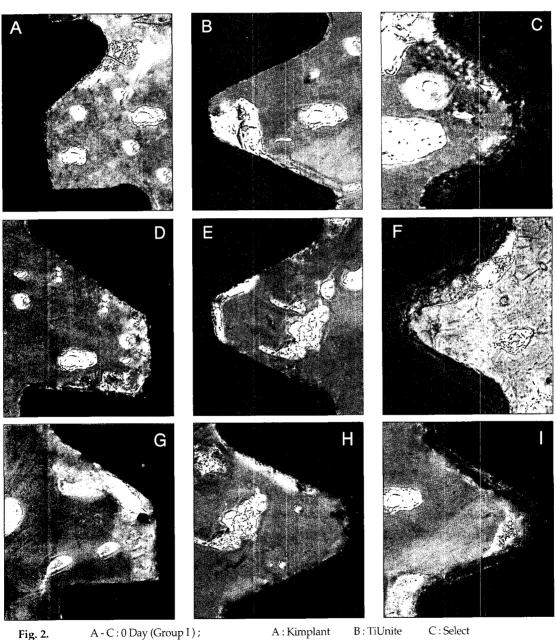
Every fixture was removed from the mandible as a form of block. The specimens and surrounding tissues were washed in saline solution and fixed in 4% paraformaldehyde and 0.1 % glutaraldehyde in 0.15mlo/L cacodylate buffer at 4°C and pH 7.4. The specimens were further dehydrated in ascending concentration 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 µm and ground to a final thickness of about 25 μm (EXAKT 310, GMBH & Co, Germany) as described by donath.12 One section was obtained for each implant and stained with hematoxylin and eosin.. In histomorphometric analysis, Kappa imagebase system was used. To calculate boneto-implant contact ratio, first we draw the connection of straight line along the fixture surface and then draw the line that was thought to be the junction of bone and fixture surface (line tool). Each point of continuous line was numbered in order $(1, 2, 3\cdots)$ and the ratio was calculated by Microsoft Excel. In calculation bone volumes inside threads, polygonal tool of system was used, which connected the boundary line of bone, vacant space, and threads. And then the ratio was calculated by Microsoft Excel.

Statistics

Mean values of bone contact ratio and bone area in each group were calculated and subjected to a repeated measure ANOVA to test for significant differences. Statistical testing was carried out at the 5% significance level.

RESULTS

All nine implants in bone blocks were analyzed. No implant exhibited clinical mobility. Further the mucosa exhibited only minor signs of inflammation with no pus discharge or severe



D-F:1 Week (Group II);

G-I: 2 Weeks (Group III);

D: Kimplant G: Kimplant E: TiUnite

H: TiUnite

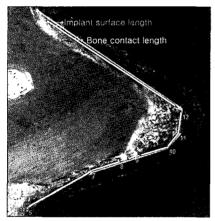
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inflammation(uneventful). But the post-operative wound healing of the all implants showed dehiscence by incision for measuring ISQ.

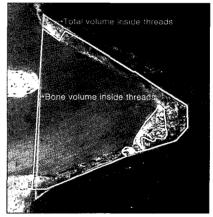
1. Histological finding

Fig. 2 A-I illustrated a ground-section of an implant with surrounding hard tissues from biopsy sampled. The peripheral portions or the

pitches were in close contact with the surrounding bone tissue, and thus hereby mechanical stability for the implant during the initial phase of wound healing was provided. In specimens, the various cells within this coagulum could be identified (Arrow;A-C; Group I). The gap between thread and bone of 1 week and 2 weeks specimens were more close contact than that of 0 day specimens.



A. Bone to implant contact ratio



B. Bone area inside threads

Fig. 3. Histomorphometric evaluation of bone-to-implant contacts and bone area inside threads : using Kappa Imagebase program.

Table II. Results from histomorphometry

A. Total Mean value of every pitch from each fixtures

(Kimplant n=12 / TiUnite n=14 / Replace Select n=13)

Group	Fixture	Bone Contact(%)		Bone Area (%)	
		Mean	SD	Mean	SD
	Kimplant	63.8	13.3	73.7	5.4
I	TiUnite	63.5	12.5	73.4	5.6
	Select	69.8	11.3	79.1	4.9
	Kimplant	67.9	9.5	79.0	6.7
H	TiUnite	68.0	8.4	77.9	5.6
	Select	70.0	7.9	79.6	6.9
Ш	Kimplant	71.9	10.2	81.3	4.5
	TiUnite	71.9	9.6	81.8	4.1
	Select	71.4	9.5	81.4	4.0

At group I, II and III: not significantly different at P<0.05 (ANOVA)

B. Data from best 4 bone response threads (n=4)

Group	Fixture	Bone Contact(%)		Bone Area (%)		
		Mean	SD	Mean	SD	
	Kimplant	65.1	6.1	75.7	5.6	
I	TiUnite	65.0	5.3	76.2	6.3	
	Select	71.5	4.3	80.9	5.3	
	Kimplant	68.9	6.4	79.7	4.5	
II	TiUnite	68.1	6.1	78.1	4.3	
	Select	70.2	5.8	80.0	5.1	
	Kimplant	72.9	8.3	82.1	3.8	
Ш	TiUnite	72.2	7.6	81.9	3.2	
	Select	72.4	7.9	81.9	3.4	

Table III. The highest values observed in each group

	Bone-to-implant contact	Bone area	
Group I	Replace Select (75.3%)	Replace Select (84.0%)	
Group II	TiUnite (75.6%)	Kimplant (84.3%)	
Group III	TiUnite (77.1%)	Kimplant (84.7%)	

Table IV. ISQ measured by Osstell[™] (≥71)

Group	Fixture	0 Day	1 Week	2 Weeks
	Kimplant	72		
I	TiUnite	72		
	Select	73		
	Kimplant	71	73	
П	TiUnite	74	75	
	Select	<i>7</i> 5	76	
	Kimplant	72	73	75
Ш	TiUnite	72	74	75
	Select	71	72	75

2. Histomorphometric analysis

Bone-to-implant contact ratio and volume of the bone engaged between threads were calculated(Fig. 3).

3. Resonance frequency analysis (Table IV)

DISCUSSION

In this study, no significant difference was found from histomorphometric analysis between Kimplant and other commercially available implants. In conclusion Kimplant were developed successfully in comparison with the results of commercially used TiUnite and Replace Select in 2 weeks study.^{13,14} In addition, it could be

guess that three different implants may be response to bone excellent enough to satisfy immediate or early loading(within 2 weeks) in clinical situation.

In the present study, when implants were installed, their own drill system was used. In case of Kimplant, screw tap in developing was used for its own screw shape of fixture. If screw tap would be more precisely developed, it could be expected that bone response of this newly developed fixture will be more excellent.

Highest values of each experiment are showed on table III. In group I, Replace Select showed the highest values in BCR and BVR. The diameter of Replace select is 4.3mm, which is larger than that of TiUnite and Kimplant. The difference in diameter may affect the results of this study. In addition, the initial bone response of hydroxyappatite coated surface is prospected well from other study.15 In group II and III of bone-toimplant contact, one of the pitches in TiUnite specimen shows the best scores. In group II and III of bone volumes inside the threads, one of the pitches in Kimplant specimen shows the highest score(84.3% and 84.7%), but the second sore was Replace Select (84.1% and 84.5%) and the difference between fixtures were minimal.

It could be concluded that from all difference tendency of this study, the variation of surgical situation(e.g. bone quality, bone quantity, insertion torque, heat generation etc) may be one of the major factors to estimate clinical success.

However, no studies describing "normal" ISQ levels have been published. By P. Balleri et al(2002) an ISQ level of 69 (range of 57~82) may describe the stability of a fully integrated implant. In this study, every measured ISQ level was more than 71 and then all the fixtures were thought to be stable.¹⁴⁻¹⁸

In this study, implants submerged without loading during 2 weeks were analyzed. In other study, 6 weeks analysis in dog was often used. But

also during 2 weeks it could be seen that in some pitch regions which were responsible for primary mechanical stability, the bone tissue exhibited signs of ongoing bone remodeling, resorption and apposition. In further study during longer period the surface reaction by woven bone formation with lamellar bone maturing should be evaluated.

The results are comparable with the histomorphometric measurements in previous study in spite of different experimental designs. ²¹⁻²⁶ It is well known that rapid ingrowths of bone into the threads during the initial healing period make sure better stability of the implants after 1st surgery and also contribute to better long-term success. ²⁷⁻³¹ However, the clinical implications of the exact healing time and mechanisms by the surface properties of implant remain subjects for further study.

CONCLUSIONS

With the limitation of this study, the experiment revealed that:

- 1. The percentages of bone-to-implant contact on the fixture in each group were not significantly different(P > 0.05).
- The percentages of bone area inside threads on the fixture in each group were not significantly different(P > 0.05).
- The ISQ level measured by OsstellTM showed clinical stability of each fixture during 2 weeks (all ISQ level ≥71).

REFERENCES

- Brånemark PI. Osseointegration and its experimental background. J Pros Dent 1983;50:399.
- Brånemark P-I, Hansson BO, Adell. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand Reconstr Surg 1977;11(suppl 16).
- Roos J, Sennerby L, Albrektsson T. An update on the clinical documentation on currently used bone-anchored endosseous Implants. Dent Update 1997;24:194-200.

- Albrektsson T, Zarb GA, Worthington P, Eriksson AR. The long-term efficacy of currently used implants. A review and proposed criteria of success. Int J Oral Maxfacial Impl 1986 I:11-25.
- Davies JE et al. The bone-titanium interface in vitro. J Biomedical Materials Research 1990;24;1289-1306.
- 6. Hazan R et al. Bone growth to metal implants is regulated by their surface chemical properties. Biomaterials 1993;14:570-574.
- Weinlaender M et al. Histomorphometry of bone apposition around three types dental implants. Int J Oral Maxillofacial Impl 1992;7:491-496.
- Ericsson I, Johansson CB et al. A histomorphometric evaluation of implant contact on machined-prepared and roughened titanium dental implants Clin Oral Impl Res 1994;5:2002-206.
- 9. Larsson C, Thomsen P. et al. Bone response to surface modified Ti implants: studies on the early tissue response to machined and electropolished implants with different oxide thicknesses. Biomaterials 1996:17(6):605-615.
- Sul YT, Johansson CB, Jeong YS, Wennerberg A, Albrektsson T. Resonance frequency and removal torque analysis of implants with turned and anodized surface oxides. Clin Oral Impl Res 2002:13:252-259.
- Meredith N, Alleybe D, Cawley P. Quantitative determination of the stability of the implant-tissue interface using resonance frequency analysis. Clin Oral Impl Res 1996;7:261-267.
- 12. Donath K. Die Trenn-Dünnschliff-Technik zur Herstellung histologischer Präparaten von nichti schneidbaren Geweben und Materialien. Der Präparator 1988;34:197-206.
- Boyan BD, Hummert T, Kieswetter K. Role of material surfaces in regulating bone and carilage cell response. Biomaterials 1996:17:137-146.
- Kim YS. 3-D finite element study on surface characteristics and stress of implants. SNUDH Dept of Pros. Unpublished.
- 15. Simunnek A. Evaluation of stability of titanium and hydroxyappatite coated osseointegrated dental implants: a pilot study Clin Oral Impl Res 13, 2002:75-79.
- Friberg B, Sennerby L, Meredith N, Lekholm U. A comparison between cutting torque and resonance frequency measurements of maxillary implants. A 20 month clinical study. Int J Oral Maxillofac Surg 1999;28:297-303.
- Sennerby L, Roos J. Surgical determinants of clinical success of osseo-integrated implants. A review of the literature. Int J Pros 1998; 11:408-420.
- Sennerby L, Roos J. RFA: Measuring implant stability and osseointegration. Compendium 1998:19;493-502

- Sennerby L, Friberg B, Linden B, Jemt T, Meredith N. A comparison of implant stability in mandibular and maxillary bone using RFA. European Commission Demonstration Project. RFA Symposium. 2000 Sweden.
- CJ Park. A study on the change of implant stability using resonance frequency analysis. J Korean Academy of Prosthodontics 2003;41:271-287.
- Albrektsson T et al. Histologic investigations on 33 retrieved Nobelpharma implants. Clin Materials 1993;12:1-9.
- Rocci A et al. Histology of retrieved immediately and early loaded oxidized implants: Light mocroscopic observations after 5-9 months of loading in the posterior mandible. Clin Impl Dent Related Res 2003;5(I): 88-97.
- Berglundh T et al. De novo alveolar bone formation adjacent to endosseous implants: A model study in the dog. Clin Oral Impl Res 2003;14:251-262
- Botticelli D et al. Appositional bone formation in marginal defects at implants: A experimental study in the dog. Clin Oral Impl Res 2003:14; 1-9.
- 25. Zechner W. et al. Osseous healing characteristics of three different implant types: A histologic and histomorphometric study in mini-pigs. Clin Oral Impl Res 2003;14: 150-157.
- YH Kim, JY Koak, IT Chang, SJ Heo. A histomorphometric analysis of the effect of various surface treatment methods on osseointegration. Int J Oral Maxillofacial Impl 2003;18(3):349-356.
- Albrektsson T et al. Bone metal interface in osseointegration. J Pros Dent 1987; 57:597-607.
- Collier JP et al. Macroscopic and microscopic evidence of prosthetic fixation with porous coated materials. Clin Orthop 1988;235:173-180.
- Pilliar RM. Porous-surfaceed metallic implants for orthopedic applications. J Biomed Material Res 1987;21:1-33.
- Sun L. Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings. A review J Biomed Materials Res 2001: 58: 570-592.
- Tal H. Spontaneous early exposure of submerged implants. II. Histopathology and histomorphometry of non-perforated mucosa covering submerged implants. J Periodontol 2000:71:1231-1235.

Reprint request to:
Chang-Whe Kim
Department of Prosthodontics, Graduate School,
Seoul National University,
28, Yeongun-Dong, Chongno-Gu, Seoul, 110-744, Korea k43c46w@dentistry,snu.ac.kr