<Original Article>

Bone Response to Anodized Titanium Implants in Rabbits

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• Abstract

Purpose: The quality of implant surface is one of the factors that influence wound healing of implant site and subsequently affect osseointegration. The objective of modification of the surface properties of an implant is to affect the biological consequence. The purpose of this study is to evaluate the biologic response of osseous tissue to anodized implants.
Materials and Methods: Two machined titanium implants for control group were installed in a tibia of each rabbit and two anodized implants for test group were installed in the other tibia of each rabbit. At the moment the implants were installed, resonance frequency analysis (RFA) values were measured. After healing periods of 1, 2, 3, and 7 weeks, the implants were uncovered and RFA values were measured again. Removal torque was measured for one implant in the test group and one implant in the control group. Histological evaluation was executed in the other implants.
Results: Both of test group and control group have the tendency of greater RFA change rate and removal torque value as healing periods became longer, but were statistically insignificant ($P>0.05$). However, in the case of the same healing period, the test group tended to have greater RFA change rate and removal torque than the control group ($P<0.05$). More active new bone formation from endosteal surface was noted on the anodized surface than machined surface in specimen after 1 week. There were no significant differences between the test group and control group in histological evaluations.
Conclusion: In summary, the anodized surface showed slightly favorable results and it is postulated that it may facilitate improved stability in bone.

• Key word : Implant, Resonance frequency analysis, Removal torque, Bone regeneration

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Introduction

Since the concept of osseointegration was introduced to dentistry, the relevant fields of dental implants have seen remarkable development. It is not a controversy any more in the field that implant as an abutment for dental restorations guarantees a better long-term prognosis. Now, many researchers and clinicians in the implant-related area are focusing on how fast osseointegration can be achieved, how fast patients recover function and aesthetics, as well as whether high success rate remains regardless of bone tissue.

Now, osseointegration became a predictable process and it is influenced by various factors. The variables related to implant include implant material, design and surface characteristics; the variable related to patients include bone tissue, medical condition, whether they smoke, previous radiotherapy and parafunctional habits. Surgical technique and practitioners' experience can additional factors affecting surgical success[1].

It is well known that the biological response of bone tissue to implant surface is closely related to various factors of the implant's surface such as morphological and physical characteristics, and chemical components[2]. Bone tissue response to various implant surfaces has great clinical significance; if the response is good, success rate of implantation can be increased and the period between implant placement and prosthesis completion can be reduced, increasing satisfaction of patients. To that end, researchers have made continued efforts to advance clinical results of implant treatment by improving the surfaces of implants[3,4]. Such improvements include: mechanically polishing the surface of titanium, the most frequent implant material, or roughening the surface of titanium by sandblasting, grit blasting, plasma sprayed coating or the addition of fine particles, followed by the formation of a highly reactive titanium oxide layer on the titanium surface. This layer is a dynamic interface, which induces deposition and growth of bone matrix. The initial thickness of the oxide layer is 2-6 nm; however, it is thickened more than 2~3 times by the subsequent interaction between bone and implant; and continues to change when imposing load[5]. The chemical composition of oxide layer can be changed by thickness, or addition of calcium, phosphorus and/or sulfur[6].

Sul et al.[3,7] reported that the surface shows no qualitative difference if the oxide layer has a thickness of 200 nm or less; if the layer has a thickness of 600 nm or above, a porous structure appears; at 1,000 nm, its amorphous crystalline structure is changed into anatase or rutile crystalline structure. That is, implants having an oxide layer with a thickness of 600 nm or above are reported to remarkably increase removal torque and histologically show significant advance of bone-implant contact ratio. Currently, many research projects are being conducted to advance implant performance by improving this oxide layer[8-14].

The purpose of the study is to evaluate the response of osseous tissue to the anodized implants by histologically observing and biomechanically analyzing the degree of osseointegration, and the biologic response between implant surface and bone tissue over time after implant placement.

Materials and Methods

1. Implant Fabrication and Surface Process

Adopting commercially pure titanium (grade 2), 96 screw-type implants with an outer diameter of 3.3 mm and a length of 7.0 mm were fabricated. Among them, 48 were processed by lathe machining without any additional surface process; others were anodized (Warentec, Seoul, Korea). Anodization was conducted in a potentiostatic mode with pulse power (660 Hz, Duty: 10%). The electrolyte solution included 0.15 M calcium acetate and 0.02 M calcium glycerophosphate. The whole process was executed at room temperature for 3 minutes; steel was used for the cathode; and the voltage was constantly maintained at 270 V. The implants to be placed were washed with distilled water and anhydrous alcohol; and then autoclave-sterilized at 134°C for 30 minutes.

2. Lab Animals and Test Group

A total of 24 New Zealand white rabbits aged 2-6 months were used, regardless of gender. Two implants with anodized surface for test group and two implants with machined surface for control group were installed in each tibia of 6 rabbits in each group, respectively. The healing period of each group was set for 1, 2, 3, and 7 weeks respectively.

3. Implant Installation

For the surgical procedure, Ketamine HCl (Yuhan, Seoul, Korea) was injected (75 mg/kg) into muscle for general anesthesia. After shaving the mesial epiphyseal region of both tibias, hemostasis and local anesthesia on the surgery site required 2% Lidocaine Hydrochloride (Yuhan, Seoul, Korea) injection for infiltration anesthesia. Skin was cut from the region 1-2 cm below the knee joint along the medial epicondyle of the tibia and softly desquamated to the periosteum. Skin and fascia were separately opened and sutured closed. Periosteum was removed gently from the surgery site but not sutured. During surgical drilling, low-speed rotation, less than 2,000 rpm, was used and sufficient normal saline was supplied for cooling down. After using a 2.0 mm pilot drill and then a 2.7 mm twist drill, the implant was installed in a self-tapping method.

Right after surgery, test animals were forced to bear weights on legs and each of them was raised separately in an isolated cage. After the scheduled healing period for each test group, test animals had an intravenous injection of excessive sodium thiopental (Korean Pharm., Seoul, Korea) and were sacrificed.

4. Resonance Frequency Analysis and Removal Torque Measurement

Right after implant installation and after healing periods, resonance frequency analysis (RFA) was conducted using Osstell (Integration Diagnostics, Goeteburg, Sweden); when a lab animal was sacrificed after the scheduled healing period, removal torques were measured using MARK-10 (MGT50, San Diego, CA, USA) for one implant in the test group and one implant in the control group.

5. Histological Observation

When the test animals were sacrificed, the implants were collected en bloc with surrounding bone. Collected specimens were fixed with 4% paraformaldehyde buffer solution and non-decalcified samples were made using Donath's method[15]. The samples polished with 10 μm were stained toluidine blue; and the tissue were observed through a light microscope (Olympus BX-50, Tokyo, Japan).

6. Data Analysis and Statistical Process

Wilcoxon rank sum test was conducted to determine if changes in RFA values, removal torques after healing period, and histomorphometric contact ratio between bone and metal vary according to the type of surface and healing period. Statistical analysis was executed using Stata for Windows (Stata Corp, Austin, TX, USA); when P-value is less than 0.05, it is deemed statistically significant.

Results

1. Histological Observations

1) After 1 Week

In general, tissue structures of the test and control groups were similar. Only a part of the upper implant was in contact with a very small portion of existing cortical bone; bone fragments fell into the bone marrow due the surgical injury. The part where the implant was not in contact with cortical bone was filled with small bone fragments or red blood cells. New bones started to form in both groups, developing into 2 aspects: first, active trabecular bone formation from endosteal surface within 1mm of the implant surface, in particular, more active bone formation on the anodized surface; secondly, coarse bone, which is petrified well, was formed within 2nd or 3rd screw threads, close to the implant or in contact with a part of the implant (Fig. 1).

2) After 2 Weeks

Broad bone formation with trabecular structure from periosteal surface was observed; occasionally, the size exceeded that of the existing cortical bone. Since, in the existing cortical bone, cutting and filling cones were observed to be perpendicular to the implant surface, it could be seen that extensive bone remodeling has occurred in the existing cortical bone near the implant. Bone absorption occurred at one end of cones and bone deposition occurred at the other end of cones. There were no bone fragments or red blood cells between the implant surface and section of cortical bone. The section of the existing cortical bone saw obvious bone absorption as case reports; implant surface saw bone deposition. Bone formation on the endosteal surface has

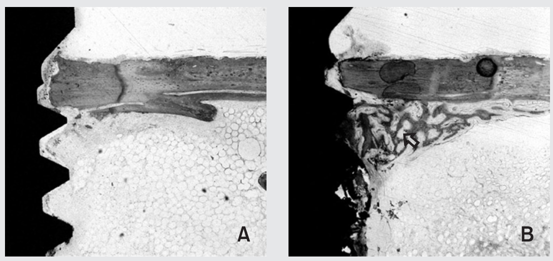

Figure 1. Photomicrograph of 1-week specimen showing new trabecular formation (arrow) in the anodized group. Machined (A) and anodized (B) (Toluidine blue, x40).

progressed further, filling more lower screw threads; bone formation from the screw threads has advanced to integrate with bone formed on the endosteal surface; making it impossible to distinguish the two bone formations. In summary, bone formations have progressed in the gap between the bone section and implant; bone formation in bone marrow has progressed further; however, there was no difference between implant surfaces in the test/control groups (Fig. 2).

3) After 3 Weeks

Observations after 3 weeks were similar to the observation after 2 weeks. Bone formation on the periosteum and endosteum had progressed further; more new bone filled the gap between the existing cortical bone section and implant surface; and bone remodeling on the existing bone was more developed. As case reports, the upper implant was covered with new bone due to the active bone formation on periosteum. Within the screw threads of the implant inserted into bone marrow, more bone was formed and the scope of bone formation expanded in a downward direction. In the marrow, newly formed bone had thicker trabeculae and bone remodeling was in progress; the density of new bone around the anodized surface seemed higher, but was not distinct (Fig. 3).

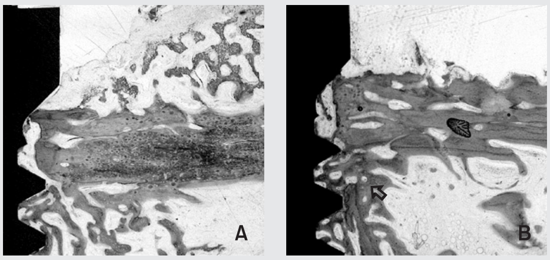

Figure 2. Photomicrograph of 2-week specimen showing cutting and filling cones observed in surface (arrow) of anodized group. Machined (A) and anodized (B) (Toluidine blue, x40).

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4) After 7 Weeks

The most distinguishing observation was that the shape of existing cortical bone has changed resulting from active bone formation of the endosteum and periosteum, and bone absorption. Since the degree of change varied by subject, while only upper and lower parts close to implant have changed the shape maintaining the shape of existing cortical bone in some cases, the shape of bone has completely changed due to active bone formation of the periosteum and bone absorption of the existing cortical bone in other cases. Bone remodeling in cortical bone continued to progress, and new bone became more compact and mature. The contact between bone and implant seemed better on the anodized surface, but not distinct. Coarse bone in the marrow completed the bone remodeling and changed into compact bone, and the size became smaller (Fig. 4).

2. Resonance Frequency Analysis and Removal Torque Analysis

Difference between changes in RFA value according to the healing period on the same surface of implant or difference between changes in RFA value according to the surface of

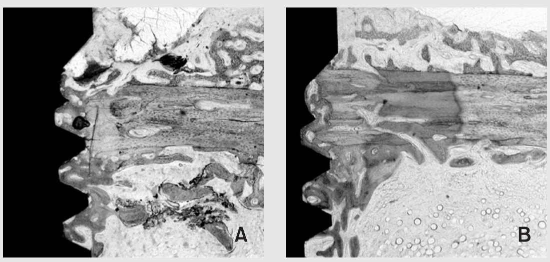

Figure 3. Photomicrograph of 3-week specimen showing periosteal and endosteal new bone formation on both surfaces. Machined (A) and anodized (B) (Toluidine blue, x40)

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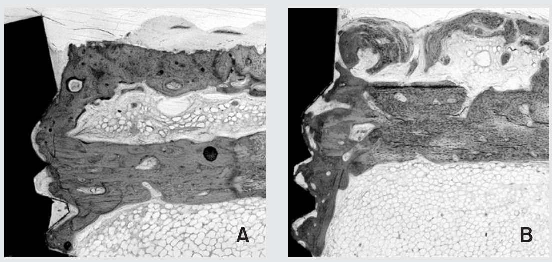

Figure 4. Photomicrograph of 7-week specimen showing ongoing remodeling and matured new bone in cortical bone. Machined (A) and anodized (B) (Toluidine blue, x40).

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Table 1. The change rate of resonance frequency analysis (RFA)

	Machined surface (%)	Anodized surface (%)
1 week	1.36±3.15	2.24±6.17
2 weeks	3.11±3.38	2.41±4.70
3 weeks	1.21±5.53	8.45±9.91
7 weeks	5.18±6.70	10.20±5.91

(RFA removal+RFA installation/ RFA installation) ×100.

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Table 2. The average removal torque of implant after healing

	Machined surface (N/cm)	Anodized surface (N/cm)
1 week	6.50	8.00
2 weeks	12.58*	13.33*
3 weeks	16.08*	17.33*
7 weeks	15.50*	20.90*

*P<0.05.

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implant for the same healing period showed no statistical significance ($P>0.05$). However, the longer the healing period was, the more RFA values increased on the same surface; for the same healing period, RFA values tended to increase more in the test group compared to the control group (Table 1).

Removal torque values showed statistical significance ($P<0.05$) between healing periods of 1, 2, 3, and 7 weeks regardless of surface type; however, there was no statistical significance between healing periods of 2, 3, and 7 weeks ($P>0.05$). However, a tendency of longer healing period to yield greater removal torque value was observed. For the same healing period, removal torque values of test groups tended to be greater than those of control groups while there was no statistical significance according to the surface process (Table 2).

Discussion

The clinical use of implants having conventional machined surfaces is widespread but the results of installation in the areas with lack of bone volume or poor bone quality have not been always satisfactory. To that end, developing new type of osseointegrating implants focus mainly on surface changes, striving to advance mechanical integration between implant surface and tissue[16,17)]. The natural oxide layer generated within the titanium implant surface exhibited excellent biocompatibility; but not chemically combined directly with bone tissue and was deemed inactive biomaterials[18)]. A recent study has reported that response of bone tissue could be improved by deforming the oxide on the titanium implant's surface[19,20)].

Implant with anodized surface shows superior results in removal torque experiment or histomorphometric analysis than that with a machined surface[21)]. This study observed such tendency; when adopting the same healing period, RFA value changes and removal torque values tended to be increased in the test group compared to the control group, confirming a stronger bone tissue response with the anodized implant.

Such differences between machined surface and anodized surface are assumed to be based on various factors; considering that the fine surface roughness of the two surfaces are similar, it seems to not have been affected by a simple surface roughness but other factors including the fine porous surface structure, crystalline structure and chemical components of oxide layer. The thickness of the oxide layer on the surface is determined depending on applied electrochemical variables; concurrently its fine form of surface is fixed, porous or not porous. Since strong response of bone tissue is observed only in porous structure, it can be inferred that bone tissue grows into such porous surface and is engaged mechanically, increasing osseointegration strength between bones and implant interface. As chemical components such as Ca, P, etc were found on the oxide layer, an inference has been raised that chemical combination of such components with bone matrix glycoprotein increases osseointegration strength[21,22)].

Anodized layers show various surface characteristics depending on electrochemical variables. The implant surface anodized by the method used in this study has characteristics such as pore size of 0.79±0.27 μm, fine surface roughness of 0.88±0.13 μm, oxide layer of 2.89±0.24 μm thick, and anatase crystalline structure. Since the implant used in this experiment shows such fine porous structure and has 2.35% Ca and 1.11% P on the implant surface, the findings are deemed affected by the possibility of chemical combination as well as mechanical combination between fine porous structure and bone tissue.

Resonance frequency is determined by the strength of the implant-tissue interface and the distance between the transducer and initial bone-implant contact. Therefore, RFA values are supposedly determined according to initial fixed condition at the installation of the implant, intraosseous depth of installed upper implant, whether lower implant contacts with lower tibia compact bone, and degree of osseointegration after healing.[23,24)]

Thus, comparing the changes between RFA values after installing and RFA values after healing rather than comparing each absolute value is deemed significant for comparative study according to surface difference.

Anodized surface is known to have relatively less reduction of stability during the healing period after implant installation compared to machined surface and to start increasing stability earlier[25)]. RFA values measured after 1-week healing period tend to increase slightly regardless of surface type; and it is observed that the longer the healing period, the larger the increase on the anodized surface.

Usually, removal torque value rather than histological measurements are effective in comparing biological responses of bone tissue because histological method evaluates osseointegration degree on a plane while removal torque values make it possible to evaluate the degree of osseointegration on functional and 3-dimensional basis.

The study observed the tendency that the longer the healing period is, the larger removal torque value will be, and that if adopting the same healing period, an anodized surface has greater removal torque value than a machined surface. Between the healing period of 1 week and the healing period of 2 weeks or longer, statistical significance of removal torque values were observed regardless of surface type, and it can be inferred that with excellent initial stability, healing period of 2 weeks is sufficient for stable osseointegration, at least to some extent, for rabbits. Given that bone remodeling cycle is 6 weeks in rabbits and 17 weeks in human beings[26)], it can be inferred that if bone tissue is in good condition and initial stability is excellent, prosthetic appliances can be connected in 6 to 8 weeks. In reality, some implants with surgical principle of connecting prosthetic appliances in 6 weeks after installing implant have been tried[27)].

The anodized surface used in this experiment is characterized by active new bone formation from endosteal surface, the same as reported in the previous literature. Along with endosteal bone formation, independent formation of coarse bone due to the closeness to or partial contact with the implant's surface in the lower screw thread area is observed, which is also duplicating the results of previous studies.

Davis observed the phenomenon that new bone forms on a dual-anodized implant surface regardless of the existing bone, and the bone contact spreads along the surface, and called it 'contact osteogenesis'; such similar phenomenon was observed in the experiment, letting us know that bone response to implant is very definite[4)]. However, when healing is completed the implant adheres stably to the bone tissue regardless of bone formation site[28)].

Comparing each value obtained from this experiment, the differences between test animal subjects are larger than the differences between test groups and control groups. Thus, for more exact experiments on biological response to anodized surface, sophisticated consideration on test animals and increase in subject number are believed necessary. Furthermore, research on the effect of changes in thickness, shape, porous structure, crystalline structure and chemical compositions of oxide layer of anodized implant on bone tissue response need to be conducted.

Conclusion

For the evaluation of bone tissue response to anodized implant, implants were installed to tibias of rabbits and subsequently osseointegration degree and biological response between implant surface and bone tissue were evaluated over time. The test group experienced histologically active bone formation, cortical bone remodeling, and new bone maturity; the analysis conducted in a biomechanical method showed the tendency of increase in RFA values and removal torque values of the test group, confirming the usefulness of clinical application of anodized implants.

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