Histological response of anodized titanium implant

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I. Introduction

Osseointegration is the essential biological basis of current dental implants[1]. Osseointegration was initially defined on the light microscopic level as "a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant"[2].

Today, by definition, osseointegtation requires the absence of a fibrous layer and implies that the biological response of the bone is not one of inertness towards a foreign material but rather one of active integration of the material with the bone as part of the body[3].

According to Giavaresi. et al.[4] osseointegration is defined not only as the absence of a fibrous layer around the implant with an active response in terms of integration to host bone, but also as a chemical(bonding osteogenesis) or physico-chemical(connective tissue osteogenesis) bond between implant and bone.

Endosseous integration can be deconvoluted into three distinct bony healing phases. The first, osteoconduction, relies on the migration of differentiating osteogenic cells to the implant surface. The second, de novo bone formation, results in a mineralized interfacial matrix equivalent to that seen in cement lines in natural bone tissue. Implant surface design will have a profound effect on osteoconduction, while the surface topography of the implant will play a critical role in bone bonding concomitant with de novo bone formation. The third healing phase, that of bone remodeling, will also, at

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discrete sites, create a bone-implant interface comprising de novo bone formation. Treatment outcomes in dental implantology will be critically dependent on implant surface designs that optimize the biologic response during each of these three distinct integration mechanisms[5].

A successful long-term implant requires biocompatibility, toughness, strength, corrosion resistance, wear resistance, and fracture resistance[6].

The surface of implantable biomaterials is in direct contact with the host bone and soft tissue and plays a critical role in determining the biocompatibility, functional compatibility, osteoinduction of bone ald osseointegration of implants. Placing of implants into bone tissue should in principle lead to bone-implant osseointegration. The bone formation that occurs during osseointegration may be an osteoblast activity which is affected by the implant surface[3].

Very few studies have been carried out to systematically investigate the role of individual surface(oxide) properties of titanium in the biological response, although recent *in vitro* and *in vivo* studies provide strong indications that biological responses to titanium are influenced both by surface structure(roughness) and chemical composition. In most studies, however, the type of surface preparation and/or characterization methods used prevent any firm conclusions as to which surface properties were the determining factor for the observed differences in biological response[7].

Differences in shape and surface characteristics of different implant systems could also influence handling, time and other resources used, and clinical success[8].

The quality of the implant surface is one of the 6 factors described by Albrektsson that influence wound healing at the implantation site and subsequently affect osseointegration.

Plasma spray-coation is one of the most common methods for surface modification. Plasma spraying is used for the application of both Ti or HA on metallic cores with a coating thickness of 10 to 40 μm for Ti and 50 to 70 μm for HA.

Blasting with particles of various diameters is another frequently used method of surface alteration. In this approach, the implant surface is bombarded with particles of aluminum oxide(Al_2O_3) or titanium oxide (TiO_2), and by abrasion, a rough surface is produced with irregular pits and depressions.

Chemical etching is another process by which surface roughness can be increased. The metallic implant is immersed into an acidic solution. which erodes its surface. creating pits of specific dimensions and shape, Concentration of the acidic solution. time. and temperature are factors determining the result of chemical attack and microstructure of the surface[2].

Sandblasting, plasma spraying and acid etching have become the three most common approaches used to alter the surface topography and increase the surface area of implants[4].

Greater surface roughness increases the implant surface area and increases the potential for biomechanical interlocking of bone into the implant surface. These characteristics are thought to enhance the gone implant interface and improve stabilization[9].

A certain degree of surface roughness may have a positive effect on adsorption of molecules, local factor production, and proliferation and differentiation of cells at the implant surface. Differences in topography and roughness can be obtained by treating the titanium surface with additive and reductive techniques[10].

Titanium plasma spray(TPS) surface has since become one of the benchmarks for rough implant surfaces, with over twenty years of clinical experience and a large number of published articles. In spite of the success of the TPS surface, numerous researchers and clinicians have been working on new and improved micro rough surfaces.

In 1998 and 1999, some major changes were introduced to the ITI. After more than 10 years of intensive research and clinical testing, a major breakthrough-the SLA (Sand-blasted, Large grit, Acid-etched) surface-was launched in mid-June 1998 at the ITI World Symposium in Boston. The SLA surface was first tested in cell cultures and animals in 1990.

The SLA surface of the ITI implant is prepared by sandblasting the implant surface with large grid(250-500 µm) corundum, washing in ultrasonic deionized water bath, and drying. The implant surface is then acid-etched in a hot hydrochloric acid/sulthuric acid(HCL/H_2SO_4) mixture, followed by thorough rinsing in deionized water befor drying in hot air. This procedure leads to a surface roughness between 20 and 40 µm[11].

The intensive testing and successfully ongoing clinical and field trials have shown that the SLA surface clearly has the potential to replace the TPS surface. Due to the macro/micro double roughness of the SLA surface, osseointegration of ITI implants has been improved and time to loading has been reduced to a maximum of 50%.

In recent years, attempts have been made to improve bone anchorage of dental implants. Thomas and Cook(1985) examined the variables that influenced the apposition of bone to an implant surface. Of 12 parameters studied, only surface characteristics had a significant effect on the integration of the implant. This observation has been confirmed in a histometric study by Buser et al. (1991) that showed a positive correlation between the percentage of bone-to-implant contacts and the roughness values of five different titanium surface tested. Implant surfaces that were sandblasted and acid etched achieved a greater bone-implant contact than the "rougher" titanium plasma sprayed(TPS) surfaces. However, this study was carried out in long bones of miniature pigs and evaluated only short-term healing periods of unloaded experimental implants[12].

A well-documented sandblasted and acid-etched surface(SLA), as a new development in this area, consistently showed better results both in histometric and biomechanical testing in comparison with alternative surfaces, such as the well-documented TPS(titanium plasma-sprayed) surface; and furthermore proved advantageous in clinical application[13].

Cochran DL et al.[12] compared TPS and acid-etched implants placed in canine mandible. They found the acid-etched surface resulted in a significantly higher degree of bone-implant contact after 3 months of healing, but 3months of loading(6 months of healing) no significant difference was found between the SLA and TPS surfaces implants. After 12 months of loading the SLA implants had a significantly greater percentage of BIC than did the TPS implants. These results are consistent with earlier studies on SLA implants and suggest that this surface promotes greater osseous contact at earlier time points compared to TPS-coated implants.

Experimental studies in animals have found stronger bone fixation for TiO_2 blasted implants than for turned implants[14].

Most metals form oxide layers when exposed to the atmosphere. The nature of this oxide depends on the metal and the conditions under which it was oxidized. Anything that comes in contact with the implant surface has the potential to change it. Assuming that the physiologic conditions of the body remain fairly constant, the behavior of a metal in the body depends on the character of the oxide layer. TiO_2 is the most stable and therefore the most commonly used under physiologic conditions.

When an implant is introduced into the body, complex reactions begin to take place at the oxide / bioenvironment interface. The oxide film grows as ions diffuse outward from the metal and inward from the environment. The oxide that forms in the body may, therefore, be somewhat different than that which forms in air. The rate of formation and composition of this film is important. Titanium, both as a pure metal and as on alloy, is easily passivated, forming a stable TiO_2 surface oxide will repair itself instantaneously on damage such as might occur during insertion of an implant. Values of 100 to 300 ppm are frequently observed in soft tissues surrounding Ti implants. At these levels, tissue discoloration with Ti pigments can be seen. this rate of dissolution is one of the lowest of all passivated implant metals and seems to be well tolerated by the body[6].

Most of the studies referred to have used machined screw implants, and it is possible that surface modification can facilitate implant healing and improve the clinical outcome. Recently, wide application in implantlogy was received with technique of anodic oxidation.

Anodic oxidation is one technique of surface modification that results in growth of the implant surface oxide to a thickness of about 1to 10 μm and a porous surface structure; an increase in oxide thickness, from 1 to 2 μm at the coronal part to7 to10 μm at the apical part of the implant. The surface exhibited nimerous pores of varying size, predominantly 1 to 2 μm, as measured at the apical part of the implant. Surface roughness increased continuously from the conical upper part to the apical end, with an average Ra value of 1.2 μm. Experimental studies have demonstrated higher bone-to- implant contact values for oxidized implants compared with those for machined implants, indicating a rapid development of a strong bone-implant fixation for the oxidized implants[15].

Titanium dioxide blasting represents a reductive technique, in which a plastic deformation of the surface is obtained by blasting particles of TiO_2 toward the surface. The technique was introduced to increase the roughness without adding foreign elements to the surface and to enable the surface configuration to be changed by using different particle sizes for the blasting procedure[10].

Gotfredsen et al.(1992) and Ericsson et al.(1994) demonstrated that titanium implants blasted with titanium-dioxide particles(TiO_2) provided better anchorage to bone than implants with conventional, machine-prepared surfaces[17].

Improved understanding of this interaction between cells and the implant surfaces would be of importance in the selection of an optimal surface texture[17].

The rough surface obtained not only improves the positive effects of coated surfaces on osseointegration but also solves the problems created by coating techniques. It was found that this method can facilitate initial bone healing at the dental implant-bone interface[1].

The purpose of the present study was to compare SLA and anodized surfaces titanium implants histologically.

II. Materials and Methods

6 rabbits were used in the study. The rabbits were anaesthetized and cleaned.

All animals were supplied with the latter an antibiotic the following 3 days after surgery.

Twenty four screw-shaped titanium implants*(12 - SLA surfaced implants and 12 - anodized surfaced implants with a length of 8 mm and a diameter of 4.3mm) were installed in every left and right femur of animals.[2]

After 12 weeks the animals were killed with overdose of a thiopental sodium.

The implants with surrounding bone were prepared and fixed for 2 weeks in 10% neutral formalin. Subsequently implants and surrounding bone with average thickness 10mm were put in 70%, 80%, 90%, 95%, 100% alcohol for 2 days and they were embedded in light-curing resin(Technovit 7200 VLC, Kultzer & Co, Germany). After polymerization, the cutting and grinding were performed in an Exakt cutting and grinding system machine. The implants were polished with 800, 1200 and 4000 grit paper until 20-50 μm of thickness, and stained with Multiple staining solution(Ethylene alcohol [64-17-5], methyl alcohol[67-56-1], Toluidine BlueO[92-31-9], Basic Fuchsin [569-61-9], water[7732-18-5]. Polysciences, Inc.).

The sections were seen by the computer connected with the digital camera(OLYMPUS B×50) and optical microscope(Uplan Apo, 2×/0,08, 4×/0,16, 10×/0.40) and the bone and the bone-to-implant contact, % were measured by TDI Scope Eye software (Techsan Co, Korea).

Statistical analysis:

Statistical analysis involved comparisons of BIC, (%) between SLA and anodized surfaces

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Table 1. BIC (%) for SLA and Anodized Titanium Implants (p>0.05).

Type of surface	SLA	Anodized surface
BIC (%) ± SD	74 ± 19	77 ± 9

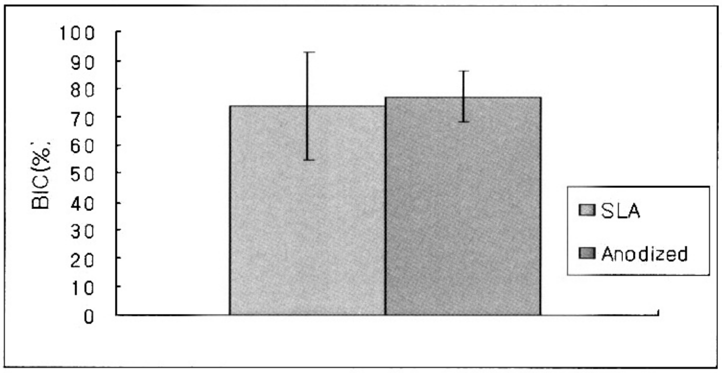

Figure 1. BIC (%) for SLA and Anodized Titanium Implants.

of titanium implants. BIC data were compared by paired student t-test ($p<0.05$).

Ⅲ. Results

The results of histomorphometric analysis (Table 1) showed a higher percentage of bone-to-implant contact for implants with anodized surface(77±9) compared with SLA implant(74 ±19). However this difference did not show statistical significance($p>0.05$).

Ⅳ. Discussion

In present study between SLA and anodized surfaces implants there are not statistically significat differences in bone implant contact % representing bone healing response. The exact mechanism as to why osteoblasts produce more bone in the presence of a microrough surface is not yet well-understood[18].

The degree of surface roughness may not be the only aspect of surface topography that effects osseointegration. The intimacy of bone contact with the implant surface may be important as may the ionic charge, surface energy and surface tension or other still undefined properties of the surface[9].

Anodic oxidation of the electropolished surfaces, which produced areas of increased roughness and a thicker surface oxide, had an enhancing effect on the rate of bone formation. Increasing the oxide thickness of rough machined implants had no significant effect on the bone response[7]. But Ivanoff CJ et al.[19] supposed, that with increasing oxide thickness there is a change in oxide crystallinity, in that the amorphous oxide changes to anatase and rutile forms, which may cre-

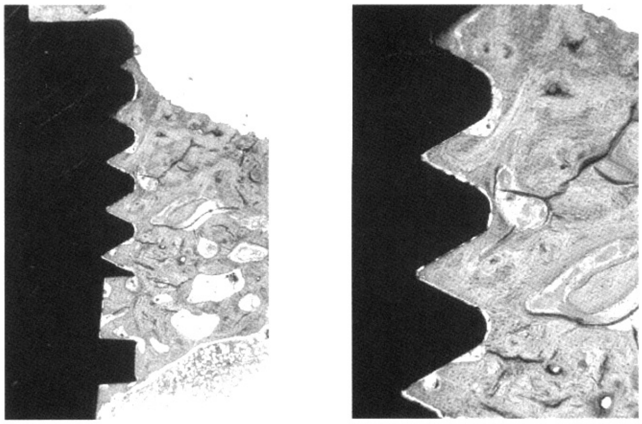

Figure 2. Histological section in mesiodistal direction of anodized implant; Multiple stain, magnification X40 (left), ×100 (right)

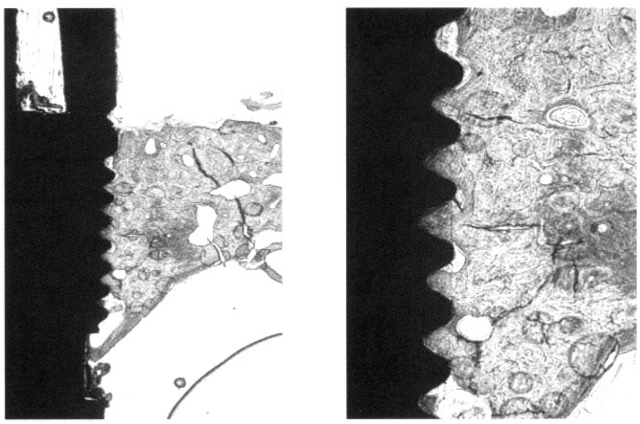

Figure 3. Histological ection in mesiodistal direction of SLA implant; Multiple stain, magnification X40 (left), ×100 (right)

ate a stronger bone reaction.

Several surface treatments(particle blasting, plasma-spray coatings, acid etching) have been proposed to improve implant surface characteristics and to increase the quantity and quality of bone at the interface, with increased interlocking[20].

Within a defined immediate loading treatment protocol, the use of oxidized surfaced implants help to reduce the risk of a stability loss in the posterior maxilla in the early healing period[21], and use of TiUnite implants was successful in treating regions of bone quality 4 and no implant was lost resulting in a cumulative success rate of 100%[22].

The preliminary results of prospective multicenter clinical study by Friberg B et al.[23] on mainly TiUnite implants showed an early cumulative survival rate(CSR) of 99.7%.

In the study by Rocci A. et al.,[24] the TiUnite implant showed bone-to-implant contact along the full length of the implant and to a level coronal to the first thread. It was concluded that successful osseointegration of a TiUnite implant placed in soft bone was also possible when the implant was immediately loaded.

Salata L. and coworkers[25] have suggested that faster development of implant stability for oxidized implants than turned components when placed in bone defects. Bone formation towards the rough surfaced implant is facilitated by a more stable connection between bone matrix and the implant surface, which can be explained by a higher degree of protein adsorption on the anodic-oxidised implants.

Glauser R et al.[26] concluded that the applied immediate loading protocol in combination with a slightly tapered implant and a modified implant surface texture was shown to be a successful treatment alternative even in regions exhibiting soft bone.

The results from study by Olsson M et al.[27] indicated that early loading can be applied to cross-arch dental bridges supported by six to eight oxidized implants in the maxilla. Authors suggested that the use of surface-modified implants may have played an important role in the favorable outcome of study.

According to Li DH et al.,[1] the rough surface of titanium dental implants created by the modified sandblasting treatment can greatly enhance the shear strength at the dental implant-bone interface and that, with this enhancement, the secondary micropores play a much more important role in implant-bone bonding.

The tissue response may not depend on only one specific surface property but rather on a number of different alterations. Bone tissue responses may greatly depend on the surface chemistry of implants, the porous oxide structures,[28] the crystallinity of titanium oxide and mechanical interdigitation[29-31]. However, it is not fully understood whether these oxide properties influence the bone tissue response separately or synergisticcally.

However, the lack of improvement of bone-bonding ability in the later stages of implantation may be attributed to the low porosity and to the superficial ingrowths of apatite-like deposits into the pores of the anodic oxidation Ti layer. In addition to the anatase surface crystal structure, other oxide properties, such as micropore size and configuration, may also play important roles in the bone-bonding ability of anodically oxidized titanium in the body[32].

However, results from our animal studies may not always be extrapolated to the clinical situation. this may be due to differences in bone anatomy, physiology and loading conditions. Therefore, it may be more relevant to study the bone tissue response to implants in human bone.

V. Conclusions

In present animal histological study new bone formation occurred along anodized titanium implant surface in apical direction and was filled within fixture threads. Anodized titanium implant showed high score of bone implant contact %. But anodized titanium implants did not show statistically significant difference with SLA titanium implant in bone response.

Ⅵ. References

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-국문초록-

양극 산화한 티타늄 임프란트의 조직학적 반응

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여러 연구들을 통해 많은 학자들이 임프란트 안정성(stability)은 표면의 특징에 달려있다고 생각하게 되었다. 표면의 구조, 에너지, 산화물(oxide) 두께와 표면성상(topography)등 임프란트의 표면의 특징은 임프란트와 골조직의 반응에서 중요한 역할을 하는 것이 알려짐에 따라 티타뮨 임프란트의 표면의 처리 방법에 큰 관심을 가지게 되었다.

그 중에서 티타늄 임프란트 표면의 산화피막화(anodization)가 한 방법으로 대두되었다. 이 방법은 전기화학적 방식으로 임프란트 표면에 거칠고(rough)두꺼우며(thick), 기공(pore)을 가지는 산화물 막을 형성하는 것으로 산화물의 두께는 coronal 부분(1-2 ㎛)으로부터 apical부분(7-10 ㎛)까지 증가하게 된다. 산화피막의 표면에는 다양한 크기의 수많은 기공이 주로 1-2 ㎛ 두께로 임프란트의 apical 부분에서 존재하며, 임프란트 표면의 거칠기는 conical 위부분에서 apical 부분까지 계속 증가한다(평균 Ra value=1.2 ㎛).

또 다른 표면 처리 방법으로는 blasting 후에 etching을 한 SLA 표면이 있다.

이 연구의 목적은 일반적으로 많이 이용되고 있는 anodized 표면과 SLA 표면의 조직학적 반응을 비교 분석하는 것이다.

24개 임프란트를(anodized surfaced implant-12개 , SLA-12개, 8mm × Φ 4.3) 6마리 토끼의 오른쪽과 왼쪽 femur에 식립하였다. 12주후에 동물들을 희생하여 EXACT cutting-grinding system을 이용하여 샘플을 절단하고 800, 1200 및 4000 번 연마제(abrasive) paper로 20-50 ㎛ 까지 grinding하였다. 샘플은 Multiple staining 용액으로 염색하여 SLA 임프란트 군과 비교하였다. 골과 임프란트 사이에 연결을 TDI 프로그램을 이용하여 %로 측정하였다.

SLA 임프란트 군 경우에는 골과 임프란트 사이의 연결이 74±19% 이고, 양극 산화한 임프란트 군 경우에는 77±9%이었다. 양극 산화한 티타늄 임프란트의 골 접촉률이 SLA 표면 임프란트 경우과 통계학적으로 유의한 차이는 보이지 않았다.3)

주요어 : 골접촉률, 양극 산화한 임프란트, 골 반응, 산화피막화