The histometric analysis of osseointegration in hydroxyapatite surface dental implants by ion beam-assisted deposition

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ABSTRACT

Purpose: This study compared the effects of coating implants with hydroxyapatite (HA) using an ion beam-assisted deposition (IBAD) method prepared with machined, anodized, sandblasted and large-grit acid etched (SLA) surfaces in minipigs, and verified the excellency of coating method with HA using IBAD.

Material and Methods: 4 male Minipigs(Prestige World Genetics, Korea), 18 to 24 months old and weighing approximately 35 to 40 kg, were chosen. All premolars and first molars of the maxilla were carefully extracted on each side. The implants were placed on the right side after an 8 week healing period. The implant stability was assessed by resonance frequency analysis (RFA) at the time of placement. 40 implants were divided into 5 groups; machined, anodized, anodized plus IBAD, SLA, and SLA plus IBAD surface implants. 4 weeks after implantation on the right side, the same surface implants were placed on the left side. After 4 weeks of healing, the minipigs were sacrificed and the implants were analyzed by RFA, histology and histometric.

Results: RFA showed a mean implant stability quotient (ISQ) of 75.625±5.021, 76.125± 3.739 ISQ and 77.941±2.947 at placement, after 4 weeks healing and after 8 weeks, respectively. Histological analysis of the implants demonstrated newly formed, compact, mature cortical bone with a nearby marrow spaces. HA coating was not separated from the HA coated implant surfaces using IBAD. In particular, the SLA implants coated with HA using IBAD showed better contact osteogenesis. Statistical and histometric analysis showed no significant differences in the bone to implant contact and bone density among 5 tested surfaces.

Conclusion: We can conclude that rough surface implants coated with HA by IBAD are more biocompatible, and clinical, histological, and histometric analysis showed no differences when compared with the other established implant surfaces in normal bone. ***(J Korean Acad Periodontol 2008;38:363-372)***

KEY WORDS: Ion beam-assisted deposition method; hydroxyapatite; rough surface implant; minipigs.

Introduction

Over the past 20 years, the number of dental implant procedures has increased steadily worldwide, reaching approximately one million dental implantations per year. The clinical implants favor both bone anchoring and biomechanical stability. The surface characteristics of dental implants are recognized as one of the most critical factors stimulating the osseointegration process[1)]. For this reason, several attempts have been made to modify the implant surface composition and morphology in order to optimize bone-to-implant contact and improve osseointegration.

Many studies have focused on the effect of increasing the surface microroughness on bone apposition[3)]. Compared with other types of surfaces, a sandblasted and acid-etched (SLA) surface has demonstrated enhanced bone apposition in histomorphometric analyses[5,7)] as well as higher removal torque values in bio-

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mechanical testing[16,24], thereby allowing a reduced healing period of 6 weeks in patients with a normal bone density, and exhibiting success rates of approximately 99 % after a follow-up of up to 5 years[2,21].

Currently, hydroxyapatite (HA) is widely used for implants as a coating material on implants for fixation and faster bone healing[9]. However, the HA coating layer has chemical nonuniformity, poor mechanical properties and low adhesion strength between the metal and HA coating[23]. To resolve these problems, coating methods using ion beam-assisted deposition (IBAD) have been developed[10].

In 2000, Lee et al. improved the bonding strength of plasma-sprayed HA coating through an interlayer coating of biocompatible (Ti, Zr, Ir) oxides and (Ti, Zr) nitrates. They are reported that the various Ca, P ratios of calcium phosphate films were formed by e-beam evaporation, and that the films had an excellent bonding strength and showed different dissolution behaviors depending on the Ca and P ratio[14].

Zhao et al. reported that the IBAD methods improved the binding strength, particularly the biological seal at the cervical level of the implant[25]. Liu et al. suggested that IBAD enhanced the tensile bond strength which was attributed to the possible chemical bonding[17].

Comut et al. reported that there were no significant differences in the orientation of the collagen fibers but there were significant effects of inflammation on the connective tissue attachment level around the IBAD implants[8]. Park et al. suggested a HA coating using IBAD may improve the bone response to a grit-blasted implant surface and have synergic effects, and a thin HA coating might have favorable effects that are independent of the causing surface roughness[20]. Hence, many authors have suggested that combining rough surface and HA deposition using an IBAD method will have synergic effects.

This study compared the effects of coating implants with hydroxyapatite (HA) using an ion beam-assisted deposition (IBAD) method with implants prepared using machined, anodized and SLA surfaces, and verified excellency of coating method with HA using an IBAD.

Materials and Methods

1. Preparation of surface

Evaporants used for coating were made by adding a 17.5% mass ratio of calcium oxide (CaO) powder (Cerac, Milwaukee, WI) to HA powder (Alfa Aesar, Ward Hill, MA). The mixture was ball milled in ethyl alcohol for 24 hours using aluminum oxide (Al_2O_3) balls as media. The powder mixtures were then sintered in air at 1,200˚C for 24 hours to make the evaporants. Roughing evaporation was carried out using a mechanical rotary pump to acquire an initial vacuum of 5×10^{-2}mmHg, which was reducted to 10^{-7}mmHg using a cryopump (Helix Technology, Mansfield, MA). Before deposition, the surface of the implants was cleaned for better adhesion using an ion beam (120V, 2A) extracted from an end-hall-type ion gun (Mark II, Commomwelth Scientific, Alexandria, VA). For evaporation, the voltage of the electron beam (Telemark, Fremont, CA) was 8.5kV. The current was initially 0.06 to 0.08A, and was increased to 0.15A. The substrate holder was rotated at a speed of 8rpm during deposition to improve the uniformity of the coating layer. The thickness of the coating layer, which was measured using a surface profiler (Model P-10; Tencor, Santa Clara, CA) was 1μm.

2. Surgical protocol

Four male Minipigs (Prestige World Genetics, Korea), 18 to 24 months old and weighing approximately 35 to 40kg, were chosen. The teeth were extracted under general anesthesia and sterile conditions in an operating room using Atropine 0.05mg/kg SQ, Rompun®(Bayer HealthCare, USA) 2mg/kg, Ketamine (Ketalar®, Yuhan Co., Seoul, Korea) 10mg/kg IV. Minipigs (Prestige World Genetics, Korea) were placed on a heating pad, intubated, administered 2% enflurane, and monitored with an electrocardiogram. After disinfecting the surgical sites, 2% lidocane HCl with epinephrine 1:100,000(Kwangmyung Pharm., Seoul, Korea) were administered by infiltration at the surgical sites. Crevicular incisions were made, and all premolars and first molar were carefully extracted on right and left sides. Prior to extraction, P2-P4 and M1 were sectioned in order to avoid root fracture. The flaps were sutured with vertical mattress 5-0 resorbable sutures (Vicryl; Ethicon, Norderstedt, Germany). On the day of surgery, the dogs received antibiotics Cefazoline(Yuhan Co., Seoul, Korea) 10mg/kg IV.

The implants were placed on the right side using the same surgical conditions as used for tooth extraction, after an 8 week healing period. A crestal incision was made in an attempt to preserve the keratinized tissue. The mucoperiosteal flaps were carefully reflected on the buccal and palatal aspects. The edentulous ridge was carefully flattened on each side of the posterior maxilla preparing the implant sites with spiral drills and taps. The implant stability was assessed by resonance frequency analysis (RFA) at the time of placement. The flaps were closed with 5-0 resorbable sutures. The post-operative care was carried out as for tooth extraction. The sutures were removed after 7 to 10 days. A soft diet was provided throughout the study period.

40 implants(ITI®, Straumann AG) in this study were divided into 5 groups(4.1 mm diameter, 10.0 mm length); machined, anodized, anodized plus IBAD, SLA and SLA plus IBAD surface implants (Fig. 1, 2). Four weeks after implantation on the right side, the same implants were placed on the left side. After 4 weeks of healing, the minipigs were sacrificed by an overdose of the anesthetic drugs, and resonance frequency analysis, histologic analysis were performed. The sections were analyzed under a microscope for any new bone formation, bone to implant contact and bone density. Block sections including the segments with implants were preserved and fixed in 10% neutral buffered formalin.

The specimens were dehydrated in ethanol, embedded in methacrylate, and sectioned in mesio-distal plane using a diamond saw (Exakt®, Apparatebau, Norderstedt, Germany). From each implant site, the central section was reduced to a final thickness of approximately 20μm by microgrinding and polishing with a cutting-grinding device (Exakt®, Apparatebau, Norderstedt, Germany). The sections were stained in hematoxiline-eosine.

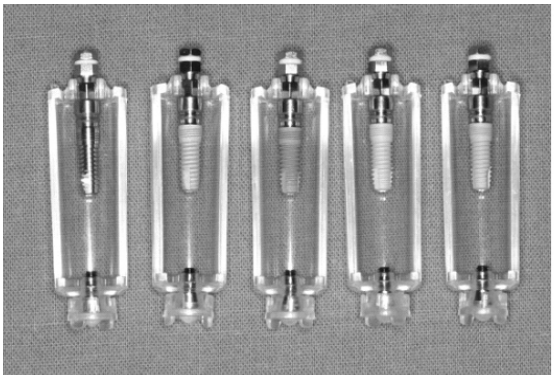

Figure 1. Five different surface fixtures. Machined, anodized, anodized plus IBAD, SLA, SLA plus IBAD.

Figure 2. Implant surgery. Machined, anodized, anodized plus IBAD, SLA, SLA plus IBAD were installed.

3. Analysis Method

1) Resonance frequency analysis (RFA)

RFA was performed to measure implant stability using an Osstell™ mentor instrument (Integration Diagnostics AB, G teborg, Sweden). Implant stability was measured in implant stability quotient (ISQ) units and was registered two times during the treatment: (1) at implant placement, (2) at sacrifice. Attach the measurement probe directly to the instrument. Attach a Smartpeg to an implant. Turn on the instrument by pressing any key. Hold the probe steadily, aiming the probe tip at the small magnet on the top of the Smartpeg, as close as possible without touching it. When the ISQ-value is detected, the instrument beeps and presents the value. Short beeps signal the measurements. The mean ISQ values were calculated for each minipig and time point.

2) Histologic and histometric analysis

The general histological findings were observed using stereoscope and microscope. The histomorphometric measurements were performed. Bone-to-implant contact was measured using the most coronal two threads and the most apical two threads and the bone density was measured within the same area

- Bone-to-implant contact(BIC)

$$= \frac{\text{Length in contact with bone}}{\text{Length of fixture(lateral side of implant sf.)}}$$

- Bone Density

$$= \frac{\text{bone area between threads}}{\text{total area between threads}}$$

3) Histomorphometric analysis

Table 3, 4 shows the histomorphometric data from the analysis. In the bone-to-implant contact and bone density, the SLA plus IBAD groups were greater than the other groups. However, statistical analysis showed no significant differences in the bone to implants contacts and bone density between the 5 tested surfaces. There was no difference between the time intervals.

4) Statistical Analysis

The means and the standard deviation for each of the 5 groups were calculated. The significance of the difference for the groups was determined by the Kruskal-Wallis test ($P<0.05$).

Table 1. RFA value of Healing Periods of 4weeks Group

Surface characteristics	RFA at implant placement		RFA at healing period of 4weeks	
	No. of implants	Mean±SD	No. of implants	Mean±SD
machined	4	78.00±2.16	4	75.25±5.50
anodized	4	77.25±2.75	3	78.33±2.89
anodized+IBAD	4	78.75±2.50	3	77.00±2.65
SLA	4	75.75±3.59	2	72.50±3.54
SLA+IBAD	4	70.00±3.74	3	76.50±3.11

Table 2. RFA value of Healing Periods of 8weeks Group

Surface characteristics	RFA at implant placement		RFA at healing period of 8weeks	
	No. of implants	Mean±SD	No. of implants	Mean±SD
machined	4	76.75±4.27	3	76.67±1.53
anodized	4	75.50±5.45	4	78.50±1.29
anodized+IBAD	4	72.00±9.76	2	73.50±4.95
SLA	4	75.25±6.90	4	78.75±3.59
SLA+IBAD	4	77.00±1.83	4	79.75±1.26

Table 3. Bone to Implant Contact

Surface characteristics	4 weeks		8 weeks	
	No. of implants	Mean±SD	No. of implants	Mean±SD
machined	4	0.43±0.38	3	0.42±0.24
anodized	3	0.24±0.12	4	0.34±0.35
anodized+IBAD	3	0.14±0.15	2	0.25±0.35
SLA	2	0.30±0.10	4	0.35±0.18
SLA+IBAD	3	0.45±0.20	4	0.75±0.62

Table 4. Bone Density

Surface characteristics	4 weeks		8 weeks	
	No. of implants	Mean±SD	No. of implants	Mean±SD
machined	4	0.22±0.19	3	0.43±0.37
anodized	3	0.20±0.09	4	0.31±0.30
anodized+IBAD	3	0.16±0.20	2	0.42±0.49
SLA	2	0.26±0.17	4	0.40±0.24
SLA+IBAD	3	0.48±0.23	4	0.50±0.19

Results

1. Clinical findings

During the postoperative healing period, healing was uneventful but 8 fixtures failed; one from each of the machined, anodized and SLA plus IBAD, and two from the SLA, and three from anodized plus IBAD surfaces. There were no signs of inflammation observed around implants.

2. Resonance frequency analysis (RFA)

RFA showed a mean implant stability quotient (ISQ) of 75.625±5.021, 76.125±3.739 ISQ and 77.941±2.947 ISQ at placement, after four weeks healing and after eight weeks healing respectively (Table 1, 2). There were no remarkable differences in RFA between the 5 groups. SLA plus IBAD group showed higher RFA value than SLA group. But statistical analysis showed no significant differences in the ISQ values between the 5 tested surfaces. There was no difference between the time intervals.

3. Histologic findings

No inflammatory reaction was observed around the implants except for the apical portion of some implants. Histologic analysis of the implants demonstrated newly formed, compact, mature cortical bone with a nearby marrow space (Fig. 3, 4, 5, 6, 7). No apical epithelial migration was observed. In the cortical portion, bone remodeling areas were present with many newly formed Haversian canals. Only in a few areas of the interface was it possible to observe an osteoblast rim. In the apical portion, newly formed bone trabeculae were present, which were composed mostly of woven bone, and only a small quantity of preexisting lamellar bone was present. Bone was in direct apposition to the titanium surface. In particular, the anodized, SLA implants coated with HA using the IBAD method showed an improved characteristic of contact osteogenesis in the soft bone, with the implant surface covered with a bone layer as a base for intensive bone formation and remodeling after 4, 8 weeks (Fig. 5, 7). Especially SLA surface implants coated with HA using the IBAD method have a greatest effects.

The HA coating did not separate from the implant surfaces coated HA using IBAD.

The junctional epithelium established the attachment to the implant surface, whereas the collagen fibers and fibroblasts of the connective tissue seal were oriented parallel to the implant.

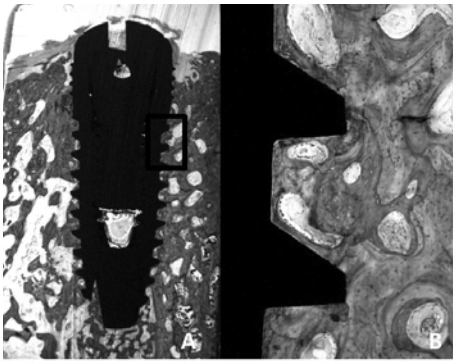

Figure 3. Histological view of the machined surface. (A) Overall view (×8). (B) Newly formed bone covers most of the rough surface of the implant and the existing bony compartment (×100).

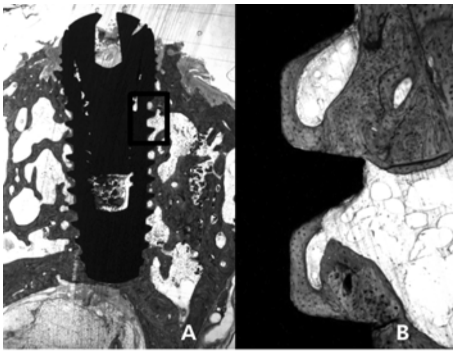

Figure 4. Histological view of the anodized surface. (A) Overall view (×8). (B) A thin rim of newly formed bone is observed (×100).

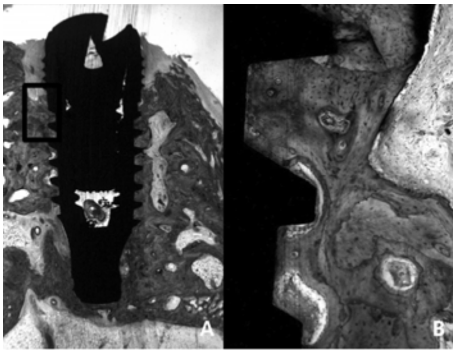

Figure 5. Histological view of the anodized plus IBAD surface. (A) Overall view (×8). (B) New bone formation occurs from the surface of the implant to the lateral direction (×100).

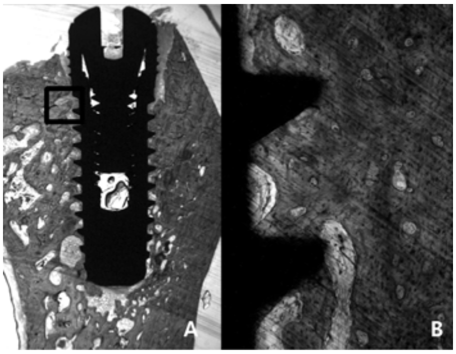

Figure 6. Histological view of the SLA surface. (A) Overall view (×8). (B) Thick dense bone is observed around the implants and nearby marrow bone (×100).

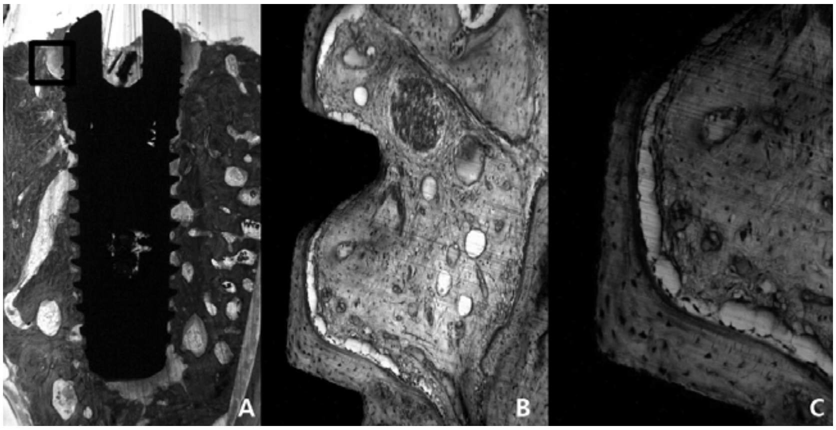

Figure 7. Histological view of the SLA plus IBAD surface. (A) Overall view (×8). (B) Improved characteristic of contact osteogenesis, with coverage of the implant surface with a bone layer as a base for intensive bone formation and remodeling (×100). (C) The osteoblasts are arranged along the implant surface followed by newly formed osteoid alongside the woven bone parallel to the osteoblasts (×200).

Discussion

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is structurally and chemically similar to the mineral constitute of the hard tissues, and is commonly used as a coating material on titanium implants to considerably improve biological affinity and activity to the surrounding bone tissues. However, the commercially available plasma-sprayed HA coating produces some defects such as poor adherence to the substrate, chemical inhomogeneity, and high porosity[11,23]. In addition, the presence of residual stress in the HA coating results in crack nucleation due to a large grain size and growth in the post-processing of the HA coatings, implantation and postoperative surgery, or even in the detachment of the HA coatings fragments, which can irritate the bone tissues. Various HA coating methods using IBAD have been developed in an attempt to solve these problems.

The minipig model was chosen because it has a bone formation rate equal to that of humans[19]. Moreover, the minipig shows similar bone quality and quantity to humans. This study used delayedplacement protocol but with a short healing period. The implant stability values were high immediately after implant placement but they decreased during the first 3 months of the healing period. The first 3 months after placement appear to be the most vulnerable phase for implant failure when unrestricted functional loading is initiated[12,21]. Some implants were exposed, which was attributed to the initiation of unrestricted functional loading with an additional effect of poor oral hygiene. There were some cases of failure. In addition, failures can be attributed by high torque values over 70Ncm and the cortical bone resorption around implants. A maximum safety margin is only achieved after a healing interval of 4 months. After this healing period, unrestricted functional loading of the implants is possible with minimal potential for implant failure[18].

Many investigators suggested that a higher removal torque value might lead to the more predictable use of shorter implants, to a support of a prosthesis with fewer implants, or to shorter healing periods. Statistical analysis did not reveal any significant differences in RFA values among the 5 tested surfaces and time intervals. This might be due to small sample size. Other studies reported that the torque value is not a reliable predictor of the implant survival during the follow-up period. In this study, cortical bone resorption was seen with over 70Ncm torque value. RFA values can be said to only be used as an indication for potential success.

No inflammatory reaction was observed around the implants except for the apical portion of some implants, which might be due to sinus perforation. The HA coating was not separated from the implant surfaces. It seemed that HA coating method using IBAD shows an increase of adhesion strength between metal and HA. Bone was in direct apposition to the titanium surface. The implants coated with HA using the IBAD method showed an improved characteristic of contact osteogenesis, with a coverage of the implant surface with a bone layer as a base for intensive bone formation and remodeling in 4, 8 weeks. In particular, the SLA surface implants coated with HA using the IBAD method showed the most significant bone formation. Significant enhancement of new bone apposition to the SLA surface implants coated with HA using the IBAD method as well as bone healing and contact osteogenesis were observed at both 4 and 8 weeks. It was reported that a fibrin network is laid upon the titanium dioxide surface and its associated adsorbed molecules, which facilitates the attachment of local osteoblasts[22]. SLA surface implants coated with HA using the IBAD method may promote direct contact osteogenesis. It was assumed that any combination effects of SLA and HA could enhance the surface reactivity with the surrounding tissues. In testing out the characteristics of implant surface, in the binding energy, the sharp edge of each peak was clearly observed. The aluminum element was detected on the sandblasted surface, while the aluminum element was not detected on SLA surface under energy-dispersive-x-ray-spectroscopy(EDS) analysis.[26]

However, further studies will be needed to test this hypothesis and determine the precise mechanism of the molecular interaction. The standard SLA surface has already led to a decrease in the healing periods from 3 months to 6 weeks[6,21]. Therefore, these results suggest that the use of SLA surface implants coated with HA using the IBAD method might result in a reduced healing period.

The effects of the IBAD deposition method on the implant stability and osseointegration were assessed in minipigs. According to the results, we could conclude that rough surface implants coated HA by IBAD demonstrated improved adhesion strength between metal and HA coating. And it showed no significant differences in clinical finding, RFA value, histologic analysis with other rough surface implants. Through this experiment, we were able to find that previous surface implants such as machined, anodized, and SLA were not statistically significant compared to the SLA+IBAD. However, the SLA surface implants coated with HA using the IBAD method could reduce healing periods of the standard SLA implants. However, the research design does not permit conclusions regarding the long-term treatment outcome with these implants. Therefore, further longer-term studies with a larger number of implants will be needed for clinical using. We could conclude that rough surface implants coated with HA by IBAD demonstrated improved biocompatibility and clinical and histological analysis showed no differences with other established implant surfaces.

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