Comparison of osseointegration between hydroxyapatite-coated and uncoated threaded titanium dental implants placed into surgically-created bone defect in rabbit tibia

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Abstract: The purpose of this study was to compare the degree of osseointegration between hydroxyapatite (HA)-coated and uncoated threaded titanium dental implants placed into a surgically created bone defect with or without an expanded polytetrafluoroethylene (ePTFE) membrane. A conventional implant site was prepared and either a HA-coated or an uncoated implant was placed in each tibia of 12 rabbits (conventional group). A bone defect approximately 3 mm in width was created on the distal end of the conventional site and either a HA-coated or an uncoated implant was placed there (bone defect group). For another group, the same procedure was performed as in the bone defect group, except the implant sites were covered with ePTFE membranes (ePTFE group). After 4 months, the rabbits were sacrificed. Specimens were prepared and examined histometrically. The results demonstrated that HA-coated threaded titanium dental implants consistently showed a greater amount of osseointegrated surface either in the conventional site or defect region with and without ePTFE membranes in this animal model. In conclusion, a HA-coated threaded titanium implant may be the choice of dental implants to establish greater osseointegration with the defect or conventional site. (J. Oral Sci. 40, 37-41, 1998)

Key words: osseointegration; threaded titanium implant; hydroxyapatite-coating; bone defect; ePTFE membrane; rabbit tibia.

Introduction

Dental implants have revolutionized treatment possibilities. Dentists can offer both fully and partially edentulous to patients. Albrektsson et al. (1) reported that titanium threaded dental implants were found to be predictably integrated into living bone by an intimate bone to titanium contact. A number of factors have been described as being important in determining the nature of the implant-bone interface (osseointegration). One important factor in determining the success or failure of an implant is the chemical properties of the outermost layer of the implant and its interaction with the living bone. Animal studies showed bone healing was enhanced by hydroxyapatite (HA) (2,3). Furthermore, HA-coated implants present the advantages of fast bone adaptation, absence of fibrous tissue layer, firm implant-bone attachment, high resistance to surgical interventions, and inhibition of ion release (4,5). Gottlander and Albrektsson (6) reported that HA-coated implants have either shown as much or significantly less interfacial bone than corresponding uncoated implants in their long-term follow up. For cylindrical, non-threaded designs, they also found significantly more bone in the HA-coated interface compared with the uncoated interface in their 6-month follow-up. However, they concluded that the effect of HA-coating seems to be uncertain with regard to threaded implants in contrast to unthreaded cylindrical designs (7).

As dentists have become more familiar with dental implant therapy, attempts have been made to place implants in what may be considered less than ideal anatomical situations in order to optimize restorative results. One of the most important prerequisites to achieve success rates with osseointegrated implants is the presence of a sufficient volume of healthy jaw bone at the recipient site. It can be successfully applied when suitable conditions exist, and now with the technique of guided bone regeneration (GBR), it is possible to fill osseous defects and insert implants (8-11). However, the question still exists as to what degree bone regeneration is achieved in areas where an immediately placed implant is not in intimate contact with the bony walls of the extraction site. It is imperative that osseointegration be verified in a standardized manner with properly controlled studies where histology can be obtained. Therefore, this study was designed to create a bone defect adjacent to the dental implant in order to simulate such defects often observed following placement of dental implants into fresh extraction sites or other anatomically challenging sites.

The purpose of this study was to compare the degree of osseointegration between HA-coated and uncoated threaded titanium dental implants placed into a surgically created bone defect with or without an expanded polytetrafluoroethylene (ePTFE) membrane.
Materials and Methods

Animals and anesthesia

Twelve adult male Japanese white rabbits (weighing 2.5 to 3.0 kg) were used in this study. They were kept in standard cages and fed with a standard laboratory diet and water. General anesthesia was induced by injecting pentobarbital sodium (Nembutal®, Abbott Laboratories, North Chicago, IL, USA) (0.3 ml/kg) via an ear vein. Local anesthesia with lidocaine-HCl (2 % Xylocain®, Astra Japan Ltd., Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) containing epinephrine 1:80,000 was used.

Implants and surgical technique

A total of 24 HA-coated and uncoated threaded titanium dental implants (HL: hex lock, Steri-Oss Inc., Yorba Linda, CA, USA) were used. The size of each implant was 3.25 × 8 mm.

The hind legs of each rabbit were shaved and washed with 70 % ethanol. The medial surfaces of both tibia were exposed via skin incision and careful subperiosteal dissection. A conventional implant site was prepared in the tibial metaphysis in accordance with the established technique outlined in the manufacturer's technical brochure. One implant was placed in each tibia. A total of 24 implants sites were prepared.

Twelve rabbits were divided into 3 groups: 1) conventional group - either a HA-coated or an uncoated implant was inserted into each prepared site (Fig. 1); 2) bone defect group - either a HA-coated or an uncoated implant was inserted into the site where approximately 3 mm width of bone defect was created surgically on the distal of each implant site (Fig. 2); and, 3) ePTFE group - after the same procedure was performed in the bone defect group, the implant sites were covered with ePTFE membranes (Oval 4, W. L. Gore and Associates, Inc., Flagstaff, AZ, USA) (Fig. 3). The surgery was performed under clean conditions and with generous irrigation using sterile saline. Thereafter, fascia and skin were sutured separately with a cut gut suture. The rabbits were administered intramuscular injections of penicillin (2,000,000 IU/5 ml; 0.1 ml/kg) postoperatively.

Preparation of specimens and histologic evaluation

After healing for 4 months, the rabbits were euthanatized with an overdose of pentobarbital. The implants with surrounding tissues were dissected out, fixed with 10 % neutral buffered formalin, dehydrated and embedded in polyester resin (Rigolac-2004, Rigolac-70F, Nisshin EM Co., Ltd., Tokyo, Japan). One sagittal undecalcified ground section (approximately 200 μm thick) from the central part of the implant and encompassing the entire bone defect/membrane and surrounding tissues of each specimen was prepared using a slow-speed diamond saw (Micro cutter, MC-201, Maruto, Tokyo, Japan). The sections were mounted on acrylic glass slabs, ground and polished to a final thickness of 100-120 μm, stained with basic fuchsin and methylene blue and examined under a light microscope (Olympus AH-2, Tokyo, Japan).

Histological examination, photography and morphometric measurement of the sections were performed using a light microscope equipped with a morphometric system connected to a personal computer (Macintosh Perfoma 588, Apple Computer Inc.,
The histomorphometric measurements of the central section obtained from each specimen were recorded using a computerized image analysis system. Slides taken at ×20 magnification were digitized using a solid state 35-mm slide scanner and a CCD linear photo diode array interfaced with the computer. The measurements were extracted from the digital images using an interactive image processing software package (Adobe Photoshop™ 2.5 J, Apple Computer Inc., Cupertino, CA, USA). The percentage of osseointegration between bone and implant surface was calculated at the 3 mm length apical to the inferior border of the periodontal collar on the distal end of each implant (Fig. 4). We measured the length of the implant facing the surrounding bone (A) and the length of surface border where the bone directly contacted the implant (B=b1+b2+b3+...). The percentage of osseointegration between bone and implant surface was calculated as follows: the percentage of osseointegration (%) = (B)/(A) ×100 (12).

Statistical analysis
Each value of the percentage of osseointegration between HA-coated and uncoated implants and among three groups was compared. Statistical analysis was performed with two-factor factorical ANOVA using StatView® Package Version 6.7 (Abacus Concepts, Inc., Berkeley, CA, USA). The significance level was 0.05 and the confidence interval was 95 %.

Results
Clinical observations
One leg of one rabbit of each group was broken and/or deteriorated. No complications were found among the other rabbits. In all, 21 implant sites were clinically stable and no clinical findings of adverse reactions or inflammation could be observed around implants.

Histologic evaluation
For all groups, generally focal areas of osseointegration were seen on both HA-coated and uncoated implants. However, for the HA-coated implants there appeared to be a considerably greater degree of osseointegration than for uncoated implants regardless of the difference of the treatment tested (Fig. 5, 6). The threads in the bone marrow were also in contact with either newly formed bone or with normal marrow tissue. No foreign-body reactions or signs of inflammation could be observed in any of the groups.

The histomorphometric analysis results are presented in Fig. 7. When comparing the percentage of osseointegration for all groups, a statistically significant difference was found for implant type (p = 0.0218). However, there were no statistically significant differences in either bone defect or ePTFE (p = 0.1238 and 0.6575, respectively).

Discussion
The results of this study demonstrated that for the HA-coated threaded dental implants there was a statistically significant increase in the degree of osseointegration relative to the uncoated implants in all groups. There are
several experimental studies demonstrating a more rapid and favorable bone response to HA-coated implants compared to uncoated ones (13, 14). The results of this study showed that the implant surface structure was a significant factor in the amount of osseointegration achieved in all groups.

There was no statistically significant difference in the use of ePTFE membranes. However, using it to treat surgically created bone defects adjacent to the implant promoted considerably better healing around both HA-coated and uncoated implants when measured histometrically than no generative treatment (the bone defect group). The presence of growth factors in the fresh extraction socket related to bone healing allows a quicker and more intense bone regeneration (15). Therefore, there was a tendency for significant differences between groups with and without ePTFE membranes, implying that the use of barrier membranes at bone defect sites is critical to maximizing the potential for bone regeneration.

In our study, the creation of the bone defects probably exposed bone matrix, which is considered to be one of the richest sources of growth factors (16). It was previously demonstrated that bone formation was activated by the release of growth factors and other bone-inducing substances. It is possible that the placement of the membrane helps to concentrate in the wound growth factors at inductive doses in the wound (8).

Zablotsky et al. (17) reported that HA-coated implants with ePTFE protection had an average of 95.2 % bone regeneration, while the grit-blasted titanium implants had 82.8 % bone regeneration. HA-coated control implants had an average of 55 % bone regeneration and un-coated titanium implants had an average of 39 % bone fill. There may be advantages to placing implants into extraction sockets and protecting them with an ePTFE membrane. The use of barrier membranes over the implants should prevent the connective tissue cells of the mucosa from entering the extraction sockets and protect the blood clot, which theoretically should give a total bone fill of the extraction socket around the implant. The results by Caudill et al. (19) supported the use of ePTFE membranes to enhance bony regeneration in artificially created bony defects around HA-coated implants in dogs.

The interface between bone and implant is dependent on the structure and nature of the bone at the recipient site. Generally, a much higher percentage of implant to mineralized tissue contact will be obtained when the implant is placed in compact bone compared to cancellous bone. Albrektsson and Sennerby (20) reported an implant surface contact with mineralized lamellar bone of up to 90 % when the implants were placed in cortical bone. The best results are reproducibly obtained when contact between the implant and the bone is most intimate at the implant placement. Schenk and Willenegger (21) described that the gap between the implant and the bone may not exceed 1 mm for reliable osseointegration. However, according to the principles of guided tissue regeneration (GTR) a much wider gap may be bridged by new bone formation. Many studies have demonstrated the value of GTR in allowing for osseous defect closure (8-11, 14-19).

In conclusion, HA-coated threaded titanium dental implants consistently demonstrated a greater amount of osseointegrated surface either in the conventional site or defect region with and without ePTFE membranes in this animal model. In clinical situations where there is a bone defect around an implant, poor bone quality, or suspected instability of a newly placed implant, a HA-coated threaded implant may be the best dental implant choice to establish greater osseointegration within the defect or conventional site. It appeared that the use of ePTFE membranes may further increase the degree of osseointegration of the newly formed bone around both HA-coated and uncoated implants. Further research will be needed to compare the degree of osseointegration of HA-coated threaded to cylindrical titanium implants.

**Fig. 6** Uncoated threaded implant in the conventional site. Note the lesser amount of osseointegration than that of HA-coated implant. This histological finding was observed in both bone defect and ePTFE groups.

**Fig. 7** Mean percentage of osseointegration by implant and treatment type for each group. * p < 0.05.
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References


