Biological performance of calcium phosphate films formed on commercially pure Ti by electron-beam evaporation

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Abstract

Thin and defect-free calcium phosphate films with a Ca/P ratio of 1.62 were formed by electron-beam evaporation. The as-deposited films had average bonding strengths to the metal implants of 64.8 MPa and the dissolution rates of 47.5 nm/h in isotonic saline solutions. The interface mechanical characteristics and histology of the as-machined, as-blasted, and calcium phosphate coating on the machined surfaces of commercially pure titanium were investigated. After a healing period of 12 weeks, the implants were unscrewed with a torque gauge instrument at the day of sacrifice. The coated samples showed a removal torque of 48.5 Ncm (SD 5.4) compared to 32.3 Ncm (SD 2.91) for the uncoated implant with the same surface roughness, and 47.3 Ncm (SD 5.8) for the grit blasted screw. The histomorphometric analyses of the calcium-phosphate-coated implants revealed a mean of 52.4% (SD 6.3) as the highest bone to implant contact. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Integration of implants in bone, without the subsequent development of an interfacial fibrous tissue, is very important. Not only is the use of a biocompatible implant material important but also other factors such as a proper implant configuration and a suitable implant surface finish, which are among several factors significantly influencing the direct bone to implant contact [1–4]. Several investigators have indicated that an implant with a rough surface might be a better candidate for implant integration than one with a smooth surface [5–8]. A rough surface provides the initial mechanical interlocking that prevents micromotion, and may be a prerequisite for direct bone apposition [5].

Titanium and its alloy Ti-6Al-4 V are attractive metals due to their strength, comparatively low stiffness, light weight and relative inertness. Titanium is currently employed in many implant designs in either pure or alloy form. Hydroxyapatite (HAp) is commonly applied to metallic implants as a coating material for fast fixation and firm implant–bone attachment [9–13]. Of the various coating methods, the plasma spraying technique is currently the primary method used commercially to produce the HAp coating on metallic implants [14–17].

However, concerns have been raised regarding the viable use and long-term stability of plasma-sprayed HAp coatings, which typically are relatively thick, highly porous with non-uniform density and especially, a poor bonding strength to the metal implants [18–19]. Furthermore, existing methods of obtaining HAp coatings have demonstrated a lack of control over the Ca/P ratio in the resultant coatings. Choi et al. [20,21] reported the formation of thin, defect-free calcium phosphate coatings with various Ca/P ratios. They demonstrated that the dissolution rate of the coating was dependent on the Ca/P ratio, and the bioactivity of the coating could be controlled.
In the present study, thin and defect-free calcium phosphate coatings with a Ca/P ratio of 1.62 was formed on CP Ti screws by electron beam deposition. In addition, the effects of the coating on the mechanical strength at the bone–implant interface of the machined surface were investigated and compared to the bone tissue reactions of the as-machined and the as-blasted surface of the implant.

2. Materials and methods

2.1. Preparation of implants

The 72 screw-shaped implants made of commercially pure Ti were machined in an identical manner. The outer diameter of the implants and the total length were 3.8 and 8 mm, respectively. Each implant had a square top to enable fixation of the removal equipment (Tohnichi, Japan). The implants were cleaned in trichlorethylene, and rinsed in absolute ethanol in an ultrasonic bath, then stored in a vacuumed desiccator for further treatment. The implants were divided into three groups. Each group had a different surface finish. They were as-machined (n = 24), calcium phosphate coating on machined surfaces (n = 24), and blasted surfaces (n = 24). For the as-blasted specimens, the implants were grit blasted with TiO2 particles of a mean particle size of 50 μm at the pressure of between 4 and 5 Kgf.

Thin calcium phosphate films were deposited to a thickness of 1 μm by electron beam evaporation. Prior to deposition, the substrates were etched with an Ar ion beam (120 V, 2 A) generated from a MarkIITM end-hall type ion gun (Commonwealth Scientific, Alexandria, VA). The details of deposition have been described elsewhere [20]. A special sample holder was employed to enable uniform deposition of the screw-shaped implants. The coating layers were analyzed by X-ray diffraction (XRD, M18XHF, Mac Science, Yokohama, Japan) and EDS (Oxford Instruments, Bucks, England). The film thickness was measured by a surface profiler (P-10, Tencor, Santa Clara, CA). Dissolution rate was measured with the surface profiler. After a half of the coating layer was covered with a water-resistant tape, the specimen was immersed in an isotonic saline solution. The dissolution rate was estimated from the film thickness dissolved during the period of time in the solution. The morphology of the sample was observed with a SEM (JSM-5310, JEOL, Tokyo, Japan).

2.2. Animals and surgical technique

Eighteen adult New Zealand white rabbits (average weight: 3 kg) were used for the experiments. They were anaesthetized with intramuscular injections of ketamine HCl (50 mg/ml, Ketalar, Yuhan Co., Korea) at a dose of 2 ml per kg body weight. Local anesthesia with 2% lidocane HCl (1:100,000 epinephrine, Yuhan Co., Korea) was administrated to the tibial metaphyses where the implants were to be inserted under aseptic conditions. Prior to surgery, the skin was carefully washed with a mixture of iodine and 70% ethanol. Each rabbit received four implants in two different surface treatments, either the as-machined surface or the as-blasted surface, or the calcium phosphate coating on the machined surface.

The two implants of identically treated surfaces were inserted in the same leg at a distance of 10 mm apart. The distal implant was used for measuring the removal torque, and the proximal one was preserved for the histomorphometric study. Holes were made with low rotary drill speeds (never exceeding 2000 rpm) and saline cooling was used. Tapping was made in the cortical layer only for the purpose of initial firm fixation. Fig. 1 shows the titanium implants inserted in rabbit tibial metaphysis. Immediately after surgery the animals were allowed full weight bearing.

2.3. Evaluations of animal test

At the day of sacrifice, the distal implants of each condition were unscrewed with a torque gauge instrument after a healing period of 12 week. When the screws were unscrewed, the removal torque force (Ncm) was directly measured with high reproducibility and low operator sensitivity. The surfaces of the unscrewed implants were examined later by a SEM.

The proximal implants were removed en bloc for qualitative optical microscopy and histomorphometric analysis. Specimens were dehydrated in graded ethanol (70%, 80%, 90%, 96%, and 100%) containing 0.4% basic fuchsin, and embedded in methylmethacrylate. A section axis was chosen parallel to the long axis of the implant. One central cut section taken from each specimen was ground and polished to a final thickness of 30 μm using a micro grinding system (Maruto Co., Japan). The surface was histologically stained for both qualitative and quantitative observation by optical microscopy (Olympus BX50, Olympus Co., Japan).

Using a CCD camera (Samsung Aerospace Ind., Korea) a computer-based morphometric assessment with Bildanalysystem AB (Styrelsens sate, Sweden) was performed.

2.4. Statistics

The data are presented as a mean value with the standard deviation in parentheses. The difference between the groups was evaluated by analysis of the variance statistics method. \( P < 0.05 \) was considered significant.
3. Results and discussion

3.1. Calcium phosphate film

Thin calcium phosphate films were deposited by electron-beam evaporation. In this method, the various Ca/P ratios of the films can be formed by evaporating hydroxyapatite targets containing different amounts of CaO. The Ca/P ratio of the film had significant effects on the dissolution rate of the coating layer, which have been exclusively studied by the present authors [20,21]. The film had a dissolution rate ranging from 0.6 to 90 nm/h in the non-heat-treated condition. This film was also highly dense, transparent, and had no distinctive features. Because of the defect-free coating layer and mixed interface zone, the as-deposited film had a very high bonding strength of 64.8 MPa compared to the bonding strength of the plasma spray coating, 5.3 MPa [20–22].

The calcium phosphate film with a Ca/P ratio of 1.62 was deposited on the implants, and the average dissolution rate in an isotonic saline solution was 47.5 nm/h as shown in Fig. 2. Since the as-deposited coating layer was amorphous as shown in Fig. 3(a), the calcium-phosphate-coated metal substrate was heated to 500°C with a ramp rate of 10°C/min and held for 1 h, then cooled down to room temperature at a cooling rate of 20°C/h. Such heat treatment procedures were enough to fully crystallize the coating layer as shown in Fig. 3(b), but did not create any cracks due to the mismatch in the thermal expansion coefficients between the coating layer and the substrate. Peaks specific to hydroxyapatite developed after heat treatment.

3.2. Removal torque

Twelve weeks after implant placement, the animals were sacrificed. Five animals died, which left 13 animals with eight implants of the calcium phosphate coating on the machined surface and eight implants of as-blasted surface, and 10 implants of as-machined surface for the removal experiment. All implants were stable in the
sense that they could not be removed using manual force without the aid of a torque gauge instrument.

The removal torque forces are shown in Fig. 4. The mean removal torque was 48.5 Ncm (SD 5.4) for the calcium-phosphate-coated implants while the as-machined implants had the mean torque of 32.3 Ncm (SD 2.91). The thin calcium-phosphate-coatings on the machined implants required a higher removal torque, approximately 1.5 times, than the as-machined ones. Calcium-phosphate-coated implants showed slightly higher removal torque force than sand-blasted implants of 47.3 Ncm (SD 5.8), but no statistical difference was obtained between the two materials.

The effects of surface roughness on bone fixation have been studied by many researchers [5–8] and it has been demonstrated that surface roughness is important for proper bone interlocking. Implants with a smooth surface were significantly less stable than when a rough surface was used. With time, the implants of a rough surface fix better in the implant bed, due to bone ingrowth into the surface irregularities [7]. The data generated in this study are consistent with previously obtained results, where the positive effects of a roughened surface were observed as compared to as-machined surface. However, a more important result is that the calcium phosphate coating on the machined surface, even though it had a smoother surface, required a higher force to unscrew the implants than the as-blasted one. Hydroxyapatite is a well-known biocompatible material [23]. It has the ability to bond to both osseous and epithelial tissues and is accepted by muscle tissue due to a composition similarity to the mineral phase of bone. The 1 μm-thick-HA coating formed by e-beam evaporation also indicated good biocompatibility and excellent bonding with bone tissue.

Fig. 5 shows SEM micrographs of the implants (a) before insertion of the as-machined implant, and after removal torque measurements of (b) as-machined, (c) calcium phosphate coating on the machined surface, and (d) as-blasted. Fig. 5(c) shows more bone tissue attached on the blasted surface, indicating more mechanical bone interlocking due to the rough surface finish. The calcium-phosphate-coated implants had a similar amount of bone tissue attached to the implant as the as-machined surface, but it required a much higher removal torque. These observations are consistent with the notion of a chemical bonding of the bone to the calcium phosphate coating. The calcium phosphate coating continuously dissolved when it contacted the tissue, and a local supersaturation of the constituent ions of the bone mineral phase, especially Ca, caused chemical bonding with surrounding bone tissues.

3.3. Histology

Fig. 6 shows the histomorphometrical data of the bone to implant contact in all threads and in the three best consecutive threads. The mean value of the bone to implant contact was 52.4% (SD 6.3) for the calcium phosphate coating on machined implants, which was much higher than 33.8% (SD 4.8) for the as-machined samples, and even higher than the 48.5% (SD 3.8) for the as-blasted ones. The corresponding values, in the three best consecutive threads were 62.5% (SD 5.2) for the calcium phosphate coating on machined implants, 54.2% (SD 5.4) for the as-blasted, and 38.2% (SD 3.5) for the as-machined implants. Thin and defect-free calcium phosphate coating certainly promoted the bony
contact, and this effect was easily seen in the three best consecutive threads.

The investigation of the implants/bone interface by optical microscopy showed new bone formation and direct bone contacts regardless of the surface condition (Fig. 7). The CP titanium implant with a machined surface had a certain degree of bone to metal contact. However, there was greater contact in the calcium-phosphate-coated implants. Surface roughness is also important for proper bone interlocking and improved bone to metal contact. The difference in the histologic findings clearly explains the observed difference in mechanical response. The higher removal torque is linked with a higher percentage of bone to implant contact.

The calcium phosphate coating provided an osteophilic substrate for bone proliferation, resulting in higher bone contact for the calcium phosphate layer on the machined surface compared to the same surface roughness of the as-machined implants. Of primary importance is the apparent mineralization associated with the osseous tissue directly on the calcium phosphate coating, in contrast to the appositional response to the blasted or machined implants. In some cases where the implants only had direct contact with the bone trabeculae by one corner (Fig. 7c), new bone was found to grow from the corner toward the other side. This suggests good osteoconductivity of the thin calcium phosphate coating.

Fig. 8 shows the bone volume in all the threads and in the three best consecutive threads. The mean value of the bone volume in all the threads (in the three best consecutive threads) was 76.4% SD 4.8 (82.4% SD 5.6) for the calcium phosphate coating on machined implants compared to 72.2% SD 5.9 (78.9% SD 5.2) for the as-blasted, and 74.8% SD 4.9 (79.0% SD 6.1) for the as-machined samples. The bone volume was not significantly affected by the surface finishing of the CP Ti implants since CP Ti is an excellent biocompatible material that illicits no acute inflammatory response. Multinucleated giant cells were observed irrespective of surface treatment (Fig. 9).
Delamination of the coating is critical and may be a major failure mechanism for the plasma-sprayed thick hydroxyapatite coating. It has been observed from push-out testing that hydroxyapatite-coated implants prepared by plasma spraying, failure occurred almost entirely (90%) at the hydroxyapatite coating/implant interface, which can influence the long-term stability of the implants [10]. Since the thin and defect-free calcium phosphate coating formed by electron beam evaporation had a bonding strength of 64.8 MPa compared to that of 5.3 MPa for the plasma-sprayed hydroxyapatite coating, the failure site after removal test was at the coating-tissue interface. Calcium-phosphate-coated implants were analyzed with X-ray photoelectron spectroscopy after removal test, and peaks originated from calcium phosphate coating not from bone fragments were observed. Since a XPS gives information on the out-most surface, the amount of survived calcium phosphate was not known. However, this suggest that the calcium phosphate coating was not completely dissolved even after 12 weeks in the animals.

If the calcium phosphate coating is applied to the blasted surface, bone tissue adaptation can be accelerated and a large improvement in removal torque can be expected when applied to the machined surface. Currently, the bone responses of the calcium phosphate coating on the blasted surface and the long-term behavior of the calcium-phosphate-coated implants are under investigation. The effects of dissolution rate on the osseointegration are also being studied.
4. Conclusions

The calcium phosphate films were deposited on screw-shaped commercially pure titanium implants by electron-beam evaporation. The film with a Ca/P ratio of 1.62 had a high bonding strength to the metal implant of 64.8 MPa and a dissolution rate of 47.5 nm/h in isotonic saline solutions. The as-deposited film was amorphous, transparent, and had no distinctive features. Peaks corresponding to hydroxyapatite developed after heat treatment.

The calcium-phosphate-coated implants required a removal torque of 48.5 Ncm (SD 5.4) compared to the un-coated, machined implants of 32.3 Ncm (SD 2.91). The surface-finished condition did not affect the bone volume. However, it significantly influenced the bone to implant contact and calcium-phosphate-coated implants had the most bone-to-metal contact.

References