Calcium Phosphate Films with/without Heat Treatments Fabricated Using RF Magnetron Sputtering*

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Abstract
Calcium phosphate coating films were fabricated on blast-treated titanium plates and screw-type titanium implants using RF magnetron sputtering. A uniform and dense coating film with a thickness of 0.5 μm could cover the blast-treated titanium plate efficiently, maintaining the surface roughness of the substrates. The as-sputtered coating films consisted of amorphous calcium phosphate (ACP) or oxyapatite (Ca₁₀(PO₄)₆O, OAP). Heat treatments of the OAp coating films were conducted in a silica ampoule or in air, and it was observed that the crystallinity of the coating films increased after the heat treatment. The bonding strength between the as-sputtered coating films, subjected to heat treatment in air, and the blast-treated titanium plates exceeded 60 MPa. An immersion test was conducted and alkaline phosphatase (ALP) activity of osteoblasts was investigated in vitro. The dissolution rate of the coating films in the 0.9% NaCl solution decreased with an increase in their crystallinity. The ACP coating film exhibited high ALP activity. As the in vivo evaluation, the coated and non-coated titanium implants were implanted into the femur of Japanese white rabbits. The percentage of bone-implant contact and the removal torque value of the coated titanium implants were greater than those of the non-coated titanium implants.

Key words: Calcium Phosphate, Titanium, Sputtering, Bonding Strength, Immersion Test, Implant, Animal Experiments

1. Introduction
Titanium and its alloys possess excellent mechanical properties, high corrosion resistance and biocompatibility¹, and have been used as a substitute for hard tissues in orthopedic and dental fields for long-term implantation. Titanium materials are considered to be the most biocompatible materials because they can be directly connected to living bones at the optical microscopic level, i.e., osseointegration³. However, it is difficult for the implant to load the stress immediately after the titanium is implanted into the bone, because a long time (about 3 months) is required to obtain a strong fixation between the
implant and the bone. The condition of the bone also affects the connection with the titanium implants\(^5\)(\(^6\)).

In order to obtain a rapid and strong fixation between a titanium implant and bone, many kinds of surface modifications and treatments have been studied, and calcium phosphate coating is one of the most effective techniques\(^7\). Although calcium phosphate coating by the plasma spraying have been clinically applied to dental implants and artificial joints, some problems related to the control of the microstructure of the coating film and the bonding strength between the implant and the coating film were reported\(^8\)(\(^9\)). It is known that a dense and uniform thin film with high bonding strength can be obtained using sputtering methods. In the previous studies, we had studied on the calcium phosphate coating films on mirror-polished commercially pure titanium and blast-treated titanium substrates using RF magnetron sputtering\(^10\)(\(^13\)). Amorphous calcium phosphate (ACP) and crystalline oxyapatite (\(\text{Ca}_10(\text{PO}_4)_6\text{O}_x\), OAp) phases were fabricated on blast-treated titanium substrates\(^13\). The crystallinity of the coating films is one of the parameters responsible for the biological performance of the implant. Heat treatment is a well-known technique for increasing the crystallinity of calcium phosphate coating films.

In this study, calcium phosphate coating films were fabricated on blast-treated titanium plates and screw-type blast-treated titanium implants using RF magnetron sputtering. The calcium phosphate coating films were heat-treated in a silica ampoule or in air. The bonding strength between the substrate and the coating film, the surface reactions of coating films in 0.9% \(\text{NaCl}\) solution and PBS(-) and alkaline phosphatase (ALP) activity of SaOS-2 cells were examined as in vitro evaluations of calcium phosphate coating films. The calcium phosphate-coated blast-treated titanium implants were implanted into the femur of Japanese white rabbits in order to evaluate the percentage of bone-implant contact and the removal torque value.

2. Materials and Methods

2.1 Fabrication of Calcium Phosphate Coating Films

Calcium phosphate coating films were fabricated on a blast-treated Ti-6Al-4V plate (10 mm × 10 mm × 1 mm) and screw-type blast-treated Ti-6Al-4V implant (φ3.3 mm × 8 mm, JMM) using RF magnetron sputtering (MS-320, Universal Systems Co., Ltd.) with hot-pressed \(\beta\)-tricalcium phosphate (\(\text{Ca}_3(\text{PO}_4)_2\), TCP) targets having a relative density of more than 99.6%. The blast treatment conditions of the plates were identical to those of the implants, and the average roughness (Ra) was 4.6 \(\mu\)m. The details of the sputtering technique and target fabrication have been reported elsewhere\(^10\). The substrates were not heated intentionally during the sputtering, and the substrate temperature was less than 373 K. The thickness of the coating film was maintained at 0.5 \(\mu\)m by controlling the sputtering.

<table>
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<tr>
<th>Deposition</th>
<th>ACP</th>
<th>OAp</th>
<th>OAp-VAC</th>
<th>OAp-AIR</th>
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<tr>
<td>RF power / W</td>
<td>100</td>
<td>150</td>
<td>150</td>
<td>150</td>
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<td>0.5</td>
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<td>50</td>
<td>50</td>
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<td>9.6</td>
<td>9.6</td>
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<tr>
<td>Post-heat-treatment</td>
<td>-</td>
<td>-</td>
<td>Silica ampoule</td>
<td>Air</td>
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<tr>
<td>time / ks</td>
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time. In order to increase the crystallinity of the calcium phosphate coating films, some coating films were heat-treated at 873 K for 7.2 ks. The coatings heat-treated in the silica ampoule and in air are hereafter referred to as OAp-VAC and OAp-AIR, respectively. The deposition conditions of calcium phosphate coating films are given in Table 1.

The phases of the calcium phosphate coating films were identified using X-ray diffraction (XRD) with a low incident angle (α-2θXRD, α = 1°). The surface and cross sections were observed using scanning electron microscopy (SEM, XL-30FEG, Philips).

### 2.2 In vitro Evaluation of Calcium Phosphate Coating Films

The bonding strength between the calcium phosphate coating film and the blast-treated titanium plate was evaluated using a mechanical strength tester (Romulus IV, Quad Group). An aluminum stud with epoxy glue (P/N 901106, Quad Group) was attached to the surface of the calcium phosphate coating film on a blast-treated titanium plate. The maximum load of pulling test of aluminum stud was recorded and used to evaluate the bonding strength between the coating film and the blast-treated titanium plate. More than five samples of one type of coating film were evaluated and the average value and standard deviations were calculated. The bonding strength of the epoxy glue used in this study was around 60-70 MPa.

The calcium phosphate-coated blast-treated titanium plates were subjected to an immersion test. Specimens were immersed in 15ml 0.9% NaCl solution or 15 ml PBS(-) at 310 K for three days. The chemical composition of the 0.9% NaCl solution and PBS(-) is given in Table 2. In order to examine the amount of dissolution from the coating films to the 0.9% NaCl solution, the concentration of calcium and phosphorus ions was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, ICPS-8100, Shimadzu). The 0.9% NaCl solution was filtered using a membrane filter with a pore diameter of 0.2 µm, and the ion concentration in the filtered solution was measured. In order to examine the apatite formation on the coating films, the calcium phosphate-coated blast-treated titanium plates were immersed in PBS(-). The specimen surface was then analyzed using XRD and SEM.

The effect of the calcium phosphate coating on the ALP activity was investigated by the cell culture test using SaOS-2 cells (RCB0428, Riken BRC). The details of the evaluation of ALP activity have been reported elsewhere(11). SaOS-2 cells (5 × 10^5) in 1 ml media were plated on non-coated and calcium phosphate-coated blast-treated titanium plates and HAp disks (CELLYARD, HA pellets, 12-000-008, Asahi Glass). The cells were cultured for 2, 4 and 8 days. The DNA contents and ALP activities of the cell lysates were examined. In this study, the ratio of the ALP activity to the DNA content (ALP/DNA) was considered to be the first-step osteogenic differentiation scale.

### 2.3 In vivo evaluation of calcium phosphate coating films

Screw-type blast-treated titanium implants were implanted into the femur of Japanese white rabbits (male, 3 kg) and the percentage of bone-implant contact and the removal torque were evaluated. ACP-coated, OAp-VAC-coated and non-coated blast-treated titanium implants were used as specimens. Both left and right side of the femurs were given three implants each other. 2 to 4 weeks after implantation, the implants from the left side of the femur were cut off with the surrounding bone. These sections were stained with toluidine blue (shown in Fig. 1) and examined using an optical microscope. Non-decalcified

| Table 2. Chemical composition of 0.9% NaCl solution and PBS(-) / mM |
|-----------------|-----|-----|-----|
|                 | NaCl| KCl | Na₂HPO₄| NaH₂PO₄|
| 0.9% NaCl       | 145.4|      |       |       |
| PBS(-)          | 136.9| 2.68 | 8.10  | 1.67  |
specimens were used for a histological examination. The percentage of bone-implant contact ($C_{B-I}$) is given by the following equation,

$$C_{B-I} = \frac{B}{A} \times 100 \quad (1)$$

where $A$ is the length of the interface between the blast-treated titanium implant and the cortical bone and $B$ is the length of the bone in direct contact with the blast-treated titanium implants. $A$ and $B$ were evaluated using an optical microscope. The blast-treated titanium implants implanted on the right side of the femur were removed using a torque calibrator (ATG24CN BTG150CN, Tonich) and the removal torque value was measured. The differences in the percentage of bone-implant contact and the removal torque value between calcium phosphate-coated and non-coated blast-treated titanium implants were statistically analyzed by using Student’s $t$ test. The statistical significance was assumed at $p < 0.05$.

3. Results

3.1 Fabrication of Calcium Phosphate Coating Films

Figure 2 shows the XRD patterns of the ACP, OAp, OAp-VAC and OAp-AIR coating films fabricated on blast-treated titanium plates. No crystalline peak was observed in the patterns of the ACP coating film. The crystallinity of the OAp coating film increased after the heat treatment, and after heat treatment in air, the formation of titanium oxides was observed.

Figure 3 shows the SEM images of the surface and cross section of the OAp and OAp-VAC coating films fabricated on blast-treated titanium plates. The surface roughness of the blast-treated titanium plate was maintained even after the coating because the thickness of the coating film was 0.5 µm. Dense and uniform coating films could cover the blast-treated titanium plate efficiently. It was observed that there were no cracks in the OAp-VAC coating film after heat treatment in the silica ampoule; moreover there was no detachment between the coating film and the blast-treated titanium plate.

3.2 In vitro evaluation of calcium phosphate coating films

The bonding strength between the blast-treated titanium plate and the coating films is shown in Fig. 4. The bonding strengths of the ACP, OAp and OAp-AIR coating films exceeded 60 MPa, and the bonding strength of OAp-VAC was around 40 MPa.

The concentration of the calcium and phosphorus ions eluted from the ACP, OAp, OAp-VAC and OAp-AIR coating films fabricated on blast-treated titanium plates immersed into the 0.9% NaCl solution for three days is shown in Fig. 5. The amounts of calcium and phosphorus ions eluted from the ACP coating films were the greatest, while those eluted from the OAp-AIR coating film were the lowest. It appears that the amount of ions eluted from the calcium phosphate coating film decreased with an increase in the crystallinity of the coating films.
Figures 6 and 7 show the XRD patterns and SEM images of the surfaces of the ACP, OAp, OAp-VAC and OAp-AIR coating films fabricated on blast-treated titanium plates after immersion in PBS(-) for three days. Reflections attributed to apatite were detected in the ACP-coated specimen (Fig. 6). The network structure of apatite was observed on the surfaces of the ACP-coated and OAp-coated specimens, while no significant change was observed in the morphologies of the OAp-VAC and OAp-AIR coatings after the immersion test.

The value of ALP/DNA of the SaOS-2 cells in the ACP-coated, OAp-VAC-coated, non-coated blast-treated titanium plates and in the HAp disk after culturing them for 2, 4
and 8 days is shown in Fig. 8. The ALP activity of the SaOS-2 cells on the ACP-coated and OAp-VAC-coated blast-treated titanium plates was significantly greater than that on the non-coated blast-treated titanium plate for all cultured periods. In the osteogenic differentiation lineage, first, the ALP/DNA values of the osteoblasts increase, followed by a gradual decline. The ALP/DNA values of the ACP coating and the OAp-VAC coating were the greatest after 4 days; these values then started decreasing after the 8th day. Therefore, it should be noted that the calcium phosphate coating enhances the osteogenic differentiation of osteoblasts on the surface of the titanium implant. Especially for the ACP coating film, since the ALP/DNA value increased as much as HAap disk, the ACP coating appears to possess the capability to accelerate osteogenic differentiation.

3.3 In vivo evaluation of calcium phosphate coating films

Figure 9 shows the percentage of bone-implant contact (CBI) for the ACP-coated, OAp-VAC-coated and non-coated blast-treated titanium implants. In the case of all
coatings, the $C_{b-1}$ increased with implantation time, suggesting that osseointegration would be developed. After 2 weeks of implantation, the $C_{b-1}$ values of the ACP-coated and OAp-VAC-coated implants were almost the same as that of the non-coated implant. After 4 weeks, however, the $C_{b-1}$ values of the ACP-coated and OAp-VAC-coated implants were greater than that of the non-coated implant without significant difference between non-coated and coated specimens.
Fig. 8 ALP activity of SaOS-2 cells on non-coated, ACP-coated and OAp-VAC-coated blast-treated titanium plates and HAp disks for 2, 4 and 8 days after they were cultured (B-L × 16.7 = U/l, 310 K) (*p < 0.05).

Fig. 9 Percentage of bone-implant contact for non-coated, ACP-coated and OAp-VAC-coated blast-treated titanium implants 2 and 4 weeks after implantation.

Fig. 10 Removal torque values of non-coated, ACP-coated and OAp-VAC-coated blast-treated titanium implants 2 and 4 weeks after implantation (*p < 0.05).
Figure 10 shows the removal torque values of ACP-coated, OAp-VAC-coated and non-coated blast-treated titanium implants from the femur of Japanese white rabbits after 2 and 4 weeks of implantation. The removal torque increased with the duration of implantation, and the values for the ACP-coated and OAp-VAC-coated implants were greater than that of non-coated implants. It was statistically improved by the coating of ACP films 2 weeks after the implantation (p < 0.05).

4. Discussion

The bonding strength between the coating film and the substrate is one of the most important parameters. The bonding strengths of calcium phosphate coating films of this study were greater than those of plasma spraying (20-30 MPa)\(^ {15-17}\) and the values of ACP, OAp and OAp-AIR coating films satisfied the ISO requirement (50.8 MPa)\(^ {18}\). A decrease in the bonding strength of the OAp-VAC coating film was observed. Some studies on the heat treatment of calcium phosphate coating films have been reported. Ong et al.\(^ {19}\) reported that there is a decrease in the bonding strength of calcium phosphate coatings with a thickness of 1 µm fabricated using ion beam sputtering after heat treatment at 873 K for 3.6 ks in air. This decrease in the bonding strength was attributed to the cracks in the coating films caused by heat treatment. These cracks developed because of the difference in the heat expansion coefficient between the coating film (13.7 \( \times 10^{-6} \) K\(^{-1} \)) and the substrate (8.4 \( \times 10^{-6} \) K\(^{-1} \)). In this study, no cracks were observed in the SEM images of the film after heat treatment (shown in Fig. 3). However, the bonding strength of OAp-VAC did decrease; this decrease can possible be attributed to microcracks in the film. Titanium oxide phases were observed in the OAp-AIR coating film. It was thought that the crystalline OAp phase would be comprised in the oxide layers and that might caused the high bonding strength of OAp-AIR.

In the immersion test, the amount of ions eluted into the 0.9% NaCl solution was the highest for the ACP coating and it decreased in the order OAp > OAp-VAC > OAp-AIR, as shown in Fig. 5. The amount of ions eluted from the films into the solution depends on the order of crystallinity of the coating film. Some studies have reported on the dissolution behavior of calcium phosphate coating films fabricated on silicon\(^ {20,21}\) and titanium\(^ {22}\) substrates using RF magnetron sputtering. These studies reported that the rate of dissolution of ions from the ACP film was higher than the rate of dissolution of ions from the crystalline film in calcium-free Hanks’ balanced salt solution\(^ {21}\) and in Kokubo solution\(^ {20,22}\). Khor et al.\(^ {23}\) carried out immersion tests in Kokubo solution using calcium phosphate films coated by high velocity oxy-fuel spraying on Ti-6Al-4V substrates, and observed the formation of apatite on the film. They reported that the precipitation rate of apatite was influenced by the calcium ion concentration adjacent to the surface of the film. They also reported that the preferential dissolution of \( \alpha \)-TCP, tetracalcium phosphate and the ACP phases in the films accelerated the precipitation of apatite. In this study, since the ACP and OAp coatings exhibited a high dissolution rate into the 0.9% NaCl solution and apatite formation was observed after immersion in PBS(-), the high dissolution rate of the ACP and OAp films may be closely related to the apatite formation on the surface.

ALP measurements have been widely conducted to assess the bone-conducting capability of implant materials. HAp is already well-known to be a bio-active implant material suitable for bone-rehabilitation. It should be noted that ACP actually increased ALP, slightly inferior but still comparable to HAp.

Some studies have reported on the in vivo investigation of calcium phosphate coatings fabricated using RF magnetron sputtering\(^ {24-26}\). Cylindrical titanium implants with ACP coating films and crystalline coating films obtained by the heat treatment of the ACP were implanted in the mandible of foxhound dogs, and the ultimate interfacial strength between
the implant and bone was evaluated\(^{(24)}\). 3 weeks after implantation, the ultimate interfacial strength of the ACP coating film was greater than that of the crystalline coating film; however, 12 weeks after implantation, the ultimate interfacial strength of the ACP coating film was almost the same as that of the crystalline coating film. They mentioned that the fact that ultimate interfacial strength of the ACP coating was high in the initial stage of implantation (3 weeks) was indicative of rapid bone formation in the presence of ACP coating with high dissolution rate. These results are in good agreement with our results, that is, the removal torque values of ACP coating obtained by us, and it should be noted that the ACP coating is effective for rapid bone formation during the initial stage of implantation. The average removal torque value of the OAp-V AC coating film was higher than that of the non-coated film; however, statistical differentiation was not observed because of the high standard deviation of the removal torque values. It is believed that this high standard deviation is caused by the low bonding strength of the OAp-V AC coating film. It appears that heat treatment of the calcium phosphate coating films is effective in increasing the crystallinity and stability in simulated body fluids. It should be noted that heat treatment of the coating film in a silica ampoule might decrease the bonding strength of the coating film.

5. Conclusions

Calcium phosphate coating films were fabricated on blast-treated titanium plate and screw-type blast-treated titanium implants using RF magnetron sputtering. The bonding strength, surface reactions in simulated body fluids and ALP activity of coating films with/without heat treatments were examined as in vitro evaluation. Further animal experiments were conducted as in vivo evaluation, yielding the following results.

1. A dense and uniform coating film with a thickness of 0.5 µm could cover the blast-treated titanium plate efficiently, and the surface roughness of the blast-treated titanium plate was maintained even after the coating. Neither cracks in the film nor detachment of the film was observed at the SEM level before and after heat treatment. It was observed that the coating film consisted of amorphous calcium phosphate (ACP) and oxyapatite (OAp) phases.

2. The crystallinity of the OAp coating film increased after heat treatment in a silica ampoule (OAp-V AC) and in air (OAp-AIR). Titanium oxides were also detected after heat treatment in air.

3. The bonding strength between the as-sputtered coating films and the blast-treated titanium plates exceeded 60 MPa, while that of the OAp-V AC coating film was around 40 MPa. The bonding strengths obtained in this study were greater than that of plasma-sprayed one, and seem to be enough for the implantation.

4. The amount of ions eluted from the films into the 0.9% NaCl solution increased with a decrease in the crystallinity of the coatings. The dissolution rate for the ACP was the highest. The high dissolution rates of the ACP and OAp films may be closely related to the apatite formation on the surface of these films in PBS(-).

5. The calcium phosphate coating improved the ALP activity of the SaOS-2 cells and it should be noted that the calcium phosphate coating enhances the osteogenic differentiation of osteoblasts on the surface of the blast-treated titanium plate.

6. The removal torque value of the calcium phosphate coating on the blast-treated titanium implant, taken from the femur of Japanese white rabbits, was greater than that of the non-coated one. This result suggests that the calcium phosphate coating films fabricated on titanium materials using RF magnetron sputtering are effective in improving the biocompatibility with the bone.
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