

Evaluation of Calcium Phosphate Coating Films on Titanium Fabricated Using RF Magnetron Sputtering

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Calcium phosphate coating films fabricated on commercially pure titanium (CP-Ti) substrates using radiofrequency (RF) magnetron sputtering were evaluated *in vivo* and *in vitro* for investigating their applications in dental and medical implants. For the *in vitro* evaluations of the calcium phosphate coating films, the bonding strength and alkaline phosphatase (ALP) activity were examined. The bonding strength of the coating films to a polished titanium plate exceeded 60 MPa. When compared with an uncoated titanium plate, the increase in the ALP activity of SaOS-2 cells (a well-characterized osteosarcoma human cell line exhibiting osteoblast-like properties) on a titanium plate coated with a calcium phosphate film was confirmed by a culture test. Titanium cylinders coated with an amorphous calcium phosphate film were implanted into the mandibles of beagle dogs. The percentage of bone-implant contact in coated titanium was greater than that in uncoated titanium. [doi:10.2320/matertrans.48.307]

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1. Introduction

Since titanium and its alloys can be directly connected to living remodeling bones at the light microscopic level, *i.e.*, osseointegration,^{1,2)} they have been widely used in dental and medical implants. In fact, almost all dental implants are made of commercially pure titanium (CP-Ti) or $\alpha + \beta$ type titanium alloys such as Ti-6Al-4V. The fixation between the dental implants and bones might be influenced by the state of the bones and the possible length of the implants. Coating of titanium implants with calcium phosphate is one of the methods used to improve their osseointegration. Currently, plasma spraying is the primary method used commercially to fabricate a calcium phosphate coating on dental implants. However, plasma-sprayed calcium phosphate coating exhibits a poor adherence to titanium substrates and nonuniformity; a critical thickness is required to ensure complete covering of the implant surface, resulting in delayed failures associated with inflammatory diseases.³⁾ We examined the fabrication of calcium phosphate thin films on CP-Ti substrates using radiofrequency (RF) magnetron sputtering with hot-pressed β -tricalcium phosphate (β -TCP) plates as the sputtering target and reported the phase, deposition rate, and preferential crystallographic orientation of the calcium phosphate films⁴⁾ and their reactivity in simulated body fluids.⁵⁾ The RF magnetron sputtering has several advantages such as a low processing temperature and excellent adherence to metallic substrates. The low processing temperature of the RF magnetron sputtering is suitable for coating calcium phosphate onto titanium alloys because the mechanical properties of the titanium alloys would be degraded by high processing coating temperatures. In

addition to that, thin calcium phosphate films fabricated with RF magnetron sputtering might be able to maintain the roughness of titanium implants such as dental implants. Although *in vivo* evaluations of RF-magnetron-sputtered calcium phosphate films on titanium have been studied,⁶⁻¹⁰⁾ the effects of the phase and crystallinity of calcium phosphate films on their performance have not been understood in detail. The stability of calcium phosphate materials is regarded to be composition- and structure-dependent.¹¹⁾ The films fabricated using RF magnetron sputtering can function as model materials in order to investigate the properties of calcium phosphate because of their dense, smooth, and homogeneous structure,¹²⁾ compared with those fabricated with other methods such as sol-gel coating, biomimetic coating or plasma spraying.

In the present study, the bonding strength and alkaline phosphatase (ALP) activity were examined for the *in vitro* evaluations of calcium phosphate films on titanium plates, and titanium cylinders coated with amorphous calcium phosphate films were implanted into the mandibles of beagle dogs in order to evaluate *in vivo* the biocompatibility of calcium phosphate films with bones.

2. Experimental

Calcium phosphate films were fabricated on CP-Ti (JIS Grade 2) substrates using RF magnetron sputtering (MS-320, Universal Systems Co., Ltd.) with hot-pressed β -TCP targets having a relative density in excess of 99.6%. A titanium plate (10 mm \times 10 mm \times 1 mm) and a cylinder (3.3 mm in diameter and 10 mm in length) were used as the substrates. The titanium plate substrate was finally polished with an Al₂O₃ paste (0.3 μ m), while the surface of the titanium cylinder substrate was as-machined. The average roughness (Ra) of

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Table 1 Deposition conditions

Evaluations		Deposition conditions				
		Substrate	Thickness/ μm	RF power/W	Total pressure/Pa	Oxygen gas concentration in sputtering gas (%)
Bonding strength	OAp	Plate	0.5	150	0.5	0
	c-OAp	Plate	0.5	150	5	0
	ACP	Plate	0.5	150	0.5	20
	ACP-1000	Plate	1	150	0.5	20
ALP activity		Plate	0.5	150	0.5	0
Animal experiment		Cylinder	0.5	75	0.5	0

the polished titanium plate was less than $0.05\ \mu\text{m}$. Before sputtering, the titanium substrates were ultrasonically cleaned in acetone for 600 s. Ar and O_2 were used as the sputtering gases, and the total gas flow rate was maintained at $3.3 \times 10^{-7}\ \text{m}^3 \cdot \text{s}^{-1}$. The substrate was not intentionally heated. The details of the sputtering technique and target fabrication have been reported elsewhere.⁴⁾ With regard to the titanium cylinder substrates, sputtering was conducted three times with 120° rotation to ensure a uniform covering of the entire substrate surface with the film. The phase of the films was determined using X-ray diffraction (XRD) with a low incident angle (α - 2θ XRD, $\alpha = 1^\circ$) or θ - 2θ XRD. The cross section of the films was evaluated using a scanning electron microscope (SEM, XL30FEG, Philips). The infrared spectra of the films were measured using a reflection-mode Fourier-transform infrared (FTIR) spectroscope (FT/IR-460 Plus, JASCO).

The bonding strength of the film to the titanium plate substrate was evaluated using a mechanical strength tester (Romulus IV, Quad Group). An aluminum stud with epoxy glue (P/N 901106, Quad Group) was set to the surface of the calcium phosphate coating film on a polished titanium plate. The thickness of coating film was either $0.5\ \mu\text{m}$ or $1\ \mu\text{m}$. The area of the surface coated with the epoxy glue was approximately $5.7\ \text{mm}^2$. After curing the specimen at 423 K for 3.6 ks in air, the aluminum stud was pulled in tension, and the maximum load was recorded and used to evaluate the bonding strength of the coating film to the titanium substrate.

SaOS-2 cells (RCB0428, Riken BioResource Center Cell Bank, Tsukuba, Japan), a well-characterized osteosarcoma human cell line exhibiting osteoblast-like properties,¹³⁾ were routinely cultured in Eagle's α -modified minimum essential medium supplemented with 10% fetal bovine serum. SaOS-2 cells (5×10^5) in 1 ml media were plated in a 24-well polystyrene culture dish (control) (Code 3820-024, Iwaki Brand, Asahi Techno Glass, Tokyo, Japan) as well as on titanium plates covered with calcium phosphate films, titanium plates, and hydroxyapatite disks (CELLYARD, HA pellets, 12-000-008, Asahi Glass, Tokyo, Japan) placed in nonadhesive (untreated) culture dishes (Code 1000-035, Iwaki Brand, Asahi Techno Glass, Tokyo, Japan). Then, the cells were cultured for 3, 7, 14, and 21 days ($n = 3$ for each condition), while medium exchange was conducted every 3 days. Cells were collected by centrifugation, washed twice with PBS (–) and dissolved in PBS (–) containing 1 mass% Triton X. The DNA contents and ALP activities of cell lysate were examined with fluorescent DNA quantitation kit (BioRad, Hercules, CA, USA) and ALP test B kit (Wako

Chemical, Osaka, Japan), respectively. 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.

A total of 12 male beagle dogs (10–14 kg) were used in the animal experiments. Amorphous calcium phosphate film with a thickness of $0.5\ \mu\text{m}$ was coated on the titanium cylinder substrate. The coated titanium cylinders were inserted into bone cavities created in the mandibles by the extraction of teeth three months prior to the experiment. The animals were sacrificed 2, 4, 8, or 12 weeks after the implantation. The sections were stained with toluidine blue and examined using an optical microscope. Non-decalcified specimens were used for a histological examination. Difference in percentages of bone-implant contact between ACP coated and uncoated titanium implants were statistically analyzed by Student's *t* tests. Statistical significance was assumed at $p < 0.05$.

Table 1 lists the deposition conditions of the calcium phosphate coating films used for in vivo and in vitro evaluations. Four types of coating films were used for the adherence test, which will be explained in the next section.

3. Results and Discussion

3.1 In vitro evaluation

Figure 1 shows the XRD patterns of the calcium phosphate films fabricated on the titanium plate, which were used for the adherence test. The calcium phosphate films were made of amorphous calcium phosphate (ACP), oxyapatite (OAp, $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$) and c-face-oriented oxyapatite (c-OAp) under different deposition conditions (see Table 1). The peak at $2\theta = 25.9^\circ$ in Fig. 1 denotes the (002) face of OAp, that is, the c-face. The thickness of the OAp and c-OAp films was approximately $0.5\ \mu\text{m}$; the thickness of the ACP film was either $0.5\ \mu\text{m}$ or $1\ \mu\text{m}$, which was controlled by varying the deposition time. Figure 2 shows an example of the cross section of the film (ACP with a thickness of $0.5\ \mu\text{m}$) fabricated on a titanium plate. The calcium phosphate film is dense and uniform, which is a characteristic of an RF-magnetron-sputtered film. Figure 3 shows the bonding strength calculated using the maximum load in the adherence test. Bonding strengths ranging from 60 to 80 MPa were obtained, which were independent of the phase or thickness of the coating films. Figure 4(a) shows the specimen surface coated with an OAp film after the adherence test. Epoxy glue is detected on the coating film. A schematic illustration of the

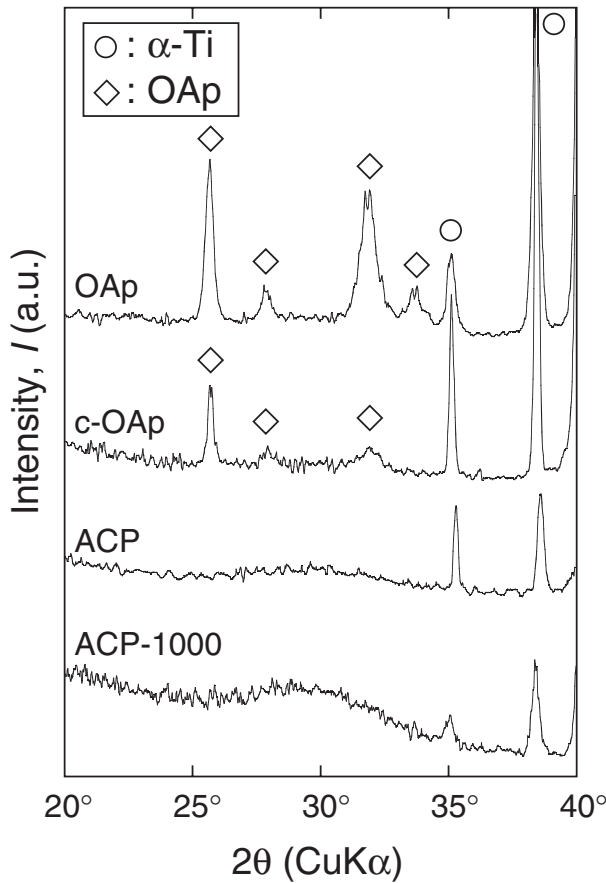


Fig. 1 XRD patterns of calcium phosphate films on titanium plate for the adherence test.

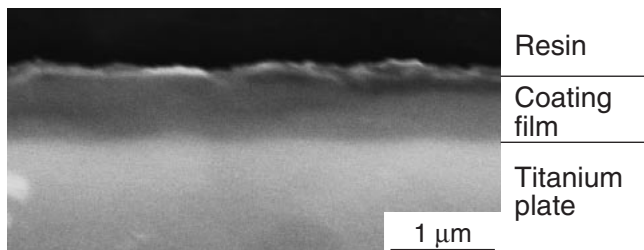


Fig. 2 Cross section of calcium phosphate film on titanium plate for the adherence test.

surface region with the epoxy glue is in Fig. 4(b). The detachment of the coating film from the titanium plate substrate was not observed for all the specimens. These results suggest that detachment occurred at the interface between the coating film and epoxy glue or in the interior of the epoxy glue. Therefore, it is improbable that the values shown in Fig. 3 reflect the bonding strength of the coating film to the substrate. However, it can be concluded that the bonding strength of the coating film to the polished titanium plate is greater than 60 MPa. Some studies have been performed on the bonding strength of sputtered calcium phosphate films.^{14–18)} Ong *et al.*,¹⁴⁾ Ding *et al.*,¹⁵⁾ and Lee *et al.*¹⁶⁾ evaluated the bonding strength of calcium phosphate films fabricated by ion-beam sputter deposition, RF magnetron sputtering, and electron beam evaporation with ion bombardment, respectively, using the same adherence testing

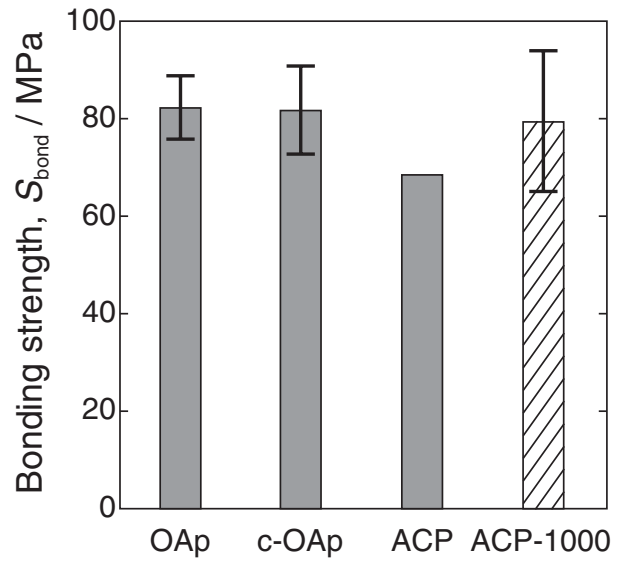


Fig. 3 Bonding strength calculated using the maximum load in the adherence test.

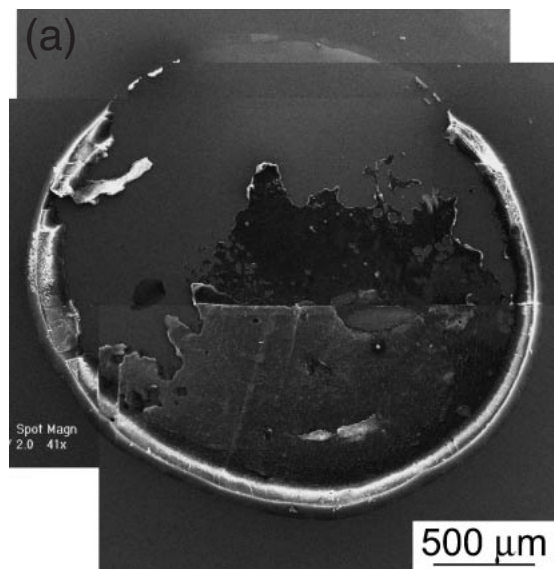


Fig. 4 (a) Scanning electron micrograph of the surface of the OAp-coated specimen after the adherence test and (b) schematic illustration of remaining epoxy glue on the surface.

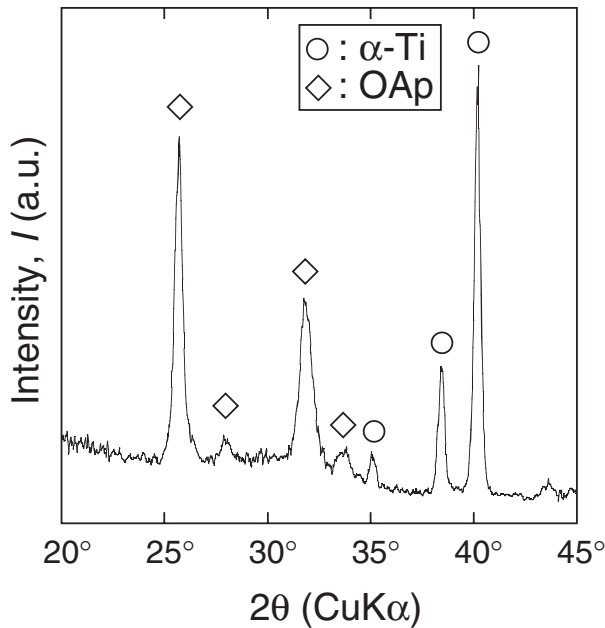


Fig. 5 XRD pattern of calcium phosphate coating film on titanium plate for the evaluation of ALP activity.

method used in this study. They reported bonding strengths ranging from 40 to 65 MPa for as-sputtered films, which are comparable to the present results. These values are greater than the bonding strength (20–30 MPa) reported for plasma-sprayed calcium phosphate films fabricated on blast-treated titanium substrates.^{19,20} It has been pointed out that the bonding strength of a calcium phosphate coating film is influenced by heat treatments and immersion in simulated body fluids.^{14,15} A study on the effect of the post-treatment of calcium phosphate coating films on the bonding strength is currently in progress.

Figure 5 shows the XRD pattern of the coating film fabricated on the titanium plate substrate, which was used for evaluating ALP activity. The phase of the film was OAp. The ALP production per DNA production of SaOS-2 cells in the OAp-coated titanium plate is shown in Fig. 6. When compared with the uncoated titanium plate, an increase in the ALP activity was observed on the OAp-coated titanium plate 3 and 7 days after the cells were cultured. ALP activity (ALP/DNA value) can be considered to be osteoblastic phenotypic marker and an indicator of the first stage osteoblastic differentiation.^{13,21} In the osteogenic differentiation lineage, first, the ALP/DNA value of the osteoblasts increases, followed by a gradual decline. Therefore, it can be pointed out that a calcium phosphate coating enhances the osteogenic differentiation of osteoblasts on the surface of titanium implants. It was reported that ALP is related to the surface roughness of substrates.²¹ The higher ALP activity on hydroxyapatite as compared to OAp-coated titanium can be partly attributed to the greater surface roughness of the hydroxyapatite than that of the titanium plate.

3.2 *In vivo* evaluation

Figure 7 shows the FTIR spectrum of the calcium phosphate coating film fabricated on a titanium cylinder used in the animal experiments. The peaks at 550–600 cm^{-1}

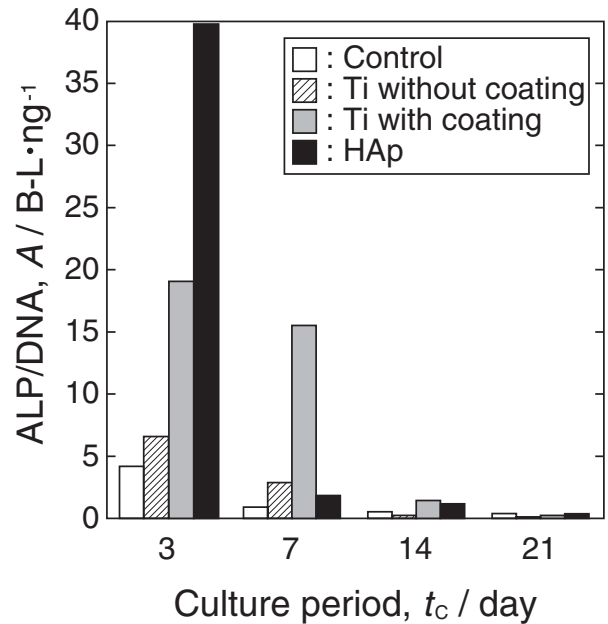


Fig. 6 ALP activity of SaOS-2 cells 3, 7, 14, and 21 days after they were cultured. ($B-L \times 16.7 = U/l$, 310 K)

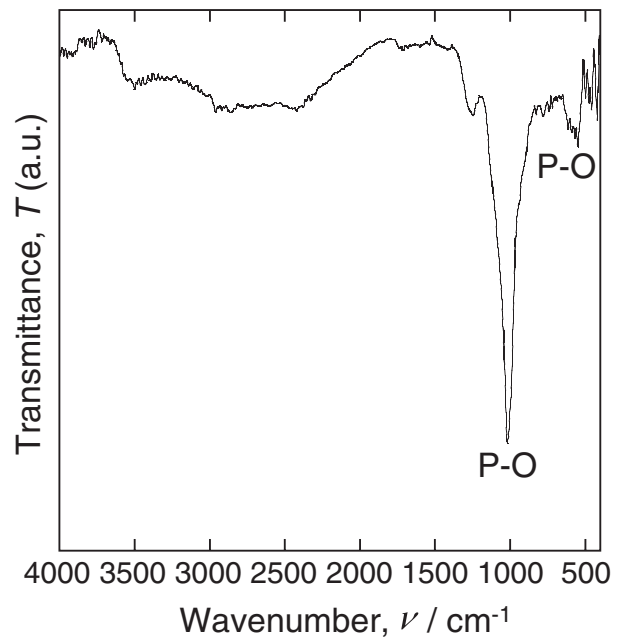


Fig. 7 FTIR spectrum of the coating film fabricated on titanium cylinder used in the animal experiments.

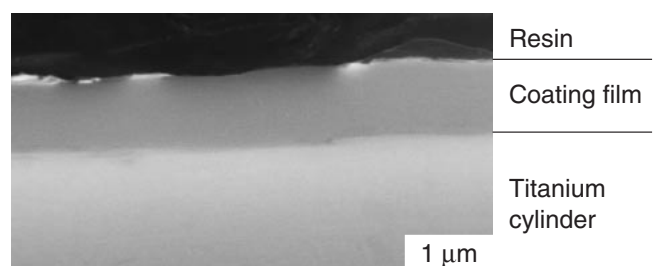


Fig. 8 Scanning electron micrograph of the cross section of the coating film on titanium cylinder.

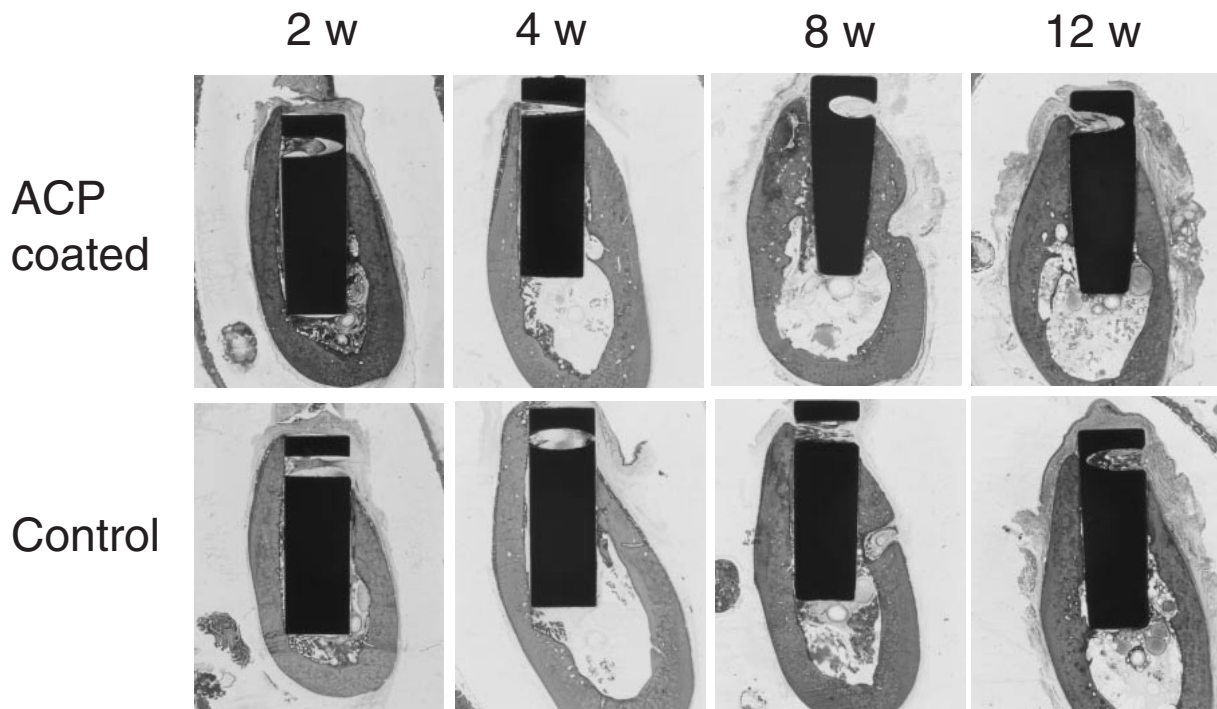


Fig. 9 Optical micrographs of the interface between the titanium cylinder and bone 2, 4, 8, and 12 weeks after implantation.

and 1050 cm^{-1} , which are attributed to the P–O bonds of the calcium phosphate materials,²²⁾ are detected. No reflections were detected in the micro-area XRD pattern of the coating film on the titanium cylinder. These results suggest that the coating film on the titanium cylinder is ACP. The low RF power during sputtering resulted in the formation of the ACP phase (see Table 1).⁴⁾ Figure 8 shows the cross section of the ACP film fabricated on a titanium cylinder. The dense and uniform film can be fabricated even on a titanium cylinder. Figure 9 shows the optical micrographs of the interface between the titanium cylinder and bones 2, 4, 8, and 12 weeks after implantation. The bone-implant contact reflects the biocompatibility of implants, which is shown in Fig. 10. The percentage of bone-implant contact for the ACP-coated specimen was greater than that for the uncoated specimen 8–12 weeks after implantation. It has been reported that the crystalline calcium phosphate coating film formed by sputtering methods enhanced the biocompatibility of titanium materials with bones.^{6–10)} In the present study, the application of sputtered ACP films as coatings on titanium implants was suggested to be effective for their rapid and strong fixation with bones. The low processing temperature required in the sputtering process of ACP films could prove as an advantage for its coating on implants made of β -type titanium alloys compared with other coating methods having high-temperature processes or post heat treatments such as sol-gel coating and NaOH treatment method, because the microstructure of β -type titanium alloys change due to the high processing temperature during coating.

4. Conclusions

The calcium phosphate films fabricated on CP-Ti sub-

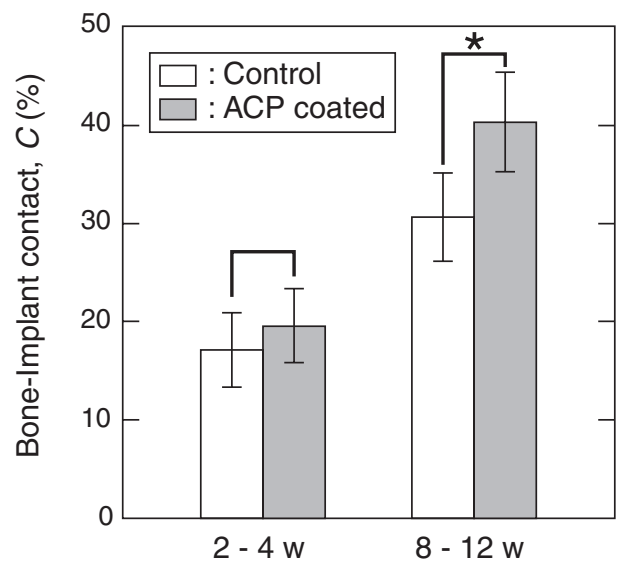


Fig. 10 Percentage of bone-implant contact for ACP-coated and uncoated (control) titanium cylinders (* $p < 0.05$).

strates using RF magnetron sputtering were evaluated in vivo and in vitro as coatings on titanium implants in dental and medical applications. The following results were obtained:

- (1) The bonding strength of the calcium phosphate coating film fabricated on a polished titanium plate was evaluated to be greater than 60 MPa, which was independent of the phase and thickness of the coating film. These values were greater than those reported for plasma-sprayed calcium phosphate films on blast-treated titanium substrates.
- (2) The oxyapatite coating film increased the ALP activity of SaOS-2 cells on the titanium plates 3 and 7 days after

they were cultured, indicating that this process is effective in enhancing the osteogenic differentiation of osteoblasts on the surface of titanium implants.

- (3) An increase in the percentage of bone-implant contact was demonstrated in titanium cylinders with an amorphous calcium phosphate coating film by means of animal experiments where they were implanted into the mandibles of beagle dogs; this increase was observed 8–12 weeks after implantation when compared with uncoated titanium cylinders. Amorphous-calcium-phosphate-coated titanium implants were suggested to effectively exhibit rapid and strong fixation with bones, particularly for dental and medical implants made of β -type titanium alloys.

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