

# Calcium phosphate coating formed in infusion fluid mixture to enhance fixation strength of titanium screws

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**Abstract** A novel technique was developed to coat a calcium phosphate (CaP) layer on titanium screws with a titanium oxide surface layer, using infusion fluids officially approved for clinical use. A calcium-containing solution, a phosphate-containing solution, and a sodium bicarbonate solution prepared from the infusion fluids were mixed at a Ca/P molar ratio of 2.0. Each screw was immersed in 10 mL of the resulting mixture at 37 °C for 2 days. A low-crystalline apatite layer (Ca/P molar ratio =  $1.681 \pm 0.038$ ) was formed on the screws. The layer consisted of a few 100 nm diameter particles fixed on the screw surface. In animal experiments, the screws were percutaneously implanted in both proximal tibial metaphyses of rabbits. The insertion torque was not significantly different between the CaP-coated screws ( $0.132 \pm 0.002$  Nm,  $n = 10$ )

and uncoated screws ( $0.140 \pm 0.002$  Nm,  $n = 10$ ) ( $p = 0.5785$ ). After the insertion torque test, the apatite layer remained on the surface of the screws, which means that the apatite layer survived the friction of screw insertion. The extraction torque of the screws in the CaP-coated group ( $0.239 \pm 0.066$  Nm,  $n = 19$ ) was significantly higher (by 29.9%) than that in the uncoated group ( $0.184 \pm 0.062$  Nm,  $n = 18$ ) 4 weeks after the operations ( $p = 0.0132$ ). Histologically, a larger amount of new bone formation was observed around the CaP-coated screws than that around the uncoated screws. Even after the removal of the screw, the CaP layer remained on the screw at the site where soft tissues were attached. The coating technique with the use of the infusion fluids is an effective method of improving bone-screw interface strength.

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## Introduction

Complications in external fixation pin tracts including pin track infection arise primarily from mechanical instability in the bone-screw interface [1]. A severe progressive reduction in the screw extraction torque occurs with time [2, 3]. This deterioration of bone-screw interface strength can result from the bone being damaged during pin insertion and fibrous tissue encapsulation around the screw.

Plasma-sprayed hydroxyapatite coatings have improved bone-screw interface strength. Hydroxyapatite, when implanted in bone, has the ability to bond directly to the bone without fibrous tissue intervention with a high biocompatibility and a low degradation [4–6]. Therefore, a plasma-sprayed hydroxyapatite coating has been reported as a suitable coating to improve the bone-screw interface: the bone-screw interface strength for stainless steel screws

with a 30 ~ 60- $\mu\text{m}$ -thick plasma-sprayed hydroxyapatite coating is 3.5–11 times higher than that for uncoated stainless steel screws [7–11]. Plasma-sprayed and flame-sprayed hydroxyapatite-coatings are applicable also to titanium, and enhanced bone formation on titanium [12–13].

Another technique for coating calcium phosphates on titanium is the biomimetic coating method in which apatite layers are coated on titanium or NaOH- and heat-treated titanium in calcium phosphate solutions supersaturated with respect to hydroxyapatite [14–17]. The NaOH- and heat-treated titanium has a sodium-containing titanium oxide layer on the surface. Recently, a supersaturated calcium phosphate solution has been prepared by mixing infusion fluids that have been officially approved for clinical use to coat dense hydroxyapatite ceramic with a calcium phosphate layer [18]. We hypothesized that the calcium phosphate solution prepared by mixing the infusion fluids can be used for producing a calcium phosphate layer on titanium screws with a titanium oxide layer, and that the calcium phosphate layer can improve the bone-screw interface strength. The calcium phosphate solution prepared by mixing infusion fluids has advantages over many other previously prepared supersaturated calcium phosphate solutions because infusion fluids are sterile and endotoxin-free, thus ready for clinical use.

The purpose of the present study was to prepare a calcium phosphate coating layer on a titanium screw having a surface titanium oxide layer using infusion fluids, to characterize the calcium phosphate coating layer, and to

compare the bone-screw interface strength between calcium-phosphate-coated screws and uncoated screws implanted percutaneously in rabbits.

## Materials and methods

### Preparation of supersaturated calcium phosphate solution

Five clinically available infusion fluids were used (Table 1) to prepare calcium-containing and phosphate-containing solutions, and an alkalizer. The calcium-containing solution (4.5 mM  $\text{Ca}^{2+}$ ) was prepared by mixing Ringer's solution (Otsuka Pharmaceutical Co., Ltd., Japan) and Conclyte<sup>®</sup>-Ca (Otsuka Pharmaceutical Co., Ltd., Japan). The phosphate-containing solution (20 mM  $\text{PO}_4^{3-}$ ) was prepared by mixing Klinisalz<sup>®</sup> B (KOBAYASHI Pharmaceutical Co., Ltd., Japan) and Conclyte<sup>®</sup>-P (Otsuka Pharmaceutical Co., Ltd., Japan). The alkalizer was Bifil<sup>®</sup> ( $\text{NaHCO}_3$ ; 166 mM) (AJINOMOTO PHARMA Co., Ltd., Japan). The calcium-containing solution (8.172 mL), the phosphate-containing solution (0.917 mL) and the alkalizer (0.911 mL) were mixed to prepare a supersaturated calcium phosphate solution with a Ca/P molar ratio of 2.0 (Table 1). Although this supersaturated calcium phosphate solution is initially a precipitate-free and transparent solution, the onset of homogeneous precipitation of calcium phosphates occurs after 15 min at 37 °C. The pH of the supersaturated calcium phosphate solution was

**Table 1** Chemical compositions of calcium-containing solution, phosphorus-containing solution,  $\text{NaHCO}_3$  solution and supersaturated calcium phosphate solution

	Calcium-containing solution (mM)		Phosphorus-containing solution (mM)		$\text{NaHCO}_3$ solution (mM)	Calcium phosphate solution (mM)
	Ringer's solution	Conclyte-Ca	Klinisalz B	Conclyte-P	Bifil	
pH (room temperature)	6.43	6.60	5.87	9.12	8.28	–
pH (37 °C)	6.64	6.91	5.90	9.11	8.35	7.98
PH (after 48 h)	–	–	–	–	–	8.72
Volume (mL)	8.135	0.037	0.898	0.019	0.911	10.000
$\text{Na}^+$	147.00	–	45.00	–	166.00	139.17
$\text{K}^+$	4.00	–	25.00	1000.00	–	7.39
$\text{Mg}^{2+}$	–	–	2.5	–	–	0.22
$\text{Ca}^{2+}$	2.5	500.00	–	–	–	3.67
$\text{Cl}^-$	156.00	1000.00	45.00	–	–	134.39
$\text{HP}_2\text{O}_4^-$	–	–	10.00	–	–	0.90
$\text{HPO}_4^{2-}$	–	–	–	500.00	–	0.94
$\text{HCO}_3^-$	–	–	–	–	166.00	15.09
$\text{CH}_3\text{COO}^-$	–	–	20.00	–	–	1.80
Xylitol	–	–	333.00	–	–	29.93

measured immediately, 1, 3, 6, 12, and 48 h after preparation. The homogeneously formed precipitate was collected after 1, 3, 6, 12, and 48 h after preparation, and analyzed by scanning electron microscopy (SEM; Model XL30, FEI Company Japan Ltd., Japan), X-ray diffraction (XRD) using a powder X-ray diffractometer (Model Rint 2400, Rigaku, Japan), and X-ray photoelectron spectrometry (XPS; Model Quantum-2000, ULVAC-PHI Inc., Japan) using Al K $\alpha$  X-ray to measure the Ca/P molar ratio. In the XPS measurements, the photoelectron take-off angle was set at 45° and a stoichiometric hydroxyapatite ceramic block was used as the reference for the analysis for the Ca/P molar ratio.

The ionic activity product and equilibrium partial pressure of CO<sub>2</sub> of the supersaturated calcium phosphate solution, and the degree of supersaturation ( $\sigma$ ) with respect to that of hydroxyapatite (HAP), octacalcium phosphate (OCP), amorphous calcium phosphate (ACP), and calcium carbonates were calculated. The method of calculation for the ionic activity product and the equilibrium partial pressure of CO<sub>2</sub> is described elsewhere [19]. The degree of supersaturation is expressed as

$$\sigma = 38; [(ionic\ product)^{1/n} - (solubility\ product)^{1/n}] / (solubility\ product)^{1/n}$$

$$= 38; (ionic\ product)^{1/n} / (solubility\ product)^{1/n} - 1,$$

where the  $n$  values are 18, 8, 5.07, and 2 for HAP, OCP, ACP, and calcium carbonates, respectively, on the basis of the chemical formulae, Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>, Ca<sub>4</sub>H(PO<sub>4</sub>)<sub>3</sub>, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>1.87</sub>(HPO<sub>4</sub>)<sub>0.2</sub>, and CaCO<sub>3</sub>, respectively (for example 10 + 6 + 2 = 18 for HAP).

#### CaP coating on titanium screws with titanium oxide layer

AO titanium cancellous screws from the company SYNTHES<sup>®</sup>, USA (# 407-030, 4.0 mm in diameter, 30 mm in length) were immersed in the supersaturated calcium phosphate solution at 37 °C for 48 h. Originally, the screws had a titanium oxide layer on the surface which was made by an anode oxidation process in a 75% phosphoric acid solution at a D.C. voltage of 57 V. The thickness of the titanium oxide layer is 142 nm, which was determined from interference color. After the immersion, the screws were taken out from the solution and dried at room temperature (CaP-coated screw).

#### Surface characterization of screw

After the immersion in the supersaturated calcium phosphate solution, the CaP-coated and uncoated screws were observed by the naked eye. The morphology and chemical

composition of the surfaces of the CaP-coated and uncoated screws were analyzed by SEM equipped with an energy dispersive electron probe X-ray (EDX) analyzer (Genesis 2000, EDAX Japan K. K., Japan). The screw specimens were coated with a thin carbon film before observation.

The layer coated on the screw surface was scraped off and analyzed by XRD analysis using a silicon zero-background plate.

To measure the Ca/P molar ratio of the coated layer, CaP-coated screws were analyzed by XPS.

#### Animal experiments

##### *Four-week implantation of CaP-coated screws*

Twenty skeletally mature male Japanese white rabbits weighing approximately 3.0 kg were used in this study. The rabbits were maintained according to the guidelines of the Ethical Committee of the University of Tsukuba and the National Institutes of Health for the care and use of laboratory animals (NIH Pub. No. 85-23 Rev. 1985).

After an intravenous injection of barbiturate (40 mg/kg body weight), the screws were implanted in both proximal tibial metaphyses of the rabbits. Small (10 mm) incisions were made in the skin at the medial proximal tibia aseptically. Then, holes 2.5 mm in diameter were drilled (# 310-250, SYNTHES<sup>®</sup>, USA) in both proximal tibial metaphyses using cordless driver surgi-mini (ISO MEDICAL SYSTEMS INC., Tokyo, Japan). The holes were created with individual taps (# 311–340, SYNTHES<sup>®</sup>, USA) for each screw. Then, the screws sterilized by ethylene oxide gas were manually inserted in the hole. Twenty CaP-coated screws were implanted in ten rabbits. Twenty uncoated screws were implanted in the other 10 rabbits. After the implantation, the skin was apposed with two 3-0 nonabsorbable sutures (Fig. 1). Postoperatively, each animal was allowed free activities in its own cage. The animals did not receive any antibiotics and treatment of their wounds. Then, all the animals were sacrificed 4 weeks after the operations.

After sacrifice, examination for pin tract osteomyelitis was carried out by roentgenographic analysis using KXO-50G (TOSHIBA, Tokyo, Japan) and manual inspection for pin loosening. The criterion for pin tract osteomyelitis was the presence of both roentgenographically detected bone destruction and pin mobility.

The extraction torque of the screw was measured for specimens without exhibiting osteomyelitis, using the torque measuring apparatus (HTG2-5N, IMADA CO., LTD., Japan).

Two specimens consisting of the screw and surrounding tissues each from the CaP-coated and the uncoated groups, respectively, were fixed in 10% neutral buffered formalin. The fixed specimens were undecalcified and embedded in



**Fig. 1** Postoperative view of implanted AO screw in rabbit proximal tibial metaphysis

methylmethacrylate (MMA) resin. The embedded specimens were cut perpendicularly to the tibial longitudinal axis and parallel to the implanted screw using a diamond saw (Crystal Cutter (MC-413) with a diamond blade and metal bond ( $\phi$  100  $\times$  0.3  $\times$  30 mm), MARUTO INSTRUMENT CO., LTD., Tokyo, Japan).

After grinding to a 30  $\mu$ m thickness, the sections were stained with hematoxylin and eosin (H&E). These sections were used for morphological qualitative analyses of the bone-screw interfaces.

All other specimens subjected to the extraction torque study were fixed in 10% neutral buffered formalin, decalcified and embedded in paraffin. The sections were sliced 5  $\mu$ m thick perpendicularly to the tibial longitudinal axis, parallel to the screw hole and H&E stained to detect new bone formation around the screw hole. All the specimens were examined by light microscopy (BX-51, OLYMPUS OPTICAL CO., LTD., Tokyo, Japan).

The removed screws were investigated by SEM and EDX analysis. The screws removed in the extraction torque study were embedded in MMA, or immersed in 10 mL of 0.1% papain (Merck KGaA, Darmstadt, Germany) solution at pH 7.4 at room temperature for 7 days. The embedded screws were cut parallel to the longitudinal axis of the screws using the diamond saw. These removed screws were coated with a thin carbon film before observation.

#### Measurements of insertion torque

Ten CaP-coated screws were implanted in proximal tibial metaphyses of 10 cadaveric rabbits. Ten uncoated screws

were implanted in the contralateral proximal tibial metaphyses. The insertion torque of the screw was measured using a torque measuring apparatus (HTG2-5N, IMADA CO., LTD., Japan). Immediately after insertion, the screws were removed and analyzed by SEM and EDX.

#### Statistical analysis

The insertion torque data between CaP-coated and uncoated screws was compared with paired Student's *t* test data at a  $p < 0.05$  significance value. The extraction torque data between CaP-coated and uncoated groups after 4-week implantation was compared with unpaired Student's *t* test at a  $p < 0.05$  significance value.

## Results

### CaP coating on titanium screws with titanium oxide layer

The supersaturated calcium phosphate solution is supersaturated with respect not only to HAP and OCP but also to ACP and calcium carbonates (Table 2). The equilibrium partial pressure of CO<sub>2</sub> for the supersaturated calcium phosphate solution is 10<sup>-2.07</sup> atm, that is, one order magnitude higher than the ambient partial pressure of CO<sub>2</sub> (10<sup>-3.52</sup> atm). The pHs of the solution immediately, 1, 3, 6, 12, and 48 h after incubation were 7.98, 7.87, 7.80, 7.62, 7.80, and 8.72, respectively. When no titanium screw was immersed, precipitates began to appear 15 min after incubation. Precipitates changed with incubation time in morphology (Fig. 2). Initially, precipitates were smooth spherical particles 170–200 nm in diameter (Fig. 2a). However, 3 h after incubation, precipitates were mostly aggregated fibers 80–150 nm long and smooth spherical particles in small amounts (Fig. 2b). Six and 12 h after incubation, only aggregated fiber precipitates were observed (Fig. 2c and d). Finally, densely aggregated fibrous

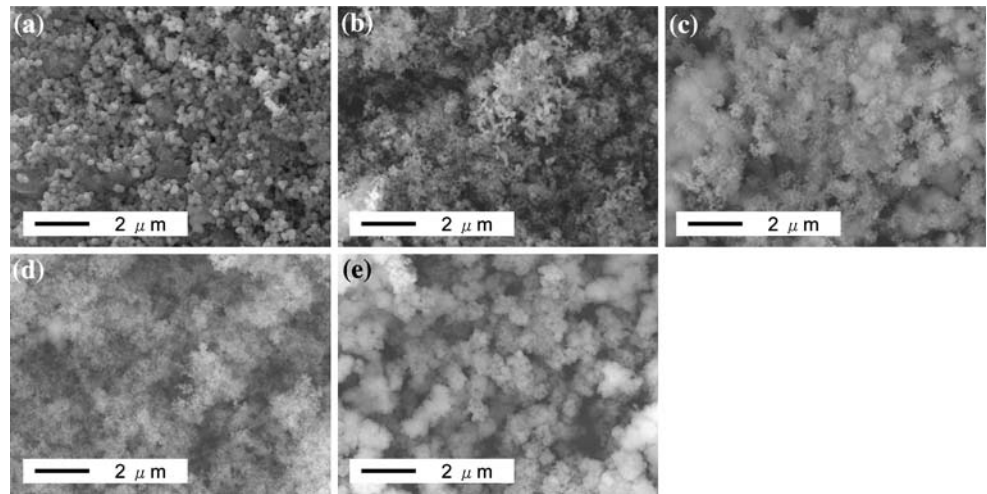
**Table 2** Negative logarithms of solubility products (K<sub>sp</sub>) for calcium phosphates and carbonates, ionic activity products (IP) of the supersaturated calcium phosphate solution for the corresponding phase, and the degree of supersaturation

Phase	–Log(K <sub>sp</sub> )	–Log(IP)	Supersaturation
HAP	115–118.7 [20, 21]	86.2	38–62
ACP	24.8 [22]	23.4	0.85
OCP	46.9–49.6 [23, 24]	42.1	2.9–7.6
CaCO <sub>3</sub> (C)*	8.6 [25]	7.7	1.9
CaCO <sub>3</sub> (A)**	8.4 [25]	7.7	1.3

\* CaCO<sub>3</sub> (C), calcite

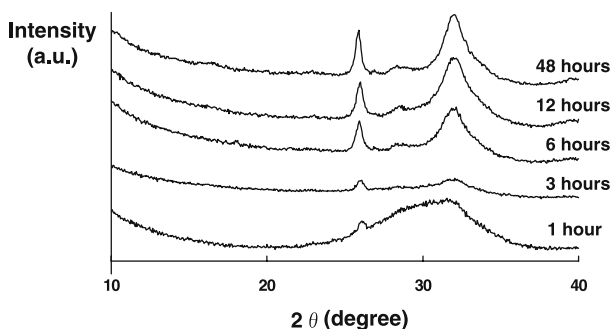
\*\* CaCO<sub>3</sub> (A), aragonite

**Fig. 2** SEM images of homogeneously formed precipitate after 1 h (a), 3 h (b), 6 h (c), 12 h (d) and 48 h (e). The crystals changed from smoky hazelike substances to small particles with time

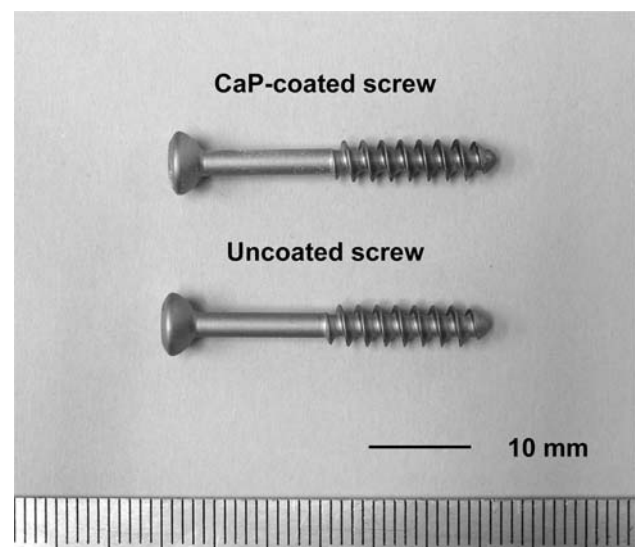


precipitates were observed 48 h after incubation. The precipitates observed after 1 h of incubation were a mixture of ACP ( $2\theta = 30^\circ$ ) and low-crystalline apatite ( $2\theta = 31.8^\circ$ ) as demonstrated by XRD analysis (Fig. 3). The amount of low-crystalline apatite gradually increased with time. After 48 h, the precipitates consisted mostly of low-crystalline apatite. The Ca/P molar ratios of the precipitates after 1, 3, 6, 12, and 48 h after incubation were  $1.364 \pm 0.003$  ( $n = 3$ ),  $1.383 \pm 0.106$  ( $n = 3$ ),  $1.405 \pm 0.055$  ( $n = 3$ ),  $1.385 \pm 0.023$  ( $n = 3$ ), and  $1.575 \pm 0.005$  ( $n = 3$ ), respectively.

By immersing the titanium screw in the supersaturated calcium phosphate solution, a low-crystalline apatite layer was formed on the screw surface after 48 h. The layer was almost transparent, and thus hardly visible to the naked eye (Fig. 4). In SEM observation, the layer consisted of particles of a few 100 nm in diameter fixed on the screw surface (Fig. 5b). Calcium and phosphorus were detected on the screw surface by EDX analysis (Fig. 5c). However, no such calcium phosphate layer was observed on the uncoated screw surface by SEM and EDX analysis (Fig. 5d and e). The XRD pattern for the surface layer scraped off



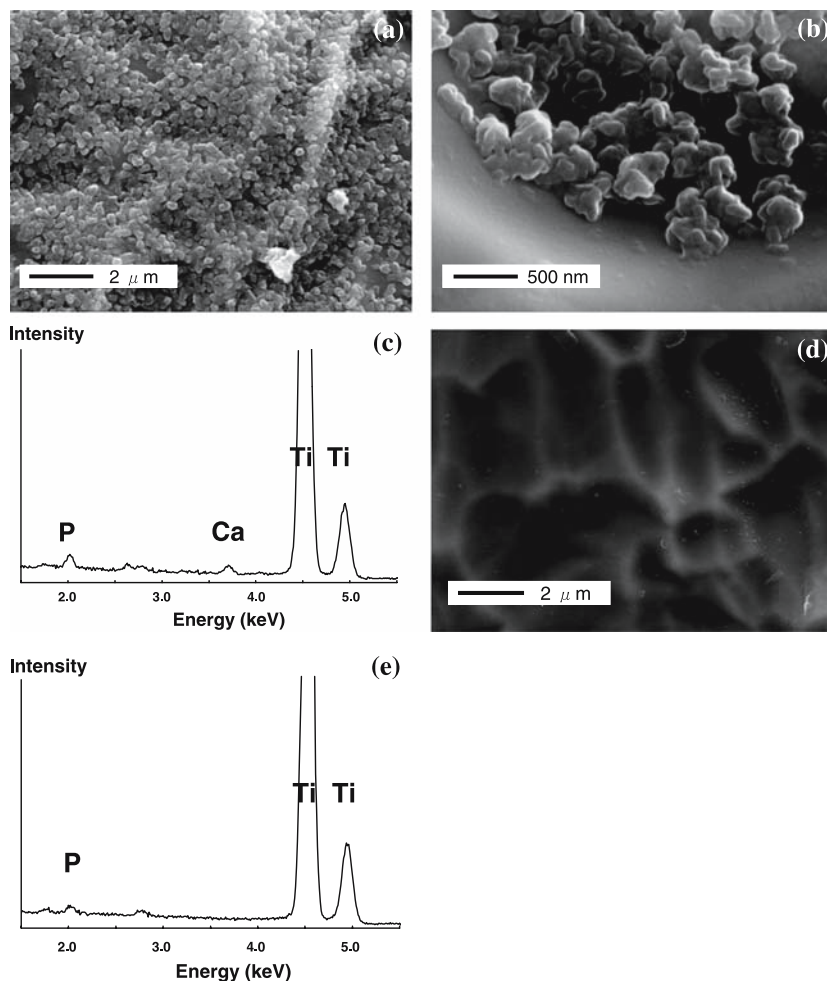
**Fig. 3** XRD patterns of precipitates homogeneously formed in supersaturated calcium phosphate solution



**Fig. 4** Macroscopic view of CaP-coated screw (upper) and uncoated screw (lower). The CaP layer was hardly visible to the naked eye

from the coated screws and placed on the silicon zero-background plate showed the presence of low-crystalline apatite: broad peaks ascribed to low-crystalline apatite were observed at  $25.8^\circ$  and  $31.8^\circ$  (Fig. 6 upper). No calcium carbonate was detected. Since only a very small amount of the sample was able to be scraped off and available for XRD measurement, the XRD pattern for the surface layer contained peaks for a silicon oxide that originated from the silicon zero-background plate. No peak corresponding to calcium phosphates was observed in the silicon zero-background plate itself (Fig. 6 lower). The XRD pattern of the low-crystalline apatite (Fig. 6 upper) resembled that of the low-crystalline apatite homogeneously precipitated after 3 h (Fig. 3). The Ca/P molar ratio of the coated layer was  $1.681 \pm 0.038$  ( $n = 3$ ), which was revealed by XPS analysis.

**Fig. 5** SEM images showing that a precipitate morphologically identical to bonelike apatite was formed on the screw surface (a). A few nm diameter particles were fixed on the screw surface (b). Calcium and phosphorus were detected on the screw surface by EDX analysis (c). No calcium phosphate was observed on the uncoated screw surface by SEM (d) and EDX analysis (e). P: phosphorus, Ca: calcium and Ti: titanium



## Animal experiments

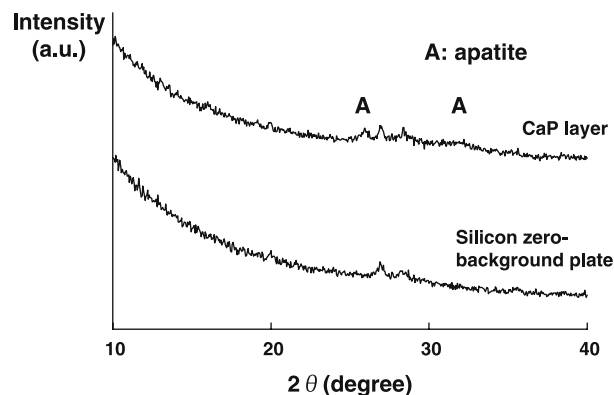
### Biomechanical results

The insertion torques were  $0.132 \pm 0.002$  Nm for the CaP-coated screws ( $n = 10$ ), and  $0.140 \pm 0.002$  Nm for the uncoated screws ( $n = 10$ ) as shown in Table 3. The difference in insertion torque was not significantly different between the CaP-coated screws and uncoated screws ( $p = 0.5785$ ). In spite of friction during insertion, the CaP layer remained both on the threaded portion and smooth stem portion of the screw, as observed by SEM and EDX analysis (Fig. 7).

Four weeks after implantation, the CaP-coated group had one limb that exhibited osteomyelitis out of the 20 limbs. The uncoated group had two limbs that exhibited osteomyelitis out of the 20 limbs. The extraction torque increased to  $0.239 \pm 0.066$  Nm for the CaP-coated group ( $n = 19$ ), and  $0.184 \pm 0.062$  Nm for the uncoated group ( $n = 18$ ) as shown in Table 3. The extraction torque for the CaP-coated group was significantly higher (by 29.9%) than that for the uncoated group ( $p = 0.0132$ ).

## Histological results

Four weeks after implantation, a larger amount of new bone was observed around the CaP-coated screw than



**Fig. 6** XRD pattern of surface layer scraped off from CaP-coated screws and placed on silicon zero-background plate (upper pattern). The XRD pattern shows the presence of low-crystalline apatite. No peak corresponding to calcium phosphates was observed in the silicon-zero-background plate (lower pattern)

**Table 3** Biomechanical results: the insertion torque and the extraction torque 4 weeks after the operations

Parameter	CaP-coated screw	Uncoated screw	<i>p</i> value
Insertion torque (Nm)	0.132 ± 0.002 ( <i>n</i> = 10)	0.140 ± 0.002 ( <i>n</i> = 10)	0.5785
Extraction torque (Nm)	0.239 ± 0.066 ( <i>n</i> = 19)	0.184 ± 0.062 ( <i>n</i> = 18)	0.0132

Values are the mean ± SD

around the uncoated screw in both the undecalcified and decalcified sections (Fig. 8).

#### Characterization of CaP-coated screws removed after four-week implantation

Calcium and phosphorous were detected by EDX analysis in most of the analyzed spots on the smooth stem portions of the screws where soft tissues were attached. Figure 9a shows the remaining CaP-coated layer on the smooth stem portions of the screws. Calcium and phosphorus were detected in the area by EDX analysis (Fig. 9b). Moreover, apatite formation was observed in some areas on the smooth stem portions where soft tissues were attached (Fig. 9c, d). On the contrary, only a small amount of, or even no calcium and phosphorus were detected on the threaded portions where bone tissues were attached. No coated layer was observed by SEM (Fig. 9e), and calcium

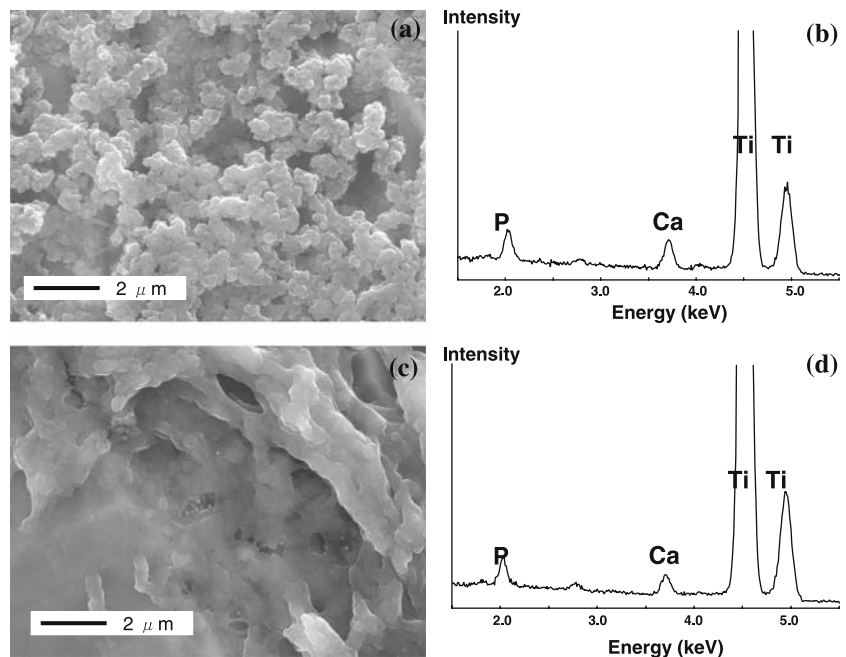
and phosphorus peaks were less intense in large areas on the threaded portions (Fig. 9f). In other areas on the threaded portions, however, thick calcium phosphate layers were observed with significant frequency (Fig. 9g and h). These results suggest that the initial CaP-coated layer remains on most of the smooth stem portions even after screw removal after 4-week implantation. It was not clear whether the initial coated layer remained on the threaded portions after screw removal after 4-week implantation.

#### Discussion

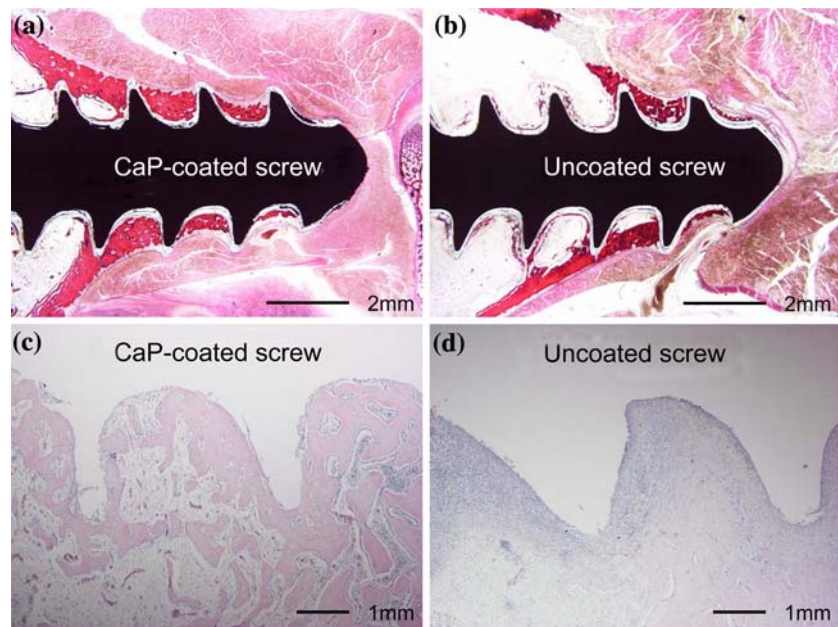
Using a supersaturated calcium phosphate solution prepared from infusion fluids, a CaP layer consisting of low-crystalline apatite was successfully formed on titanium screws having a titanium oxide layer on the surface. The supersaturated calcium phosphate solution has an equilibrium CO<sub>2</sub> partial pressure that is one order magnitude higher than that of ambient CO<sub>2</sub> partial pressure. Therefore, CO<sub>2</sub> gas is released from the solution during the incubation at 37 °C for 2 days. The release of CO<sub>2</sub> leads to an increase in the solution pH and, thus, an increase in supersaturation with respect to calcium phosphates. Although the solution was initially supersaturated with respect to calcium carbonates, no precipitation of calcium carbonate was detected probably due to the release of CO<sub>2</sub> that can lead to a decrease in the ionic activity of CO<sub>3</sub><sup>2-</sup> ions.

The difference in crystallinity and Ca/P molar ratio between homogeneously precipitated apatite and coated

**Fig. 7** Apatite layer remaining on surface of CaP-coated screws where soft tissues attached (**a** and **b**) and bone tissue attached (**c** and **d**) after the insertion torque test as observed by SEM and EDX analysis. P: phosphate, Ca: calcium and Ti: titanium



**Fig. 8** Histological sections of undecalcified ( $\times 12.5$ ) and decalcified ( $\times 40$ ) specimens 4 weeks after operations, stained with H&E. A larger amount of new bone formation was observed around the CaP-coated screw (a and c) than around the uncoated screw (b and d)



apatite indicates the presence of some sort of chemical interaction or reaction between the apatite and the titanium oxide layer. Since the solution is supersaturated with respect to ACP, ACP was precipitated in addition to low-crystalline apatite from the solution at the initial stage when no screws were immersed in the solution. The amount and crystallinity of the low-crystalline apatite increased with time. On the other hand, when the screw with a titanium oxide surface was immersed in the solution, the crystallinity of the low-crystalline apatite on the surface remained very low even after 2 days. Moreover, Ca/P molar ratio was different after 2 days between the homogeneously precipitated apatite and coated apatite. Therefore, there must be some sort of chemical interaction or reaction between the low-crystalline apatite and the titanium oxide. The chemical interaction or reaction would be an origin of adhesion strength of the low-crystalline apatite to the titanium oxide. Originally, the AO titanium screws had approximately 147 nm titanium oxide layer on the surface. Note that specific types of titanium oxide have induced apatite formation in the simulated body fluids [26].

The improvement in bone-screw interface strength observed in the CaP-coated screws compared with the uncoated ones was primarily due to extensive new bone formation around the CaP-coated screws. No improvement in initial insertion torque was observed in the CaP-coated screws. However, the osteoconductivity of the CaP coating facilitated bone remodeling and direct bony coverage on the screw surface. Therefore, the bone-screw interface strength was improved not by an improvement in initial insertion torque but by the osteoconductivity of the CaP coating. A similar improvement in interface strength was

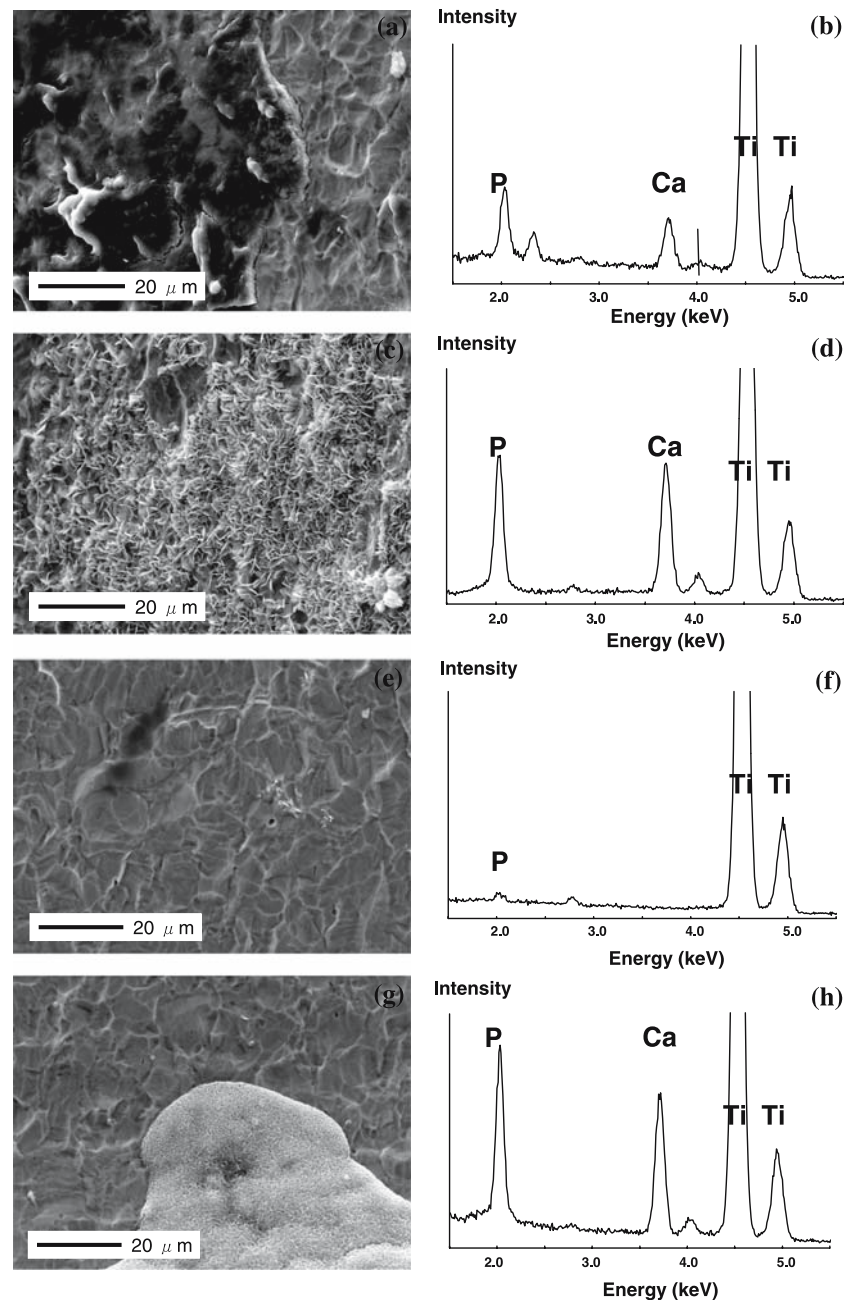
reported for stainless steel pins with a plasma-sprayed hydroxyapatite layer compared with stainless pins with a plasma-sprayed titanium layer [10].

It should also be stressed that the CaP-coated layer has a good stability and adhesion strength. The coated layer survived the friction of insertion in the insertion torque study. Almost no deterioration or degradation of the CaP-coated layer was found after the extraction study after 4-week implantation at the site where the coating was in contact with soft tissues. Therefore, this CaP coating is resistant to friction during screw insertion, and to the initial inflammatory reaction in the wound healing process. The CaP-coated layer at the site where the layer was in contact with bone was removed in many areas in the extraction study after 4-week implantation. Therefore, the adhesion strength between the CaP layer and the screw cannot be higher than that between the CaP layer and the bone tissue. In spite of such a level of adhesion strength, however, this CaP layer could still be successfully used to improve the initial interface strength and stability of other screw-shaped implants in both external and internal fixation techniques. The adhesion strength between the CaP layer and the screw could be a result of the chemical interaction or reaction that induced a delayed increase in the crystallinity of the low-crystalline apatite.

Clinically, it is important to achieve a stronger bone-screw interface early in the course of treatment with external fixation. This time period corresponds to the crucial time of fracture instability, particularly in osteoporotic bone. A stable bone-screw interface also may be particularly useful for long-duration treatment or when the surgeon decides a change from static to dynamic fixation. The



**Fig. 9** Coated layer remaining on screw in area where soft tissues were attached as observed by SEM (a) and calcium and phosphorus detected in area as observed by EDX analysis (b). Apatite formation was observed on the screw in the area where soft tissues were attached as observed by SEM and EDX analysis (c and d). No coated layer was observed by SEM (e), and calcium and phosphorus peaks were less intense in the area where bone tissue was attached (f). In other areas on the threaded portions, thick calcium phosphate layers were observed with significant frequency by SEM and EDX (g and h). P: phosphate, Ca: calcium and Ti: titanium



lack of stability of the bone-screw interface can lead to loosening of the screw and infection, and, thus, affects the clinical results of treatment [2]. The higher strength of fixation associated with the CaP-coated screws might result in a lower screw tract infection rate [12]. A substantial improvement in the stability of the interface between the bone and the screws can be obtained in many other techniques in orthopaedic surgery. Moreover, the CaP-coated layer is expected to improve adhesion of soft tissues to the screws as was demonstrated by percutaneous devices consisting of or combined with hydroxyapatite [27–32]. Since the CaP coating remains for as long as 4 weeks after

the operations, the CaP coating could also be used as a vehicle for the slow release of drugs such as antibacterial agents and growth factors to prevent pin tract infections and to further improve bone-screw interface strength. Drugs and proteins co precipitated with calcium phosphates were reported previously [18, 33–36].

The CaP-coated screws can be produced from other infusion fluids officially approved for clinical use, which contain calcium and phosphate ions separately.

In conclusion, a calcium phosphate layer was successfully formed on a titanium screw with a titanium oxide surface by only using officially approved infusion fluids.

The advantage of using infusion fluids to prepare the supersaturated calcium phosphate solution is that infusion fluids are sterile, endotoxin-free, and have a low regulational barrier for clinical application. The CaP-coated titanium screws have a 29.9% higher bone-screw interface strength than the uncoated titanium screws. Therefore, the use of CaP-coated screws is an effective method of increasing bone-screw interface strength. Further studies with screws being loaded, studies on pin tract infection rate and long term results are necessary.

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