

Márta Szekeres  
Gabriella Fodor  
András Fazekas  
Márta Radnai  
Kinga Turzó  
Imre Dékány

## Formation of octacalcium phosphate by heterogeneous nucleation on a titania surface

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M. Szekeres (✉) · G. Fodor · I. Dékány  
Department of Colloid Chemistry,  
University of Szeged, Aradi vt.1,  
6720 Szeged, Hungary  
E-mail: szekeres@chem.u-szeged.hu

A. Fazekas · M. Radnai · K. Turzó  
Department of Dentistry and Oral Surgery,  
University of Szeged, Tisza Lajos krt. 64,  
6720 Szeged, Hungary

I. Dékány  
Nanostructured Materials Research Group  
of the Hungarian Academy of Sciences,  
Aradi vt.1, 6720 Szeged, Hungary

**Abstract** Biocompatibility of the surfaces of titanium dental implants can be improved by covering them with calcium phosphate crystals, which makes the surface bioreactive. Possibly the most effective bioreactive foreign material that improves osteointegration and adsorption/binding of extracellular proteins and structural proteins is crystalline octacalcium phosphate  $\{2 \times [\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 2.5\text{H}_2\text{O}] \text{ or } \text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}, \text{OCP}\}$ . In this work the building up of OCP crystals on the surface of  $\text{TiO}_2$  anatase is examined in the process of heterogeneous nucleation from constant-composition solutions of  $\text{CaCl}_2$  and  $\text{KH}_2\text{PO}_4$  at constant pH (pH 6.8) and ionic strength ( $I=0.05 \text{ M}$ ), in dense titania suspensions. Constant relative supersaturation with regard to the calcium phosphate formation was maintained by the controlled addition of the reagent solutions,

according to the desired speed of crystallization. The surface saturation value of calcium ion adsorption was measured by detecting the pH decrease during  $\text{CaCl}_2$  addition in a separate experiment. The OCP crystallization was also conducted on the surface of an evaporated titanium layer, and on titanium metal disks. The surface of the disks was modified by the laser ablation method in order to increase the oxide layer thickness. Calcium phosphate crystals formed on the surface of the modified titanium disks, but not in an appreciable amount on the surface of the evaporated titanium layer.

**Keywords** Octacalcium phosphate nucleation · Heterogeneous nucleation · Constant-composition kinetics method · Surface modification · Biocompatibility

### Introduction

Calcium phosphate ceramic coatings are commonly used to improve biocompatibility of the surfaces of titanium implants [1], which are initially covered by a bioinert native titanium oxide layer [2]. Calcium phosphate surfaces are considered to be bioactive since they have a higher potential for attachment of osteoinductive agents than other more inert implant materials. Calcium phosphate coatings can be created by a variety of different

procedures, such as sol–gel coating, ceramic coating [1], invert glass-ceramic coating [3], ion-beam sputtering [4], the plasma spraying technique [5], and electrochemical techniques [6]. In spite of their routine industrial application the coatings produced by these techniques are easily destroyable upon implantation.

Heterogeneous nucleation and growth of calcium phosphate phases on oxide surfaces provides strong binding between the implant surface and the apatite coating. Nucleation and crystallization of various types

of calcium phosphates on titanium oxide and silica surfaces have been investigated by several authors [7, 8, 9, 10, 11, 12, 13]. Combes et al. [8] found that in the nucleation of dicalcium phosphate on the surface of  $\text{TiO}_2$  at pH 5.5 the first stage is phosphate ion adsorption. At this pH the titania surface is positively charged, promoting anion adsorption. Dicalcium phosphate dihydrate [8], octacalcium phosphate  $\{2 \times [\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 2.5\text{H}_2\text{O}] \text{ or } \text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}, \text{OCP}\}$  [7, 10] and hydroxyapatite (HAP) [10] crystalline phases were successfully grown on titania particles using the constant-composition kinetics technique. Glow discharge plasma treated titanium surfaces also nucleated calcium phosphate phases from the precursor electrolyte solution [12]. Immersion of sol-gel-derived titania-coated titanium [9] and chemically modified titanium into simulated body fluid [11] or supersaturated HAP solution [14] resulted in HAP and bone-like apatite formation. It has been shown that strong chemical binding is possible between apatite crystals and different implant materials and adhesives applied in dentistry [15]. OCP is considered by many authors as the most probable precursor of HAP formation in *in vivo* biomineralization [16]. It was found that even in the presence of  $\beta$ -tricalcium phosphate seeds from solutions supersaturated with respect to  $\beta$ -tricalcium phosphate, HAP and OCP, the OCP phase grows in preference [16]. Therefore we investigated the formation of OCP on titania and titanium surfaces in a heterogeneous nucleation process using the constant-composition kinetics technique.

The nucleation and crystallization rate in heterogeneous nucleation processes is a function of many different parameters, such as the degree of relative supersaturation, interfacial tension, structural match between the substrate and the embryos, and the size and concentration of foreign particles [7, 10, 17]. The concentration of foreign particles in crystallization experiments is usually held at low values to ensure that the interfacial adsorption of precursor ions does not decrease appreciably the bulk concentration. The bulk concentration starts to decrease, consequently, only owing to surface precipitation, at the end of the induction period. We focused our investigation on crystallization at high foreign particle concentration to increase the OCP production efficacy that would approach industrial needs.

Applying the results of OCP formation in a titania suspension, we attempted to prepare coatings on macroscopically flat titanium surfaces in a heterogeneous nucleation process with the perspective of coating implant surfaces.

## Materials and methods

OCP was precipitated on titania particles and flat titanium surfaces.  $\text{TiO}_2$  anatase (Bayertitan-A, Bayer,

Germany) was free of organic and inorganic impurities, with a  $\text{TiO}_2$  content of 99%. Thin titanium films were prepared on microscope slide glass surfaces by evaporation of commercially pure titanium (Fluka). Titanium (99.99% pure) was obtained from TISTO, Germany. The salts  $\text{KH}_2\text{PO}_4$ ,  $\text{CaCl}_2$ ,  $\text{NaCl}$  and  $\text{KOH}$  were analytical grade reagents (Reanal Hungary). Ultrapure water from a Milli-Q RG water purification system was used throughout.

Calcium phosphate crystals were prepared using the constant-composition kinetics method [18] with the crystallization kinetics parameters given by Wu and Nancollas [7] at  $37 \pm 0.1$  °C during continuous stirring (Electromantle MA) in a  $\text{N}_2$  atmosphere. The precursor solutions  $\text{KH}_2\text{PO}_4$  and  $\text{CaCl}_2$  were added to 200 ml of their metastable solution (with respect to calcium phosphate precipitation), containing 0.5 g titania powder. The speed of titrant addition was controlled using a Radelkis OP-936 pipetting unit attached to Radelkis OP-930 automatic burettes (Radelkis, Hungary). The value of the relative supersaturation was chosen as  $\sigma = 1.46$  for the given titrant addition rate corresponding to a crystal growth rate of  $4.93 \times 10^{-8} \text{ mol s}^{-1} \text{ m}^{-2}$ . The calcium-to-phosphate ratio was 1.33:1, equal to that of the OCP crystalline phase. Constant pH and ionic strength (pH  $6.8 \pm 0.1$ ,  $I = 0.05 \text{ M}$ ) were maintained during the synthesis by parallel addition of  $\text{KOH}$  and  $\text{NaCl}$  solutions. The pH was monitored using a Radelkis OP 208/1 pH-meter (the precision is  $\pm 0.002$  pH, the reproducibility is 0.001 pH), with an OP 0808P combined glass electrode. The induction time was 210 min.

Titanium films were prepared on microscopic glass plates by the vacuum evaporation technique. The plates were cleaned with chromic acid and distilled water before use.

Dental implant material, a 99.99% pure titanium rod (TISTO, Germany) was machined to 2-mm-thick, 8-mm-diameter discs and the surface was pretreated with aluminium-oxide sand blasting. The discs were subjected to laser irradiation (ArF excimer laser,  $\lambda = 193 \text{ nm}$ ) on areas of  $1 \times 2$ -mm with a mean pulse energy of 1.6 mJ and 15 ns duration. The probes were heated at 450 °C for 3 days before use in the calcium phosphate deposition experiments.

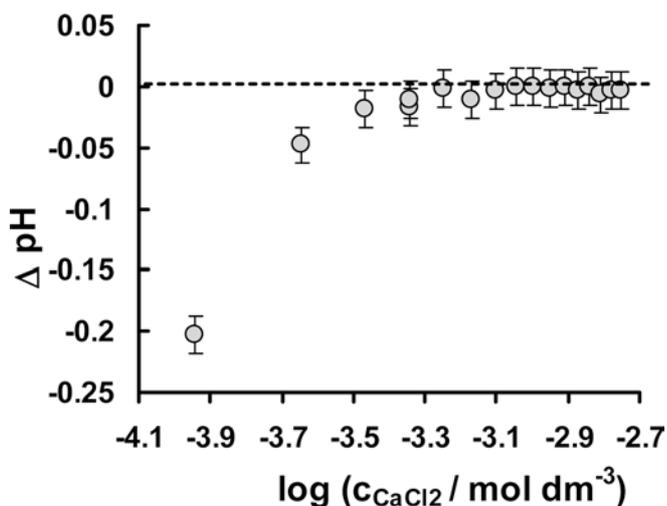
The presence of the phosphate phase on the titania and titanium surfaces was investigated by Fourier transform (FT) IR spectrometry, X-ray diffraction (XRD) and scanning electron microscopy. The FT-IR measurements were made at the Department of Physical Chemistry of the University of Szeged (VibLab) by using a Bio-Rad Digilab Division FTS-65A/869 type FT-IR spectrometer, with a deuterated triglyceride sulfate detector. The resolution was  $4 \text{ cm}^{-1}$ . The diffuse reflection spectra of the powder samples were measured in KBr solid solution. The samples prepared on the surface of the evaporated titanium layers were investigated by

measuring the reflection absorption spectra at a tilt angle of  $30^\circ$ . The crystal structure was investigated by XRD in a Philips diffractometer ( $\text{Cu}_{K\alpha}$  radiation,  $\lambda = 0.1542$  nm, W1830-type generator, PW1820-type goniometer and PW1711-type position-sensitive detector). Scanning electron micrographs were made at the Department of Phitology, University of Szeged, by using a Hitachi S-2400-type scanning electron microscope.

## Results and discussion

### Crystallization on anatase in concentrated suspension

The process of crystallization through heterogeneous nucleation is generally studied at low nucleating foreign particle concentration. The surface has to be saturated with precursor ions of the nucleating crystal before crystallization starts. In very dilute suspensions, adsorption of precursor ions on the relatively small total surface does not reduce the solution concentration appreciably and the level of supersaturation remains practically unchanged. In our OCP crystallization procedures the suspension concentration is about 40 times larger than that in conventional experiments. The amount of  $\text{CaCl}_2$  necessary to saturate the surface with calcium ions prior to crystallization was calculated from a calcium ion uptake experiment (Fig. 1). The calcium ion uptake is related to the pH decrease of the solution at constant ionic strength due to an ion-exchange process at the solid surface. The hydrated surface of  $\text{TiO}_2$  anatase is covered by dissociable amphoteric OH groups in aqueous suspension. The pH of point of zero charge determined in potentiometric acid–base titrations is at

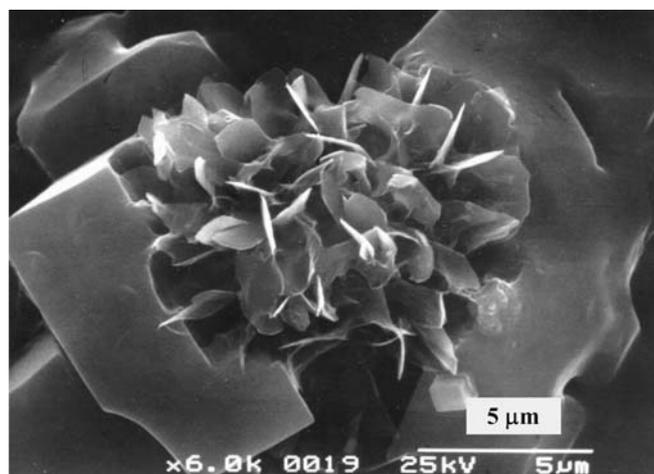


**Fig. 1** pH change in titania suspension ( $0.5 \text{ g } 200 \text{ cm}^{-3}$ , pH 6.8) in  $0.05 \text{ M NaCl}$  due to addition of  $\text{CaCl}_2$  solution. The dotted line designates the pH value of 6.8, to which the pH was reset after each  $\text{CaCl}_2$  portion

pH  $6.0 \pm 0.3$  [19]. In the OCP crystallization experiments at pH  $6.8 \pm 0.1$  the titania surface is negatively charged, so it promotes calcium ion adsorption. Divalent calcium ions adsorb specifically, resulting in deprotonation of surface OH groups. From the pH decrease the number of protons released can be calculated and is supposed to be approximately equal to the number of adsorbed calcium ions [20]. The pH change in the titania suspension is shown as a function of calcium ion concentration in Fig. 1. After addition of a portion of  $\text{CaCl}_2$  solution the pH was reset to 6.8 with KOH solution. The pH decrease stopped at  $0.57 \times 10^{-3} \text{ M CaCl}_2$  ( $\log c = -3.25$ ): the calcium ion adsorption reached a plateau value. From this experiment, the calculated calcium ion adsorption at saturation was  $0.228 \text{ mmol g}^{-1}$  at pH  $6.8 \pm 0.1$ . Streaming potential measurements (not shown) also support the concept of  $\text{Ca}^{2+}$  adsorption in the previously mentioned  $\text{CaCl}_2$  concentration range: the negative streaming potential value of the  $\text{TiO}_2$  anatase sample decreases continually to zero upon  $\text{CaCl}_2$  addition.

As the  $\text{Ca}^{2+}$  adsorption takes place during the induction period, after adding the anatase suspension to the metastable supersaturated solution of  $1.900 \times 10^{-3} \text{ M CaCl}_2$  and  $1.429 \times 10^{-3} \text{ M KH}_2\text{PO}_4$  [7] in  $0.05 \text{ M NaCl}$  at pH 6.8, we increased the concentration of the OCP precursor ions by 44% (by  $0.57 \times 10^{-3} \text{ M}$  for  $\text{CaCl}_2$  and  $0.62 \times 10^{-3} \text{ M}$  for  $\text{KH}_2\text{PO}_4$ ) to allow adsorption and to keep the relative supersaturation at  $\sigma = 1.46$ . Following the induction period, 210 min, the OCP precursor solutions and the NaCl and KOH solutions were added gradually. The titration speed was calculated using the crystallization data in Ref. [7].

A scanning electron microscope picture of OCP crystals nucleated on the surface of anatase at high suspension concentration is shown in Fig. 2.



**Fig. 2** Scanning electron microscope image of octacalcium phosphate (OCP) crystals on  $\text{TiO}_2$  anatase particles at  $\times 6,000$  magnification

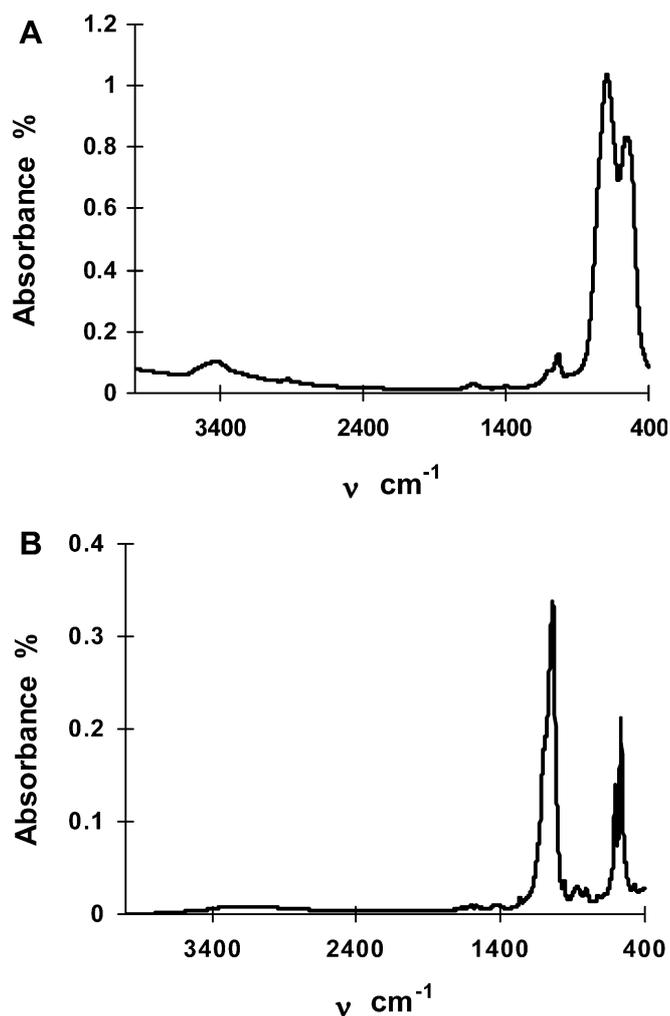


Fig. 3 Fourier transform (FT) IR absorption spectra of a OCP crystals nucleated on  $\text{TiO}_2$  anatase particles and of b reference hydroxyapatite (HAP, Merck)

Blade-shaped OCP crystals are grown on the cubic crystals of anatase particles.

Fourier transformed IR spectra of the OCP crystals formed on the surface of  $\text{TiO}_2$  anatase particles are shown in Fig. 3a. The FT-IR spectra of a reference HAP (Merck) is shown in Fig. 3b. The absorption bands around  $1,050\text{ cm}^{-1}$  and around  $600\text{ cm}^{-1}$  in the diffuse reflectance FT-IR spectra of the OCP/anatase sample (Fig. 3a) indicate the presence of the phosphate. The first absorption band corresponds to vibrations in  $\text{PO}_4$ ,  $\text{HPO}_4$  and  $\text{H}_2\text{PO}_4$ . IR absorption bands were found near  $600\text{ cm}^{-1}$  by other authors as well for HAP, dicalcium phosphate dihydrate and OCP [7, 8, 9]. The IR spectra of the reference HAP also shows absorption at  $1,050$  and  $600\text{ cm}^{-1}$ . The broad absorption band characteristic of OCP can be distinguished at  $3,400\text{ cm}^{-1}$ , which is explained by the presence of either  $\text{O}_3\text{PO-H}\dots\text{OPO}_3\text{H}$  type interphosphate hydrogen bonds or adsorbed water

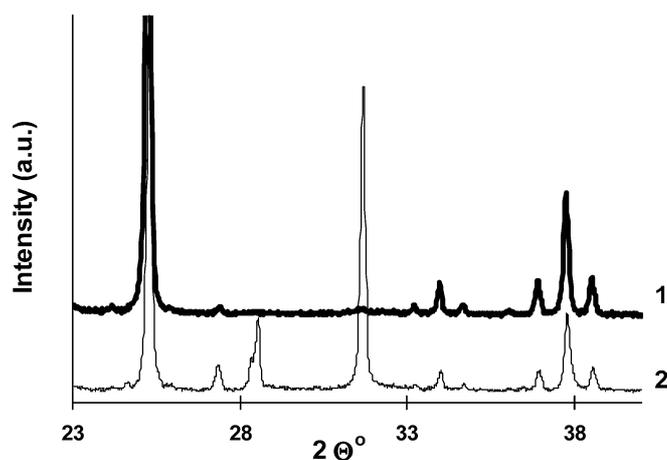


Fig. 4 X-ray diffraction pattern of OCP crystals (1) and HAP crystals (2) nucleated on  $\text{TiO}_2$  anatase particles

molecules in the crystal structure [21]. The broad band between  $800$  and  $600\text{ cm}^{-1}$  in the OCP/ $\text{TiO}_2$  spectra can be assigned to the anatase content [22]. The XRD pattern of the OCP/ $\text{TiO}_2$  sample is shown in Fig. 4 (diffraction pattern 1). We could not observe the characteristic reflection of the 100 crystal spacing of OCP at  $d=18.68\text{ \AA}$  ( $2\theta=5^\circ$ ), possibly because the OCP crystals are very thin [21]. The reflection peaks in the XRD pattern at  $2\theta=25.3$ ,  $36.9$ ,  $37.8$  and  $38.6^\circ$  are characteristic of  $\text{TiO}_2$  anatase [23]. The group of three lines centred at  $2\theta=34^\circ$  is present in the X-ray pattern of the original anatase sample as well.

Using a lower crystallization rate,  $0.9\times 10^{-8}\text{ mol s}^{-1}\text{ m}^{-2}$  [4], we synthesized a HAP phase on the anatase surface as well, to use as a reference sample to the XRD pattern of the OCP/anatase sample. The HAP diffraction lines in the HAP/anatase sample can be identified at  $2\theta=28.3$ ,  $28.6$  and  $31.7^\circ$  (Fig. 4, diffraction pattern 2), the last line corresponding with the diffraction from the 211 plane of HAP ( $I_{\text{rel}}=100\%$ ) [23]. The absence of these reflections in the X-ray pattern of the OCP/anatase sample demonstrates that the HAP phase did not form during OCP precipitation. The scanning electron micrograph of the HAP/anatase sample is shown in Fig. 5.

Crystallization on an evaporated titanium layer and a titanium disc

The surface of the evaporated titanium film oxidizes in atmospheric conditions. The deposition of calcium phosphate could be expected to some extent on the native oxide layer. In our experiments we did not observe formation of OCP crystals on the evaporated titanium film. A picture of one of the very few calcium phosphate deposits is shown in Fig. 6. The difference IR

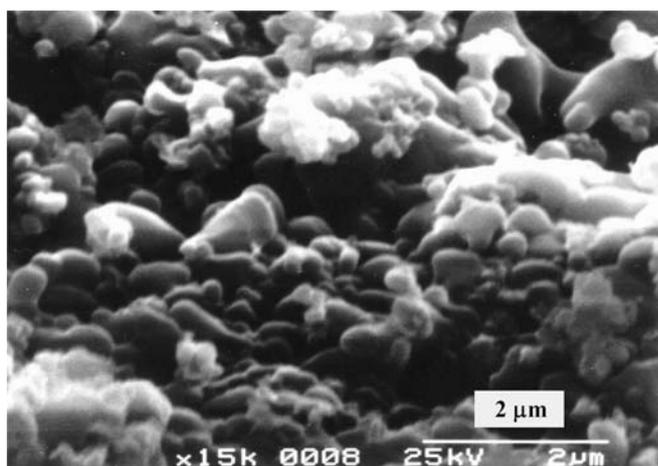


Fig. 5 Scanning electron microscope image of HAP on  $\text{TiO}_2$  anatase particles at  $\times 15,000$  magnification

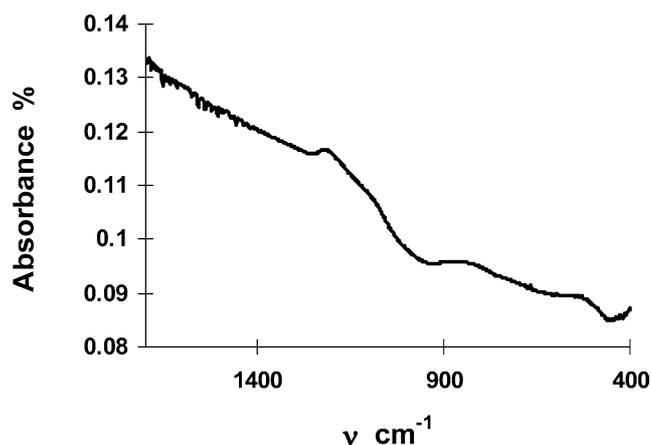


Fig. 7 FT-IR spectrum of calcium phosphate deposited onto an evaporated titanium layer

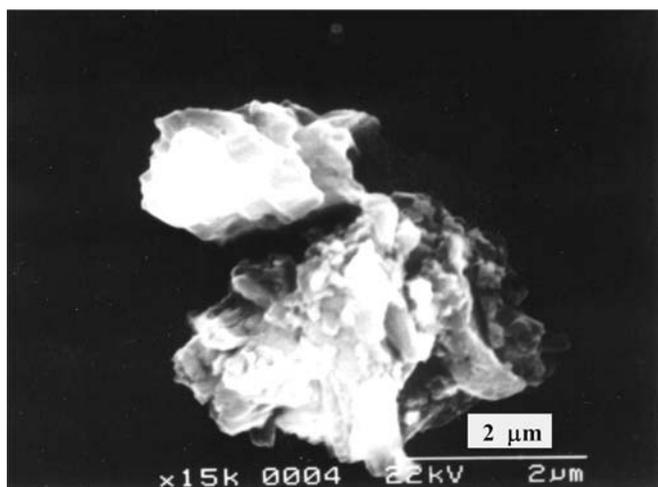


Fig. 6 Scanning electron microscope image of a calcium phosphate deposit on an evaporated titanium layer at  $\times 15,000$

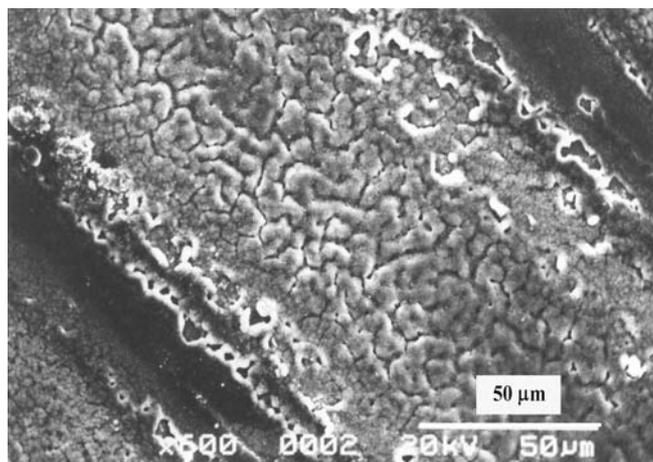
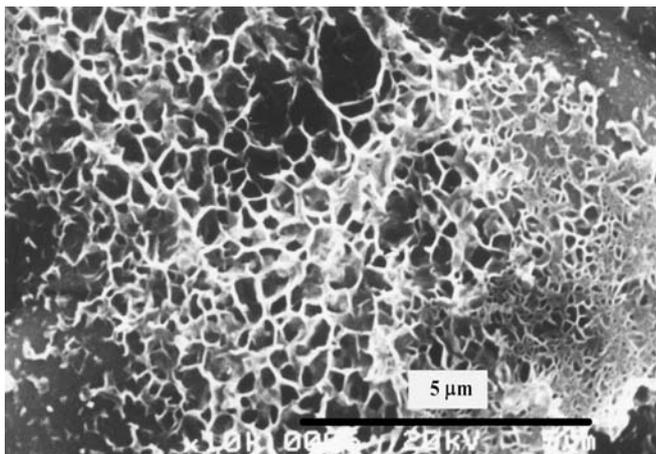


Fig. 8 Representative scanning electron microscope image of a titanium plate surface after laser ablation and heat treatment at  $450\text{ }^\circ\text{C}$  for 3 days, at  $\times 600$  magnification

absorption reflection spectra constructed by subtraction of the spectra of the original titanium layer is shown in Fig. 7. Small peaks can be seen in the ranges characteristic of the calcium phosphates, at  $600$  and  $1000\text{--}1300\text{ cm}^{-1}$ .

Titanium discs were pretreated before calcium phosphate crystallization, using the laser ablation technique. A laser flux of  $1.4\text{--}5\text{ J cm}^{-2}$  was used and the pulse number was varied from 10 to 1,000. After laser treatment the samples were heated at  $450\text{ }^\circ\text{C}$  for 3 days. The surface of the pretreated titanium plates showed the morphology seen in Fig. 8. Laser ablation and heat treatment leads to the formation and thickening of the titanium oxide layer, evidenced from X-ray photoelectron spectroscopy measurements [24]. The thickness of the surface oxide layer doubled during 30 min of irra-

diation. Typical calcium phosphate formation on the pretreated parts of the titanium surface is shown in Fig. 9. Phosphate deposits did not form on the parts of the titanium discs that had not been exposed to laser radiation. This suggests that there is a minimum thickness of the surface oxide layer necessary to initiate the calcium phosphate formation in a heterogeneous nucleation process. The presence of the native oxide layer or simple heat treatment to increase the oxide layer thickness is insufficient for calcium phosphate deposition. We conclude that surface treatment with the laser ablation technique is a promising method for modification of titanium implant surfaces and the resulting thick oxide film would serve as a nucleating surface for OCP deposition. We plan systematic studies on the optimal surface modification for OCP nucleation.



**Fig. 9** Scanning electron microscope image of calcium phosphate nucleated on the surface of a titanium plate pretreated by laser ablation and heating at 450 °C for 3 days

## Conclusions

Heterogeneous nucleation of OCP crystals on the surface of TiO<sub>2</sub> anatase particles was performed in dense titania suspensions at neutral pH. The degree of calcium

ion adsorption, necessary to saturate the titania surface prior to OCP nucleation, was determined from the decrease in the suspension pH due to the displacement of surface protons by Ca<sup>2+</sup>. The kinetics of OCP crystal growth on the surface saturated with Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> in the dense titania suspensions corresponded with that in dilute suspensions published previously by Wu and Nancollas [7]. Scanning electron microscopy, XRD and FT-IR investigations verified the presence of the OCP phase.

Macroscopically flat surface evaporated titanium films and titanium discs were used to nucleate OCP crystals. It was observed that the thickness of the titanium oxide layer on titanium is the determining factor in the nucleation. Evaporated titanium films were covered with a native oxide layer, the thickness of which proved to be insufficient for the formation of a phosphate coating. In contrast, formation of CaP phases could be observed on titanium discs treated by laser ablation. The surface of the titanium discs did not nucleate the phosphate phases if it had not been treated with the laser previously.

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