



A new simplified calcifying solution to synthesize calcium phosphate coatings

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ABSTRACT

In this work we set up new experimental conditions to deposit biomimetic coatings of different calcium phosphates onto titanium (Ti) substrates using over-simplified, economically convenient, slightly supersaturated solutions. The new supersaturated solutions, CaPPs, contain just calcium chloride and phosphate buffer, without addition of further salts and organic buffers usually employed for biomimetic coatings. The product precipitated at pH 7.2 and 37 °C was constituted of spherical aggregates of poorly crystalline hydroxyapatite (HA), similar to that obtained using HEPES as buffer system. Reduction of starting pH, which was varied from 7.2 to 6.6, promoted the precipitation of the kinetically favored phase, octacalcium phosphate (OCP), together with HA. Furthermore, OCP could be obtained as a single phase by increasing the Ca/P molar ratio of the calcifying solution from 1/1 to 2/1. Temperature reduction from 37 °C to 25 °C promoted the co-precipitation of calcium monohydrogen phosphate dihydrate (DCPD) together with HA and OCP, in agreement with the solubility isotherms of the different calcium phosphates. Variation of the experimental conditions was utilized to synthesize the coatings of spherical aggregates of poorly crystalline HA, petal-like crystalline OCP, as well as coatings containing both HA and OCP, onto titanium substrates in a few hours.

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1. Introduction

Short and long-term performance of dental and orthopedic implants is related to early osteointegration [1–3]. Calcium phosphate coating of metallic implants is generally accepted to be advantageous for clinical success. In fact, it allows retaining the excellent mechanical properties of titanium (Ti) and its alloys, widely employed in this field, while providing the surface of the implant with the bioactivity of calcium phosphates. A variety of physical techniques are commercially applied to this purpose [4–7]. However, physically deposited coatings exhibit several inherent drawbacks that can be overcome by wet-chemical deposition techniques [8]. In particular, the biomimetic approach involves deposition from slightly supersaturated solution at physiological, or nearly physiological, values of pH and temperature. The method, which leads to the deposition of a poorly crystalline hydroxyapatite (HA) phase similar to the inorganic phase of bone, is economic and can be applied to complex-shaped materials [9,10]. Moreover, it can be used to co-precipitate ions, drugs, macromolecules, proteins and growth factors [11–17]. The first formulation of a calcifying solution, known as simulated body fluid (SBF), was developed by Kokubo et al. [18] in 1990. Since then, a number of compositions have been proposed in order to accelerate the biomimetic deposition of poorly crystalline HA on metallic substrates. Most methods rely on buffer systems, such as TRIS (tris-hydroxymethyl-aminomethane)-HCl or HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethane sulphononic acid)-NaOH

for the control of pH. Lactic acid/sodium lactate was also employed as buffer system [19]. We previously developed a slightly supersaturated calcium phosphate solution (CaP), characterized by a very simple composition and buffered with HEPES, which can deposit coatings of poorly crystalline HA on metallic substrates in a few hours [20]. In this work, we explored the possibility to further simplify the composition of the CaP solution, in order to reduce the coating cost without provoking significant lengthening of the deposition time. To this aim, we removed HEPES buffer and carbonate ions from the composition of the calcifying solution, and employed a phosphate buffer solution as source of phosphate ions. Furthermore, we investigated the influence of concentration, pH and temperature on the composition of the inorganic deposits. Selected conditions were used to modulate the crystalline phases deposited on Ti substrates from hydroxyapatite to octacalcium phosphate (OCP).

2. Materials and methods

2.1. Preparation of the reference CaP calcifying solution

The reference CaP calcifying solution was prepared as previously reported [20]. Briefly, the reagent grade chemical $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in double distilled water and buffered at pH 7.2 with HEPES (Ca solution). The reagent grade chemicals $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, and NaHCO_3 were dissolved in double distilled water and buffered at pH 7.2 with HEPES (P solution). CaP supersaturated calcifying solution was freshly prepared by mixing equal volumes of the Ca and the P solutions at 37 °C. The ionic concentration of the CaP solution is reported in Table 1 [20].

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Table 1
Composition and starting pH of the different calcifying solutions.

Solution	Ca/P (molar ratio)	[Ca ²⁺] (mM)	[phosphate] (mM)	[CO ₃ ²⁻] (mM)	Buffer solution	pH
CaP	1/1	2.5	2.5 ^a	18	Hepes	7.2
CaPPC	1/1	2.5	2.5 ^b	18		7.0 7.0 6.8 6.6
CaPP1	1/1	2.5	2.5 ^b	–		7.2 7.0 6.8 6.6
CaPP2	1/1	5	5 ^b	–		7.0 6.8 6.6
CaPP3	2/1	5	2.5 ^b	–		6.6 6.8

^a Concentration of PO₄³⁻ ions for CaP solution.

^b Concentration of H₂PO₄⁻/HPO₄²⁻ for CaPP solutions.

2.2. Preparation of the new CaPP calcifying solutions

The new calcifying solutions, CaPPs, were prepared using 0.1 M phosphate buffer (PBS) solutions at different pH values as sources of phosphate ions without addition of the HEPES solution.

The 0.1 M phosphate buffer solutions at pH values 7.2, 7.0, 6.8, and 6.6, were prepared by mixing proper amounts of 0.2 M NaH₂PO₄ and 0.2 M Na₂HPO₄ solutions.

The reagent grade chemical CaCl₂·2H₂O was dissolved in double distilled water (Ca solution). The P solution was prepared using the proper amounts of phosphate buffer solutions, in order to obtain the required phosphate concentration.

NaHCO₃, when used, was added to the P solution.

CaPP solutions were freshly prepared by mixing appropriate volumes of the Ca and the P solutions at 37 °C, or at 25 °C, as reported in Table 1.

2.3. Powder samples

In order to better evaluate the effect of the different preparation methods on the inorganic phase precipitated from the calcifying solution, preliminary data were obtained on the powders precipitated in the absence of Ti substrates: after mixing the P solution with the Ca solution the temperature was kept constant either at 37 °C or 25 °C for 6 h, then the precipitate deposited on the bottom of the flask was filtered, washed with double distilled water and air-dried at 37 °C overnight.

XRD measurements were performed using a PANalytical powder diffractometer equipped with a fast X'celerator detector. CuK α radiation was used (40 mA, 40 kV). The 2 θ range was from 3 to 50° with a step size of 0.033° and time/step of 10 s/step. Morphological investigation was performed using a Philips XL-20 scanning electron microscope operating at 15 kV. The samples were sputter-coated with gold before examination.

2.4. Substrate coatings

Ti disks Grade 2 (diameter 15 mm; thickness 0.5 mm, NextMaterials, Milano, Italy) were cleaned by means of soaking in an acidic mixture, containing 30% HNO₃ and 3% HF in distilled water (10 ml/2 disks), then rinsed with distilled water and dried at 37 °C overnight, prior to use.

Coating of the Ti substrates was performed by immersion of the disk-shaped samples in the calcifying solutions at 37 °C for 6 h. In some experiment, the calcifying solution was refreshed after 3 h. Coated samples were accurately rinsed in double distilled water, and dried at 37 °C overnight.

XRD measurements were performed on the coatings using a PANalytical powder diffractometer equipped with a fast X'celerator

detector. CuK α radiation was used (40 mA, 40 kV). The 2 θ range was from 3.8° to 11° and from 24° to 41° with a step size of 0.083° and a time/step of 1000 s/step.

Morphological investigation of the coatings was performed using a Philips XL-20 scanning electron microscope operating at 15 kV. The samples were sputter-coated with gold before examination.

For the observation of cross-sectional microstructure, the specimens, embedded into Methylmetacrylate (Merck Schuchardt, Hohenbrunn, Germany), were cross-sectioned with a Leica SP 1600 diamond saw (Leica, Milano, Italy). The specimens were polished, and sputter-coated with gold before SEM investigation.

Energy dispersive X-ray spectrometry (EDX) analyses were performed in order to evaluate the Ca/P ratio of the deposits.

3. Results

3.1. Powder samples

3.1.1. Characterization of the precipitates obtained at 37 °C

The new calcifying solutions containing just CaCl₂ as source of calcium ions, and NaH₂PO₄/Na₂HPO₄ buffer as source of phosphate ions, allowed to obtain fast precipitation of calcium phosphates and

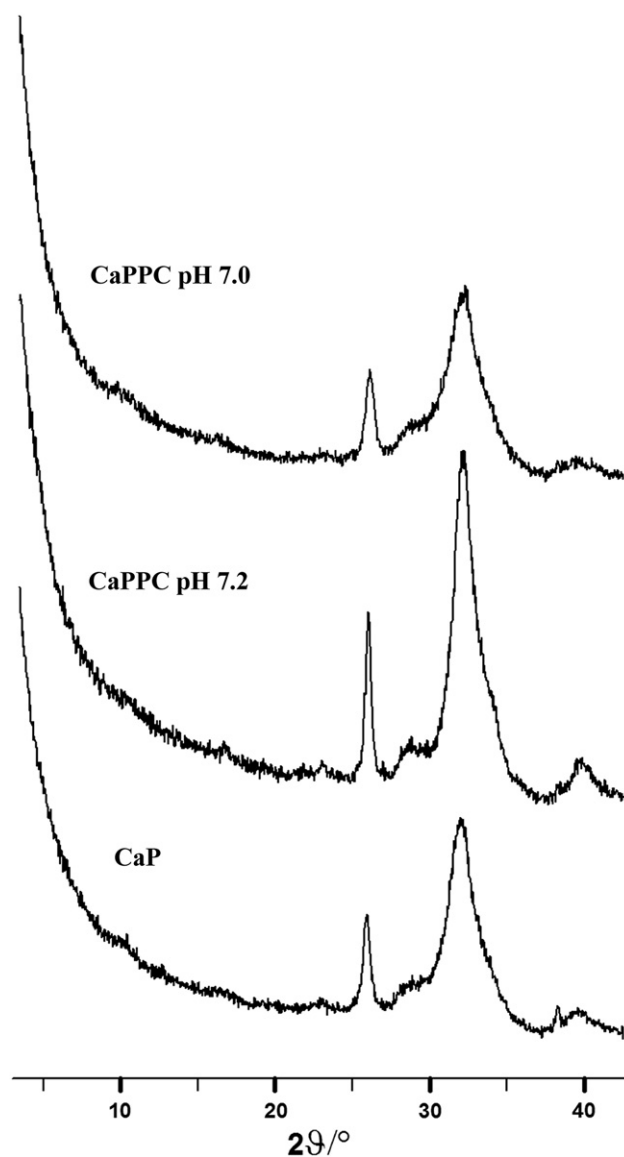


Fig. 1. Powder X-ray diffraction patterns of the powder samples obtained at 37 °C.

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