CHARACTERIZATION OF BILAYERED BRUSHITE/HYDROXYAPATITE COATINGS ON TITANIUM SUBSTRATE

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Abstract
The two-stage process of the calcium phosphate layer deposition – the plate-like brushite particles electrochemically deposited in the first stage and electrophoretically coated by the hydroxyapatite layer in the second stage – on titanium substrate was studied in this paper. The results showed that compact calcium phosphate layers of different thickness, roughness and morphology can be prepared by this method. Because of large shrinkage of the second hydroxyapatite layer during annealing, only a thin coating of hydroxyapatite on brushite forms the final calcium phosphate layer without any cracks. The main phases after thermal treatment of depositions in argon at given temperatures (from 800 to 960°C) were $\beta$TCP, $\beta$-Ca$_2$P$_2$O$_7$ and TiO$_2$. The phosphorus enriched regions (probably Ti$_x$P$_y$ phases) are formed up to 3 µm far from the titanium substrate surface in the annealing process which causes a reduction in the content of phosphorus in calcium phosphate coatings. Calcium phosphates in annealed depositions can be transformed to hydroxyapatite by hydrolysis at pH=10.8. The results showed good bioactivities of coatings in SBF.

Keywords: brushite, hydroxyapatite, composite coating, electrochemical, titanium alloys, scanning electron microscopy.

INTRODUCTION
The high degree of biostability of titanium and its alloys, which are widely used as implant materials for failed hard tissue because of good mechanical properties, corrosion resistance and compatibility with bone, is attributed to the natural formation of a protective, stable oxide layer on the surface.

In spite of the above advantages of these materials, it is required to improve its properties from the point of view of their lifespan and bioactivity, which is low for metal implants. The bioactivity is the ability of material to promote a healing process or the formation of new tissues in a body. The bioactivity of implants such as corrosion resistance can be enhanced by the deposition of a layer of biocompatible material such as, e.g., calcium phosphates. Upon the coat of calcium phosphate (as, e.g., hydroxyapatite) on the surface of titanium alloys, many surface treatment techniques have been used, including plasma spraying, biomimetic method (immersion in physiological fluids) [1,2,3], the sol–gel methods [4,5], electrophoretic deposition or electrochemical (cathodic) deposition [6-10]. Magnetron and plasma spray coating of hydroxyapatite make it possible to create
considerably strong and compact layers, but the starting stoichiometry of hydroxyapatite is changed as a result of the hydroxyapatite decomposition whereas the resulting components need not be biocompatible (e.g. CaO, phosphides of vanadium or titanium) [11,12]. A more detailed view about methods that allow surface modification of titanium or titanium alloys can be found in ref. [13]. The chemically or electrochemically deposited hydroxyapatite layers can be decomposed to other types of calcium phosphates too, mainly in cases where good mechanical properties and optimal adhesion with the substrate surface are obtained by the additional thermal treatment. A cheaper and faster method of coating of compact calcium phosphate layers is electrochemical deposition where conditions of electrolysis (e.g. electrolyte temperature, applied potential) are crucial from the point of view of the stoichiometry of the deposited calcium phosphate. The main products of electrochemical coating are hydroxyapatite, brushite and octacalcium phosphate [7,14,15]. Brushite is one of the most common formed products of the electrochemical deposition on metal substrates from aqueous solutions of calcium and phosphates at room temperature. It is strongly bonded to the surface of the metal substrate and consequently can be transformed to hydroxyapatite using the hydrothermal method usually at higher pH values [16-19]. Very compact and strong layers (double layered coatings) on titanium substrates have been prepared by a two stage process of depositions by the various methods – plasma spraying method [20], where hydroxyapatite was deposited during the first step and consequently biphasic calcium phosphate was applied; the electrophoretic hydroxyapatite deposition, where the second layer was deposited after annealing the first layer [21]; dip-coating of gel suspensions of hydroxyapatite or bioglass-hydroxyapatite [22] etc. The advantage of these methods is the preparation of top layers with a pure calcium phosphate phase (mainly pure hydroxyapatite) which differ in properties from lower layers. Lower layers provide good adhesion with the metal surface and they allow diffusion controlled reactions between Ti ions from the oxide layer on a titanium surface and calcium or phosphate ions from the calcium phosphate layer.

In the present paper we studied the method of preparation of calcium phosphate coating by the two-stage process – the electrolytic deposition of brushite and electrophoretic deposition of hydroxyapatite. This preparation method of calcium phosphate coating had not yet been studied. The morphology, phase composition and bioactivity of calcium phosphate layers were analysed after their thermal treatment above 800°C in argon. Brushite provides sufficient adhesion with titanium substrate and its higher brittleness, roughness and solubility in aqueous solution may be affected by the interaction with the second hydroxyapatite layer.

EXPERIMENTAL

Nanohydroxyapatite (HAP) for electrophoresis was prepared by the precipitation of 0.5 M Ca(NO$_3$)$_2$ and 0.5 M (NH$_4$)$_2$HPO$_4$ aqueous solutions (molar ratio of Ca/P = 1.66). The Ca(NO$_3$)$_2$.4H$_2$O solution was slowly dropped to an aqueous solution of (NH$_4$)$_2$HPO$_4$ in 1.5 hour. The pH at the end of the precipitation was kept at 11 by adding NH$_3$(aq) (1:1). The rotation of the stirrer was around 450 rpm and the reaction was done at 25°C. Ageing time was 72 h and the final hydroxyapatite powder was washed with distilled water, filtered and dried at 110°C for 2 h.

Electrolyte for electrochemical deposition of brushite (CaHPO$_4$.2H$_2$O) was prepared by dissolution of 2 g CaCO$_3$ in HNO$_3$ (analytical grade, 1+2). A diluted HNO$_3$ solution was slowly dropped in to CaCO$_3$ till it was fully dissolved (the HNO$_3$ excess in solution from the point of view of Ca(NO$_3$)$_2$ stoichiometry did not exceed 5%). This method allows achieving an accurate concentration of Ca$^{2+}$ ions in solution without
realizing additional chemical analysis (CaCO$_3$ is a primary standard for the preparation of calcium standard solutions). CO$_2$ was eliminated from the solution by boiling for 5 min. The pH solution was approximately 1.8. 1.8 mL of 8.5% H$_3$PO$_4$ (analytical grade) was added to Ca$^{2+}$ solution and pH was adjusted to 2.4 by the addition of NH$_3$ (aq, 1+2) (at this value begins the precipitation of calcium hydrogen phosphates – saturated solution). Total volume of electrolyte was 50 mL (after filling up with distilled water) and the concentrations of calcium and phosphorus were 0.4, respectively 0.24 mol/L.

The surfaces of titanium alloy (Ti2Al3V) sheets of 10 x 20 x 2 mm were abraded on 500 SiC grit paper and polished with 1 μm diamond paste. The sheets were rinsed with acetone in an ultrasonic bath for 5 minutes. Substrates were anodized in 8.5% H$_3$PO$_4$ (analytical grade) for 2 min at a current density of 10 mA/cm$^2$ [9], washed with distilled water and ethanol. Titanium substrate was used as cathode and Pt basket as anode to complete the electrolytic cell. Electrochemical deposition of brushite was done at a current density of 10 mA/cm$^2$ for 5 min. We used about 10 times more concentrated electrolyte than in [6,7] and approximately a similar concentration as in ref. [23] to depress reactions of the electrochemical decomposition of water or reduction of hydrogen phosphates to phosphates on the Ti cathode that can be more intensive at higher current densities (for details see ref. [6,7,19]). Note that the high concentration of dihydrogen phosphates (other types phosphates are not stable at pH = 2.4) are present at given conditions (as result from the distribution diagram [24]) and reduction of dihydrogen phosphates to hydrogen phosphates is the main electrochemical reaction. After brushite deposition, samples were rinsed with distilled water and ethanol. Electrophoresis of hydroxyapatite was carried out in ethanol - or isopropanol-hydroxyapatite suspensions (2 g HAP/100 mL of liquid) at various current densities (2.5-100 μA/cm$^2$) for 2.5 or 5 min [9]. Suspensions were ultrasonically dispersed for 5 min before electrophoresis. Samples were dried at 70°C for 2 h and annealed at 800, 850 or 960°C in Ar (99.999%) for 10 min. The heating rate was 15°C/min.

Bioactivity of calcium phosphate layers was analysed from the mass increment samples after their immersion into simulated body fluid (SBF, prepared according to ref. [25]) at 40°C for different times. The samples were weighed on analytical balances with an accuracy of ±0.03 mg. The SBF solution was exchanged after 2 days from the immersion of samples. Dissolution and transformation of calcium phosphate layers to hydroxyapatite were studied by analysis of the amount of phosphorus into NH$_3$(aq) solutions (pH=10.8) that is released from coated samples after their immersion into these solutions at various times. The analysed solutions were filtered over the membrane filter (Millipore, <0.2 μm, polyvinylidenfluoride) for the separation of calcium phosphate particles which can be exfoliated from coatings. The concentration of phosphorus in the ammonium solution was determined by the colorimetry as P-Mo-V complex.

The phase composition of calcium phosphate layers was analysed using X-ray diffraction (XRD) analysis (Philips, X’Pert Pro, CuKα radiation) and infrared spectroscopy (SPECORD M80, 1 mg sample + 400 mg KBr). Morphology of deposited calcium phosphate particles and microstructure of coatings were observed by scanning electron microscopy (SEM, TESLA BS 340) equipped with an EDX analyser (LINK ISIS, Oxford Instruments) after deposition of gold or graphite on sample surfaces (in the case of EDX analysis – line scan).

**RESULTS AND DISCUSSION**

Morphologies of deposited calcium phosphate layers on titanium substrate after annealing at 850°C for 10 min are shown in Fig.1.
Fig. 1. Morphologies of deposited calcium phosphate layers on titanium substrate after annealing at 850°C/10 min. in argon. (a – brushite layer (10 mA/cm²), b – brushite + HAP (ethanol, 100 μA/cm²), c – brushite + HAP (ethanol, 25 μA/cm²), d – brushite + HAP (ethanol, 10 μA/cm²), e – brushite + HAP (isopropanol, 12.5 μA/cm²), f – brushite + HAP (isopropanol, 5 μA/cm²).

Original pure brushite layer (Fig.1a), which consists of plate-like particles with average size of 10 μm, did not change its starting morphology and no cracks were visible in the microstructure after thermal treatment at 850°C (at 960°C, as well). The microstructure of the surface of the calcium phosphate layer was significantly affected by the deposition of the second hydroxyapatite layer from ethanol-HAP or isopropanol-HAP suspensions at various current densities. Because of higher current densities which could be achieved in ethanol-HAP suspension, thicker coatings could be obtained by electrophoresis. The massive sintering of the outer HAP layer deposited at 100 μA/cm² was found during annealing at 850°C, which causes the formation of large scale cracks (up to 50 μm) at the sample surface (Fig.1b). The thickness of the second layer was approximately 30 μm. In cracks, the brushite layer can be
visible with partially changed morphology, which confirms only very weak interaction between particles of both layers. Sintering of HAP nanoparticles (specific surface was 75 m²/g) in the layer is preferred to mutual interaction between different deposited phases. The dimensions and area fractions of cracks decrease with reduction in the thickness of HAP coating. A thin unbroken HAP deposition was obtained by the electrophoresis of ethanol-HAP suspension at 10 μA/cm² and sintering at 850°C/10 min (Fig.1d). In the case of electrophoretic deposition of the second hydroxyapatite layer from isopropanol-hydroxyapatite suspension at a current density of 12.5 μA/cm², the formation of cracks was observed in this layer after thermal treatment at 850°C (Fig.1e). Despite lower thickness of the HAP layer (about 5 μm) sintering of nanohydroxyapatite particles was again the favourable process in the layer during annealing. A full, very thin HAP coating of brushite was obtained at 5 μA/cm², where practically all brushite plate-like particles in the first layer were covered by HAP particles (Fig.1f). Roughness of the bottom brushite deposition was significantly lowered but the surface area of the coating is sufficient for nucleation and growth of new calcium phosphate particles such as for the growing of cells. The coarsening of the original hydroxyapatite and brushite particles at 960°C can be seen in Fig.2a,b, where globular particles of HAP grew from size from < 1 μm (at sintering temperature of 850°C) up to 2 μm, edges of brushite plate-like particles were smoothed after intensive interaction with HAP particles at a higher temperature, and particle surfaces were significantly modified. From comparison of morphologies of brushite particles in the layer after sintering at 960°C with the same coating after hydrolysis in NH₃(aq) solution (Figs.2c, d), the result is that the shape and size of brushite particles were unchanged after brushite conversion. Similarly, the microstructure of the hydroxyapatite layer sintered under the same conditions was unmodified after hydrolysis (see Fig.2b and 2e).

XRD analysis of deposited coatings after thermal treatment at 800 and 960°C are shown in Fig.3. As it is known, brushite decomposes around 500°C to calcium pyrophosphate (Ca₃P₂O₇) and it is no surprising fact that this compound was one of the components that was found after thermal treatment of brushite. According to XRD of brushite and brushite/HAP layers after annealing at 800°C, the main compounds in the layers are β-tricalcium phosphate (βTCP), β-Ca₃P₂O₇ and TiO₂ (rutile) where βTCP was the major phase. No additional peaks were present in diffraction patterns in contrast to coatings annealed at 960°C. In this deposition, the βTCP was again the major phase, but besides peaks corresponding to rutile and β-Ca₃P₂O₇ phases, other peaks at 2θ ≈ 36.89, 42.75 and 62.13° from reflections of planes of undefined phases were found. In refs.[26,27], reflections from TiₓPᵧ phases were observed in XRD patterns of composites prepared by the sintering of powder Ti and hydroxyapatite (or bioglass) mixtures at temperatures from 1000°C in argon. Lines from this phases were located approximately at the same 2θ’s that were found in our coatings (sintered at 960°C) for undefined phases. Line scan EDX analysis of deposition cross-sections are presented in Fig.4. From the comparison of analysis it shows that phosphorus concentration was significantly increased in 1-2 μm region bellow the Ti surface of the original brushite deposition annealed at 960°C, whereas the Ca concentration is very low in this region. In samples prepared at 800°C, only the small rise in P content in this Ti layer was observed. The above results confirm the formation of phosphorus enriched regions close to the titanium substrate surface after thermal treatment above 800°C. From XRD analysis, the result is that no hydroxyapatite and CaTiO₃ patterns were observed in the XRD of bilayered coating, thus, brushite and hydroxyapatite interacted as we expected at given temperatures. Infrared spectra confirm the formation of βTCP and Ca₃P₂O₇ phases at 960°C in Fig.5, where the IR
spectrum of bilayered deposition and spectrum of pure βTCP (prepared by calcination of the mixture of CaCO₃ and brushite at 1050°C) are shown.

Fig.2. Effect of annealing temperature and hydrolysis (at pH = 10.8 (NH₃ aq) and temperature of 40°C for 24 h) on morphology of coatings. (a – brushite + HAP (isopropanol, 5 μA/cm²), annealed at 850°C; b - brushite + HAP (isopropanol, 5 μA/cm²), annealed at 960°C; c - brushite (10 mA/cm²), 960°C; d - brushite (10 μA/cm²), 960°C, after hydrolysis; e - brushite + HAP (isopropanol, 5 μA/cm²), 960°C, after hydrolysis.
Fig. 3. XRD diffraction analysis of deposited coatings after thermal treatment: a – pure brushite layer before annealing, b – brushite coating annealed at 800°C, c – brushite coating annealed at 960°C, d - brushite + HAP (isopropanol, 5 μA/cm²) annealed at 800°C, e - brushite + HAP (isopropanol, 5 μA/cm²) annealed at 960°C, Ti: titanium substrate (JCPDS 01-1197), ▼ βTCP (JCPDS 09-0169), ■ β-Ca₃P₂O₇ (JCPDS 20-0024), X: undefined TiₓPᵧ phases, R: TiO₂ rutile, ● brushite.

Fig. 4. Line scan EDX analysis of coatings cross-sections. (a - brushite + HAP (isopropanol, 5 μA/cm²) annealed at 960°C, b - brushite layer annealed at 800°C, c - brushite + HAP (isopropanol, 5 μA/cm²) annealed at 800°C).
Dissolution behavior and the conversion of prepared coatings to hydroxyapatite were observed by the immersion of samples after annealing at 960°C into NH₃(aq) solution at pH=10.8 and 40°C. A similar method for brushite conversion was used in ref. [19] and some details about kinetics of brushite transformation are shown in [28]. The amounts of phosphate ions released from samples to solutions during transformation were determined. The change in the phosphorus concentration in the solution after immersion of samples into solution are shown in Fig.6. The concentration of phosphorus in solution after immersion of the sample with original brushite coatings very slowly increased with time up to 24 h from start of hydrolysis, but little change was found after only 8 h. After the rapid dissolution step of calcium phosphate bilayered deposition, the gradual decrease in phosphorus concentration in solution can be seen on dependence. In the first stage, the dissolution and hydrolysis of the top porous calcium phosphate layers with a larger specific surface of particles than in the case of pure brushite deposition, begin. This fact is probably responsible for the higher phosphorus concentration in solution during this step. Note, the excess of phosphorus from the point of view of phosphorus content (or Ca/P ratio) in hydroxyapatite was present in deposited unsintered layers, which needs to be released from coatings during hydrolysis. Amounts of phosphorus in depositions were strongly decreased by the formation of TiₓPᵧ phases in the sintering process which would verify only a small amount of phosphate ions released during hydrolysis from the sintered original brushite layer. Deposition of HAP on the brushite layer caused a rise of Ca/P ratio in coatings, and the amount of phosphorus, which can be released from bilayered depositions by hydrolysis, decreases. These facts can explain the gradual lowering of phosphorus concentration in solution during conversion of calcium phosphate phases in the coating to hydroxyapatite and lower the final phosphorus concentration in solution after 24 h from the start of hydrolysis. Thus, after the partial dissolution of the top calcium phosphate layer, the recrystallization and precipitation of hydroxyapatite particles begin. Because of higher calcium contents in bilayered depositions and the decrease in phosphorus content by the formation of TiₓPᵧ phases, which is the reason for another increase in the Ca/P ratio in coatings, the released phosphate ions react subsequently with calcium phosphate phases with low phosphorus content in depositions and hydroxyapatite is formed. IR spectra of depositions after conversion of calcium phosphates to hydroxyapatite are very
similar to the spectrum of pure nanocrystalline hydroxyapatite as is shown in Fig. 7. Antisymmetric ($\nu_3$) and symmetric ($\nu_1$) P-O stretching vibrations of PO$_4$ group at 1050, 1100 and 962 cm$^{-1}$, O-P-O bending ($\nu_4$) vibrations of PO$_4$ group at 565 and 603 cm$^{-1}$, $\nu_2$ and $\nu_3$ mode of CO$_3^{2-}$ at wavenumbers of 870 and 1400-1500 cm$^{-1}$, librational mode at 630 cm$^{-1}$ of OH hydroxyapatite group and band at 1650 cm$^{-1}$ from the vibration modes of adsorbed water can be found in spectra [29,30]. As it has been shown, no changes in the morphology of coatings were observed after the hydrolysis of deposited layers (see Fig.2).

Fig. 6. The change in the phosphorus concentration in solution during hydrolysis of depositions at pH = 10.8 (NH$_3$ aq) and temperature of 40°C for various times. (• brushite + HAP (isopropanol, 5 μA/cm$^2$) annealed at 960 °C, ♦ brushite layer annealed at 960°C).

Fig. 7. IR spectra of coatings prepared at 960°C after conversion of calcium phosphates to hydroxyapatite by hydrolysis. (pure hydroxyapatite – upper curve, bilayered deposition (●), brushite coating).
The biomimetic formation of hydroxyapatite on calcium phosphate coatings after thermal treatment at 800°C was studied in the SBF solution. New dense calcium phosphate layers were deposited after 4 days from immersion on sintered layers, whereas the average particle sizes were $\approx 5 \mu m$ in deposition on the original brushite layer and $\approx 3 \mu m$ in coating on bilayer (Fig.8).

The new hydroxyapatite particles have a globular shape. The layers are thin, which confirms the partially visible particles from the first brushite layer in Fig.8c (detail), but they fully cover the bottom calcium phosphate layers. The mass increase of samples during growth of new hydroxyapatite particles is shown in Fig.9. Slower growth rate was found on bilayered depositions mainly during the first two days after immersion into SBF solution which can be caused by higher dissolution of this layer at this stage (see Fig.6). Growth rates were very similar in subsequent days. We assumed that more nucleation sites are created on bilayered coatings, which may confirm smaller average particle size in the new hydroxyapatite layer in comparison with the original brushite coating. The hydroxyapatite particles created on the original brushite deposition can grow for a longer time and their growth rate is probably faster than the nucleation rate of the new hydroxyapatite nucleus at the surface. IR spectrum of calcium phosphate bilayered depositions after biomimetic growth (Fig.5) is composed of bands which correspond to all above presented bands from vibrations of the PO$_4$ group, carbonate group, adsorbed water and additional bands from Ca$_2$P$_2$O$_7$ phase (the content of this phase in coatings prepared at 800 °C was significantly higher than in coatings annealed at 960°C) can be observed in spectrum. Besides the band around 530 cm$^{-1}$ of calcium deficient hydroxyapatite [31], small changes in shape of bands at 570 and 600 cm$^{-1}$ can be found in spectrum. No clearly distinguished peak at 630 cm$^{-1}$ from the OH hydroxyapatite group is visible in spectrum.

![Fig.8. Morphologies of calcium phosphate coatings prepared at 800°C after biomimetic treatment in SBF solution after 4 days. (a - original brushite layer, b – bilayered deposition (brushite + HAP (isopropanol, 5 μA/cm$^2$), c – treated brushite layer (a), d – treated bilayered deposition (b)).](image)
Fig. 9. The changes in masses of coated titanium substrated during biomimetic growth of new hydroxyapatite layers. (a – origin brushite coating, b – bilayered coating).

In Figures 10 a,b are shown EDX spectra of brushite layers before and after biomimetic treatment that verify that no additional elements were incorporated into calcium phosphate coating during biomimetic growth. The results of EDX analysis confirms lower phosphorus content in comparison with pure brushite non treated deposition (Figs.10 a, b) and the same content of calcium and phosphorus in the new calcium phosphate layers (Figs. 10 b, c). Note, the Ca/P ratio was close to 1.6 in new calcium phosphate layers, but EDX analysis do not allow for the exact determination of calcium and phosphorus content in these depositions – it is only a semiquantitative analysis.
Fig.10. EDX spectra of the calcium phosphate layer after biomimetic growth for 4 days. (a – pure brushite non-treated layer, b – brushite layer annealed at 800 °C after biomimetic treatment, c – bilayered depositions annealed at 800°C after biomimetic growth).

CONCLUSION

The study of the two-stage process of calcium phosphate layer deposition – the plate-like brushite particles electrochemically deposited in the first stage and the electrophoretically coated hydroxyapatite layer in the second stage – on titanium substrate shows that compact calcium phosphate layers of different thickness, roughness and morphology can be prepared by this method. Because of large shrinkage of the second hydroxyapatite layer during annealing, only thin coatings of hydroxyapatite on brushite form the final calcium phosphate layer without any cracks. The main phases after thermal
treatment of depositions in argon at given temperatures (from 800 to 960°C) were βTCP, β-
Ca$_3$P$_2$O$_7$ and TiO$_2$. Besides this, significant amounts of Ti$_x$P$_y$ phases are formed up to 3μm
far from the titanium substrate surface at 960°C, which reduce the content of phosphorus in
calcium phosphate coatings and consequently affect the kinetics of transformation of
calcium phosphates to hydroxyapatite during hydrolysis process at pH=10.8. The results
showed good bioactivities of coatings in SBF.

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REFERENCES
Med., vol. 12, 2001, p. 529
14, 2003, p. 521
2004, p. 1187
767
51, 2007, no. 1, p. 15
vol. 13, 2002, p. 253
[12] Lusquinos, F., Pou, J., Arias, JL., Boutilguiza, M., León, B., Pérez-Amor, M.,
229
2006, p. 2573
2006, p. 543