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BSA/HA Coating on Titanium Alloy for Direct Bond Formation

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A biomimetic bovine serum albumin protein/hydroxyapatite coating was prepared by electrochemically co-precipitation on TiAlZr surface. Coating prepared in the presence and in absence of BSA were characterized by Scanning electron microscopy (SEM), Fourier Transformat Infrared microscopy (FTIR), and contact angle measurements. The calcium ion release content was determined using the inductively coupled plasma spectrophotometer (ICP-MS). The advantages of this chip and convenient electrochemical procedure of bioactivation is discussed over the commonly used procedures which prepared phosphate coatings modulate their properties without imitation of the mode in which hydroxyapatite bone crystals are formed in the body.

Keywords BSA/HA coating; Ca ion release; electrochemical incorporation

Introduction

Different kinds of materials have been used as artificial bone fillers such as metal [1] which are too hard, ceramic materials [2], too brittle to be a suitable bone substitute, and natural or synthetic polymer [3] too soft to provide a right mechanical support. One drawback of these biomaterials is that they are bioinert, in fact they have almost no influence in the surrounding living tissue and are unable to induce bone formation and accelerate the healing process. Titanium and titanium alloys intensively studied [4,5] in the last decade, have remarkable biocompatibility and stability in bioliquids [6–8], but despite various physical chemical procedures of surface modification [9,10], devoted to enhance their osteointegration, such alloys remain inert without a biomimetic coating. It has been suggested that a biological bonding between bone tissue and titanium alloys implanted surface can be obtained by using a bioactive surface modification obtained usually with the component of bones such as collagen and hydroxyapatite [11,12]. Titanium alloys prostheses coated with hydroxyapatite layer have been used with promising results in joint reconstruction.

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Recently, the biological active molecules, such as osteogenic agent and growth factor, have been co-precipitated with apatite crystals onto metal implants [13]. In this work a bovine serum albumin protein-containing calcium phosphate coating was prepared by electrochemically assisted co-precipitation, a biomimetic and chip procedure of bioactivation.

Materials and Methods

Ti alloys prepared by R&D, Consulting and Services, Bucharest, Romania by vacuum melting has a composition as following: 88% Ti, 4,51% Al, 6,67% Zr.

The TiAlZr alloys substrate was cut into discs with a diameter of 10 mm. All specimens were polished to a mirror finish with 0.05 μm Al_2O_3 powder and then cleaned in deionized water [14].

Electrodeposition was carried out in a standard three-electrode cell in which a platinum foil was used as the auxiliary (counter) electrode and a saturated calomel electrode (SCE) as the reference electrode. A Valtalab 40 potentiostat/galvanostat operating in potentiostatic mode was used. For electrochemically assisted co-precipitation of protein and calcium phosphate were needed tree steps:

(I) Electrochemically assisted precipitation of calcium phosphate. The precipitation was carried out at cathodic current o f 0.5 mA/cm^2, with the cathodic potential ranging from -1 to -1.6 V. The electrolyte solution consisted of 0.042 mol/l $Ca(NO_3)_2$ and 0.025 mol/l $NH_4H_2PO_4$. The pH of solution was around 4.5 and the solution temperature was 80°C.

(II) Converted to hydroxyapatite the coating obtained at step I. The coated specimen was thermal treated at 210°C for 10 h and then the coated specimen was immersed in BSA solution (5 mg/ml BSA in phosphate buffer solution) for 10 h at 37°C to enhance protein adsorption.

(III) The electrode was place into the electrochemical cell containig 5 mg/ml BSA in a solution of 0.042 mol/l $Ca(NO_3)_2$ and 0.025 mol/l $NH_4H_2PO_4$. The current was maintained at 0. 35 mA/cm^2 for 1 hour and the temperature of solution was 37°C.

Surface analysis have been investigated by scanning electron microscopy (SEM). The surfaces changes in the topography were observed using Environmental Scanning Electron Microscope FEI/Phillps XL30 ESEM, with pressure 0.7 Torr.

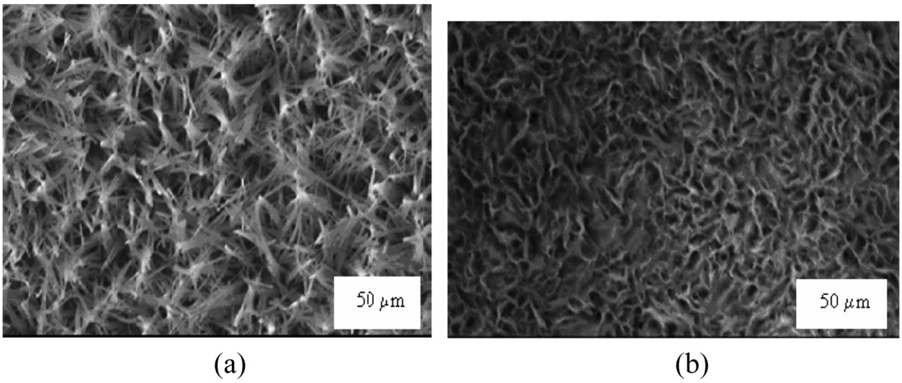
FTIR has been used to map the topographic distribution of the coating components at different times of electrochemical deposition. Infrared Microscopy Spectral data were recorded by a Perkin-Elmer equipment.

Contact angle measurements, using a Contact Angle Meter – KSV Instruments CAM 10, were carried out in order to evaluate the wettability of the surface-modified. An equal volume of distilled water was placed on every sample by means of a micropipette forming a drop on the surface.

The calcium ion release content was determined using the inductively coupled plasma ICP-MS, ELAN DRC-e Perkin Elmer SCIEX U.S.A. was used. The detection limit is 0.001 $\mu g \cdot g^{-1}$.

Results and Discussion

SEM imaging of the coating obtaining after step I is presented in Figure 1a and 1b shows the coating after step III.



(a) (b)

Figure 1. SEM of coating (a) after step I, (b) after step III.

Figure 1a revealed a highly porous coating comprised of needle-like crystals. This porous structure may have resulted from the generation of hydrogen bubbles produced in the cathodic reaction during the electrodeposition [15]. After step III a striking difference in the crystal morphology appeared with leaf-like crystals having a shorter and flatter morphology. The coating also acquired a sponge-like, smoother surface. In this cases BSA play a role in reducing the size of hydrogen bubble [16].

Comparing to other bioactivation method as plasma spraying deposition, the main advantage of this deposition is that it imitates the mode in which hydroxyapatite bone crystals are formed in the body. The small crystal units, as can be seen in Figure 1b are more easily degraded by osteoclasts than are particles obtained by plasma spaying and after coprecipitation protein are incorporated in crystal lattice, rather than deposited on the surface [17]. That reveals the role of first and second step electrochemical deposition as carriers for organic molecules with bone healing properties, which can not be added during plasma spraying procedure due to the generated temperature which is over 10.000°C.

The FT-IR analysis (Fig. 2), in the transmittance mode, for the coatings shows the peaks at 560, 602, 865 and 1100–1033 cm^{-1} attributed to PO_4^{3-} ions. The peak at 630 cm^{-1} is due to hydroxide ion and the peak at 872 cm^{-1} is due to HPO_4^{2-}. The spectrum also shows the broad bands in the 3600–2500 cm^{-1} range due to the OH stretching frequency of water and at 1620 cm^{-1} from the OH bending frequency of water. After step III the FT-IR spectrum shows a mixed composition of HA (peak at 3572 and 634 cm^{-1} from OH^-) brushite (peak at 872 cm^{-1} from HPO_4^{2-} and 3600–2500 cm^{-1} from H_2O) and BSA (peak 1530 cm^{-1} from Amide II band and 1620 cm^{-1} Amide I band coincides with OH bending mode of H_2O.

The adsorption of proteins onto calcium phosphate layer is believed to be governed by electrostatic interactions between Ca^{2+} ions, PO_4^{3-} ions and several different functional groups of the organic molecules [17–19].

The contact angle for TiAlZr uncovered was 72°. After step I of electrochemical deposition the contact angle was 52°. After electrochemical co-precipitation of protein and calcium phosphate (step III) the contact angle decreas to 32°. The decrease of contact angle from week hydrophilic as 72° to 32°, is due to the biomimetic coating of metal implants with protein incorporated into calcium phosphate [17], a imitation procedure of the mode in which hydroxiapatite bone crystals are formed

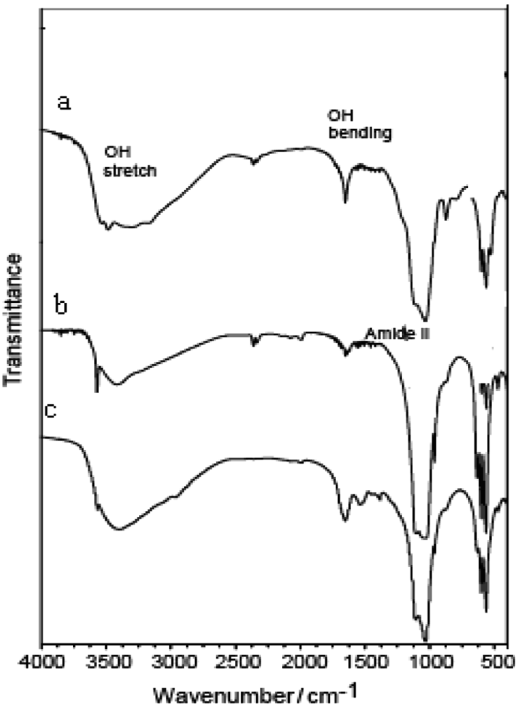


Figure 2. FTIR spectra of coating prepared by electrochemically assisted precipitation at (a) 80°C in solution without BSA, (step I) (b) 37°C after 10 h in solution with BSA (step II), (c) after step III.

in the body. The good wettability of the treated surfaces is an interesting aspect in order to obtain chemical interaction with the physiological fluids.

Dissolution Rate of Coating in NaCl 0.9%

Calcium Ion Release Concentration. The dissolution of Ca ions of coating containing BSA is more slowly than those prepare in the absence of protein (Fig. 3). The rate of

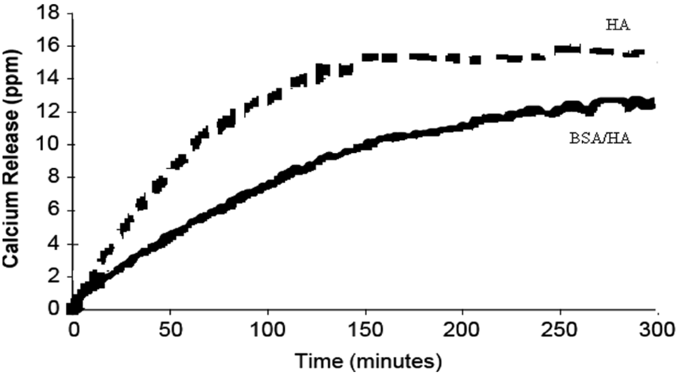


Figure 3. Ca ion release from coating prepared in the absence or presence of BSA.

Ca release from coating with BSA = 0,05 ppm/min, and were solubilized in 120 min. For the coating without BSA the rate of dissolution was 0.15 ppm/min and were completely solubilized after 275 min.

Conclusion

The following conclusions can be dran from this study:

1. A porous HA layer with needle-like morphology can be obtained using an electrochemical deposition process.
2. Protein BSA can be successfully incorporated into the a mineral matrices coating metal implants.
3. The biomimetic method for coating metal implants with protein incorporated into calcium phosphate induced a strong decrease of contact angle from week hydrophilic as 72° to 32°, a value which represents a better balance hydrophilic/hydrophobic, usually associated with better osteointegration.

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