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Effect of the deposition temperature on corrosion resistance and biocompatibility of the hydroxyapatite coatings

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ABSTRACT

Hydroxyapatite (HAP) ceramics belong to a class of calcium phosphate-based materials, which have been widely used as coatings on titanium medical implants in order to improve bone fixation and thus to increase the lifetime of the implant. In this study, HAP coatings were deposited from pure HAP targets on Ti6Al4V substrates using the radio-frequency magnetron sputtering technique at substrate temperatures ranging from 400 to 800 °C. The surface morphology and the crystallographic structure of the films were investigated by atomic force microscopy (AFM), scanning electron microscopy (SEM) and X-ray diffraction (XRD). The corrosion resistance of the coatings in saliva solution at 37 °C was evaluated by potentiodynamic polarization. Additionally, the human osteosarcoma cell line (MG-63) was used to test the biocompatibility of the coatings. The results showed that all of the coatings grown uniformly and that the increasing substrate temperature induced an increase in their crystallinity. Corrosion performance of the coatings was improved with the increase of the substrate temperature from 400 °C to 800 °C. Furthermore, all the coatings support the attachment and growth of the osteosarcoma cells with regard to the in vitro test findings.

1. Introduction

At present, the most popular materials used for medical devices can be listed as 316L stainless steel, cobalt–chromium alloys and titanium-based alloys. Unfortunately, these materials exhibit tendencies to fail after long-term usage due to various reasons such as high modulus compared to that of bone, low wear and corrosion resistance, lack of biocompatibility [1–5]. Moreover, these materials proved to have stiffness largely greater than that of the cortical bone, leading to a high rigidity, which induces stress-shielding phenomena, and ultimately the failure of the implants. Compared with the Co–Cr type alloys and stainless steels, Ti and its alloys exhibit stiffnesses closer to the bone, but still higher [6].

Until now, to improve the corrosion resistance and bioactivity of the metallic implants, various bioactive coatings have been proposed for the implant surfaces [7–9]. Bioactive hydroxyapatite has received a substantial interest because of its chemical similarity to the calcium phosphate minerals in biological hard tissue, and its ability to form strong chemical bonds with bone [10]. However, the hydroxyapatite presents a low flexural strength (100–120 MPa), a high compressive strength (350–450 MPa) and a low fracture toughness (<1.0 MPa $m^{1/2}$) compared to cortical bone which has a compressive strength of 67 MPa and a fracture toughness less than 6 MPa $m^{1/2}$ [3,11]. Therefore, hydroxyapatite ceramic materials cannot be used as heavy-loaded implants, but it is a good candidate to enhance the bonding property between metallic implants and bone [10]. Hydroxyapatite on metallic implants has been prepared by a variety of techniques including plasma spraying [12], pulsed laser deposition [9], magnetron sputtering [7,9], electrophoretic deposition [13], sol–gel [14], and electrodeposition [15,16]. Thermal plasma spraying is a high temperature processing technique, suitable for thermally stable coatings and substrates, so producing HAP layers of poor mechanical properties [17]. Although the plasma spraying is an easy and safe method for coating the Ti alloy with HAP films, a high residual stress, a low level of crystallinity, a low level of porosity, and non-uniform distribution porosity limit the application in biomedical area [18]. The aqueous deposition methods could be a good solution because they are low-temperature processing techniques, able to coat any exposed surface, but are time consuming due to the low solubility of the apatite [17]. The

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common limitations of the HAP coatings prepared by these techniques are due to their high porosity, poor thickness uniformity, insufficient crystallinity, and poor adhesion to the metallic surfaces. To prevent these drawbacks, the magnetron sputtering technique can be used, because it offers a flexible approach to surface improvement of metal implants. This technique facilitates the deposition of dense and well-adhered films with controlled elemental composition [19] by selecting appropriate values of the deposition parameters (discharge power, gas flow rate, working pressure, substrate temperature, deposition time, substrate bias voltage) [9]. In order to obtain HAP coatings with the best bioactivity properties it is required a good control of the morphology, surface roughness, and crystallinity of the coatings [9]. The major drawback of this technique is that it produces HAP coatings with low crystallinity, requiring post-deposition annealing treatment [20].

The aim of the current study is to evaluate the influence of the deposition temperature on the characteristics of the HAP coatings prepared by RF magnetron sputtering. Crystalline structure, morphology, mechanical properties, corrosion resistance and biological properties of the obtained HAP coatings were investigated. In magnetron sputtering, these properties are sensible to the deposition parameters. The coatings were prepared at substrate temperatures ranging from 400 to 800 °C. For the coatings deposited at temperatures between 600 °C and 800 °C, the post deposition annealing process, required for changing coatings to a crystalline form, was eliminated.

2. Experimental details

Si wafers (100) and Ti6Al4V alloy discs (10 mm diameter) were used as substrates. The Ti6Al4V alloy discs were mechanically ground using SiC abrasive paper (grit 4000), then polished with diamond paste until reaching a mirror-like surface finishing with an average roughness of 40 nm (measured by a surface profilometer from a 4 mm scan). The coatings were prepared using a RF magnetron sputtering unit equipped with one cathode made of HAP (1 in. diameter, 99.9% purity, Kurt Lesker Ltd.). Before introducing the substrates inside the deposition chamber, they were ultrasonically cleaned in an isopropyl alcohol to remove all the surface contaminants. Prior deposition, the substrates were etched by 1000 eV Ar+ ions for 10 min. The deposition parameters were: the base pressure = 1.3×10^{-4} Pa, Ar working pressure = 6.6×10^{-1} Pa, target power fed = 50 W, substrate bias voltage = −60 V, substrate temperature = 400, 500, 600, 700, 800 °C, deposition time = 360 min. The thickness of the coatings was of about 450 nm.

The surface morphology and the elemental composition investigations of the coatings were carried out using a Scanning Electron Microscope coupled with an Energy Dispersive Spectrometer (FEI InspectS). The surface morphology was also evaluated by an Atomic Force Microscope (AFM) (INNOVA, Vecoo), operating in the tapping mode, from $10 \times 10\ \mu m^2$ area scans. Crystalline structure, phase composition and texture were determined by means of X-ray diffraction (XRD) analysis with Rigaku MiniFlex II diffractometer, using Cu Kα radiation.

The corrosion behavior of the coatings was evaluated in Fusayama artificial saliva solution (composition: 0.4 g l^{-1} NaCl, 0.4 g l^{-1} KCl, 1 g l^{-1} urea, 0.69 g l^{-1} NaH_2PO_4, 0.795 g l^{-1} $CaCl^*2H_2O$, pH = **5**) at 37 °C, by using a VersaSTAT 3 Potentiostat, according to the ASTM standard G 59–97 (reapproved 2003). A conventional electrochemical cell with three electrodes was used for corrosion measurements: a Pt counter electrode, saturated calomel (SCE) as a reference electrode and the sample (1 cm^2) as a working electrode. The open circuit potentials (OCP) were measured in the test solution during 1 h sample immersion. The polarization curves were obtained by sweeping the potential from −1 V vs. OCP to 2 V vs. SCE at a scan rate of 0.1667 mV/s.

The corrosion current density (i_{corr}) and corrosion potential ($E_{i=0}$) were calculated by graphical extrapolation of the anodic and cathodic branches at overpotentials of about ±50 mV [21]. Additionally, by plotting the polarization curve as a linear potential-current density plot, the polarization resistance (R_p) was determined as the slope of the curve at $i = 0$.

MG 63 human osteosarcoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% inactivated foetal bovine serum (FBS) supplemented with 1% penicillin, streptomycin and neomycin (Sigma). The cells were incubated at 37 °C, 5% CO_2 with 100% relative humidity. For biological tests, 25×10^3 cells/cm^2 were seeded and cultured for 3, 5 and 7 days on studied samples. Viability of MG63 cells was measured by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Osteosarcoma cells were cultured in 24 well culture plates on tested samples for 3, 5 and 7 days, respectively. At the end of the experiments, the MTT solution was added to fresh culture medium without phenol red and serum at 10% of the total culture volume according to the manufacturer's instructions. After 3 h incubation at 37 °C in CO_2 incubator, 10% anhydrous isopropanol/0.01 N HCl was added to each well and the absorbance at 590 nm of solubilized MTT formazan products was measured using a TECAN spectrometer. For each experiment, multiply by threes was run and repeated at least three times. The colonization capacity of the cell after 5 days in culture on the investigated surfaces was evidenced by optical microscope equipped with an epi-fluorescence and G1-B filter [Hoechst nuclear staining (blue) and actin staining with phalloidin (green)]. The micrographs were captured with a Sony DSC-S75 Digital camera. The paired Student's *t*-test was used for all statistical analyses.

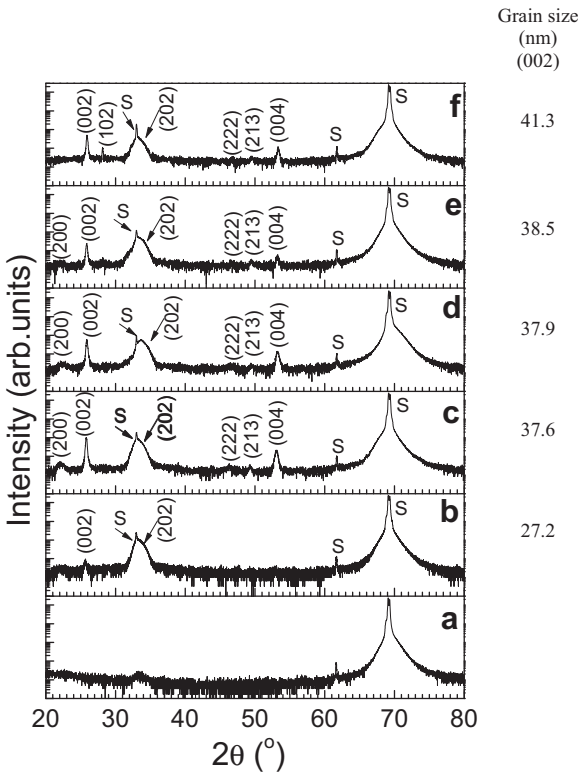

Fig. 1. X-ray diffraction patterns of the Si wafer substrate (a) and HAP films deposited at 400 °C (b); 500 °C (c); 600 °C (d); 700 °C (e); 800 °C (f).