Available online at www.sciencedirect.com
ScienceDirect
Journal of Bionic Engineering 12 (2015) 583–591

Influence of Doping Ions on the Antibacterial Activity of Biomimetic Coating on CoCrMo Alloy

Georgeta Totea, Daniela Ionita, Ioana Demetrescu

Faculty of Applied Chemistry and Materials Science, *Department of General Chemistry*, *University Politehnica of Bucharest*, 1-7 *Polizu*, *district* 1, *Bucharest Ro*-011061, *Romania*

Abstract

In this study biomimetic fluoridated phosphate doped hydrophilic coatings with various ions on CoCrMo alloy were prepared by electrodeposition. Cu and Zn ions were chosen for doping because of their well known antibacterial activity. The structures of the coatings were identified using Fourier-transform Infrared (FTIR) analysis. X-ray Diffraction (XRD) analysis was performed to evaluate crystallite dimensions of the specimen surface. The contact angle was measured in order to establish the hydrophilic/hydrophobic balance and to compute surface energy. All studied samples have a hydrophilic character which is weaken after doping. The time evolution of ions releasing from the coatings was evaluated with an inductively plasma mass spectrometer after immersion in saline phosphate. The hemolytic experiments indicate that except the fluoridated coatings doped with Zn which is slightly hemolytic, all other samples are non hemolytic. The test for antibacterial activity for *Staphylococcus aureus* and *Pseudomonas aeruginosa* indicated that the fluoridated biomimetic coating doped with various positive ions increases bacterial growth inhibition level significantly. Fluoridated phosphate coating doped with Cu has best antibacterial activity.

Keywords: phosphate coating, electrodeposition, CoCrMo alloy, doping ions, antibacterial activity

doi: 10.1016/S1672-6529(14)60148-7

1 Introduction

Due to its passive film formed on the surface in various bioliquids[1] CoCrMo alloy has remarkable corrosion resistance together with a good biocompatibility and mechanical properties, which recommends this alloy as a good choice for biomedical applications. Taking into account that Ni is an allergenic element, and could be carcinogenic as well[2], especially the type of Ni-free alloy with a composition according to ASTM F-75 standard[3] is more used. Numerous and various ways in improving its performance in exploitation in dentistry and orthopaedics are in continous development[4–6]. A part of them are oriented to biomimetic coatings[7] already known as good options for other substrates such as stainless steel[8], magnesium alloys[9], and titanium[10,11]. In all such cases after learning from nature design to copy nature, the answer of the living body was a friendlier one. Biomaterials based on calcium orthophosphate have their wide range of applications in medicine[12], and among them, synthetic hydroxyapatite (HAP,$Ca_{10}(PO_4)_6(OH)_2$) is the most promising favorite candidate as biomimetic coating, knowing that the bone is a natural composite with hydroxyapatite and due to its biocompatibility, bioactivity, and osteoconductivity. Despite such merits, it needs to point out that such biomimetic coatings depending on fabrication procedures have demerits, such as dissolution and degradation leading to the weakening of the coating-substrate bonding[13]. Doping the coatings with various ions by substituting OH^- groups was performed successfully on various alloys[14,15], changing the surface properties in a positive direction, firstly increasing stability. It was suggested that a fluoridation level of 0.5–0.625, is the best compromise among cell response, apatite deposition and dissolution resistance[14]. The effect of fluoride in oral cavity is the prevention of caries which is mainly attributed to the process of demineralization/remineralization at the tooth-oral fluid interface. The shift from demineralization to remineralization is starting at ppm levels of fluoride in saliva. Increasing the

Corresponding authors: Ioana Demetrescu
E-mail: i_demetrescu@chim.upb.ro; ioana_demetrescu@yahoo.com

fluoride content in saliva and plaque to the amount at which carries can be prevented is a strategy of oral hygiene and could be combined with an antibacterial treatment. The fluoridated hydroxyapatite has small bacteria inhibition rate, introducing various ions could be an efficient procedure in improving antibacterial properties. The incorporation of antibacterial ions in fluoridated hydroxyapatite coating (FHA) has been performed using Cu, Zn and Ag. Functionally graded hydroxyapatite coatings doped with antibacterial components on cheaper substrates, such as stainless steel, have been developed in recent years[8,16]. Previous studies reported the electrochemical elaboration of doped biomimetic phosphate coatings on stainless steel[8,16], but did not motivate the reasoning for their choice in selecting doping ions. The aim and the novelty of the present paper are to find the doping ions of biomimetic coating with best influence on antibacterial activity on CoCr alloys. A comparison between the elaboration and behaviour of the fabricated phosphate masses on stainless steel and CoCrMo alloy is discussed as well.

A schema including the influence of doping ions in antibacterial activity of phosphate masses is proposed.

2 Materials and methods

2.1 Biomimetic coating elaboration

Before coating, the sample plates were prepared according to our previous research[8].

The coating process was performed at 30˚C, in electrochemical cell having platinum plate as anode, Ag/AgCl as reference electrode, and CoCrMo alloy as cathode. The composition of working electrode (wt.%) was Co 63.5, Cr 27, Mo 5.5, Fe 2.2, Si and Mn 1. VoltaLab PGZ 301 potentiostat/galvanostat controlled by a computer equipped with VoltaMaster4 Software was the electrodeposition equipment (cronopotentiometric procedure). For fluoridated phosphate coating, the electrolyte contained a mixture with 0.025 mol·L^{-1} $(NH_4)2HPO_4$, 0.42 mol·L^{-1}, $CaCl_2{\cdot}2H_2O$ and 0.012 mol·L^{-1} NH_4F while fluoridated hydroxyapatite/titanium dioxide nanocomposite coating was elaborated previously by an electrochemical deposition technique, adding F(−) ions, nanoscaled TiO_2 particles and 6% H_2O_2 into the electrolyte[17], the present recipe is a modified one[14]. Other working conditions were related firstly to the anodizing time as 30 minutes at 30˚C, keeping the pH at 4.8 and adding H_2O_2 3% for H_2 gas release and production of HO^-, according to well known process of H_2O_2 reduction. Doped coatings were obtained after introducing in the initial electrolyte 50 ppm of $ZnCl_2$ for the coating doped with Zn and 50 ppm $CuCl_2{\cdot}2H_2O$ for the coating doped with Cu. A mixture of 50 ppm from both components in equal parts was incorporated for the situation of a double doping with Cu ad Zn as well.

2.2 Biomimetic coating characterization

2.2.1 Physical chemical characterization

Structure analysis with identification of characteristic chemical bonds was performed with an ATR Perkin Elmer FTIR equipment. The identification and quantification of crystalline phases, including the determination of network parameters, was performed with a D8 DISCOVER (Bruker Axs), as presented in a previous work[8].

The hydrophilic-hydrophobic balance was evaluated from wettability measurements which have been performed to determine the Contact Angle (CA) values with a 100 Optical Contact Angle Meter (CAM 100). Each determination value is the average of minimum three measurements and all the tests were carried out with an accuracy of ± 1˚ at 25 ˚C. The liquid used to measure the contact angle was distilled water.

The evolution in time of ions release from biomimetic coatings was measured with an inductively plasma mass spectrometer (ICP-MS) equipment Perkin Elmer ELAN DRC-e in the working conditions presented previously[8]. The instrument was working with liquid sample introduction by a micro-nebulizer and calibration was against aqueous multi-element solutions for internal standardization.

2.2.2 Biological characterization

The tests, including the blood acquisition and positive and negative control sample preparation, were described in previous papers[8,18]. Drabkin method[19] in determination of biomaterial cytotoxicity over the red blood cells was used. Hemolytic activity was determined according to ASTM F 756-00[20] and ISO 10993-4(ISO 10993-4:2002/Amd1: 2006[21]). The formula used for calculation of Hemolytic Index (*HI*) was[8]:

$$HI\%=\frac{(OD_{\text{sample}}-OD_{\text{negative controle}})}{(OD_{\text{positive control}}-OD_{\text{negative controle}})}\times 100, \quad (1)$$