Materials Science and Engineering C 40 (2014) 435–444

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Ion release and surface oxide composition of AISI 316L, Co–28Cr–6Mo, and Ti–6Al–4V alloys immersed in human serum albumin solutions

Shima Karimi, Akram M. Alfantazi

Department of Materials Engineering, The University of British Columbia, 309-6350 Stores Road, Vancouver, BC V6T 1Z4, Canada

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form 7 March 2014
Accepted 3 April 2014
Available online 13 April 2014

Keywords:
Biomaterial
Oxide film
Immersion test
Protein
Ion release
Surface concentration

ABSTRACT

The long-term weight loss, ion release, and surface composition of 316L, Co–28Cr–6Mo and Ti–6Al–4V alloys were investigated in a simulated body environment. The samples were immersed in phosphate-buffered saline (PBS) solutions with various human serum albumin (HSA) concentrations for 8, 14, and 22 weeks. The specimens initially lost weight up to 14 weeks and then slightly gained weight. The analysis of the released ions was performed by induced coupled plasma-optical emission spectrometer (ICP-OES). The results revealed that the precipitation of the dissolved Fe and Co could cause the weight gain of the 316L and Co–28Cr–6Mo alloys. The surface chemistry of the specimens was determined by X-ray photoelectron spectroscopy (XPS). The XPS analysis of Co–28Cr–6Mo alloy showed that the interaction of Mo with HSA is different from Mo with bovine serum albumin (BSA). This was also observed for Na adsorption into the oxide layer of Ti–6Al–4V alloy in the presence of HSA and BSA.

1. Introduction

Ion release as a consequence of body fluid interacting with a metal–oxide surface can disturb cell behavior. This disturbance can change the pH and oxygen partial pressure of the tissue adjacent to the implant [1]. Ion release increases the possibility of systemic toxicity and cancer risk. The most hazardous ions released from surgical alloys were Co from the CoCrMo, Ni from stainless steel and V from the Ti–6Al–4V alloys [2]. The amount of metal dissolution and formation of corrosion products can be identified by performing immersion corrosion experiments in simulated body environments. The presence of main components of the body fluid is required to successfully simulate body environment. Body fluids contain Cl^{-}, Ca^{+2}, Na^{+}, PO_3^{-3} as well as organic acid anions. Some of the organic complex compounds of human fluids are phospholipids, cholesterols, natural fats, glucose, and proteins [3]. Corrosion of biomaterials is usually studied in the presence of these species to simulate a real body environment.

In a 5-day immersion corrosion study, the oxide layer composition of a high carbon CoCr alloy was compared in several simulated body fluids such as human serum, fetal bovine serum (FBS), synovial fluid, and phosphate-buffered saline (PBS) solutions [4]. The deposition of calcium phosphate impeded the release of the migrated Co and Cr from the bulk materials [4–6]. The deposition of calcium and phosphate on the Co–36.7Cr–4.6Mo alloy in Hanks' solution containing 10% FBS was also determined over a 7-day immersion test [7]. The presence of Ca^{2+} and Mg^{2+} increased bovine serum albumin (BSA) adsorption on 316L, whereas the BSA adsorption was independent of Na^{+} [8]. The ion release of 316L and high carbon CoCr powders was also measured by atomic absorption spectroscopy (AAS) up to five days of incubation in human serum [9]. The predominant corrosion product was organometallic complexes. These complexes formed either at the metal–solution interface or in the solution. For instance, Cr and Ni interactions with protein were at the interface and Co bonded to serum in the solution [9].

Applied load and chemical composition of metallic bio-implants can influence metal dissolution. Ion release of wrought CoCrMo alloys (F75 and F1537) and a spark plasma sintered CoCrMo alloy in a solution of 25 wt.% bovine serum, 0.01 wt.% Na azide and distilled water under a rotational motion and load of 5 N was measured [10]. The rate of Co release was faster than Mo and Cr from these alloys. The release of Co from F75 had the lowest value. The amounts of Cr and Mo released from the sparked plasma sintered alloy were smaller due to the lower carbide content in this alloy as opposed to F75 and F1537 [10]. Metal release from CoCrMo (F75), Ti–6Al–4V (F136) and commercially pure Ti (F67) in 4 mg L^{-1} of human serum under a constant rocking motion for 1 week was measured by Hallab et al. [11]. Cr dissolution from the CoCrMo alloy steadily increased over the course of 4 days. The Ti release rate was relatively fast within the first hour and then leveled off for the next 100 h. Yan et al. stated that the presence of protein increased the metal ion release in static environments but decreased ion release in sliding systems because bovine serum lubricated the counter bodies [12].

Protein adsorption depends on the substrate, protein type and concentrations [13–19]. For instance, the adsorption of BSA on pure Cr

E-mail address: shimak80@gmail.com (S. Karimi).

http://dx.doi.org/10.1016/j.msec.2014.04.007
0928-4931/

and Mo in a 0.1 M phosphate buffer ($Na_2HPO_4 + NaH_2PO_4$) solution was characterized by Pradier et al. [20]. This study showed that the amount of adsorbed phosphate and protein on the Cr substrate was higher than on the Mo inlay. The authors addressed these differences by the variation of hydroxylation and charges of metallic samples [20].

Numerous simulated body fluids have been used in corrosion studies to understand the cause of implant failure due to the high dissolution rate of metallic biomaterials. To the best of our knowledge, only few papers have considered immersion corrosion studies in PBS solutions containing human serum albumin (HSA) [4,9,11,21]. HSA is the main component of the human circulatory system which can bind to a variety of ligands. The aims of these studies were to analyze the ion release, protein adsorption and oxide composition of biomaterials immersed in HSA for different immersion periods. However, these studies were not conducted in phosphate buffered saline solutions which are a typical *in-vitro* testing environment. In this paper, we present the long-term study of ion release and surface concentration of 316L, Co–28Cr–6Mo and Ti–6Al–4V alloys along with weight loss measurements in PBS solutions with several HSA concentrations (0–4 g L^{-1}). Long-term immersion of the samples into the solutions was used to measure the weight loss. In addition, similar materials and methods to our previous work on BSA–PBS [15] were employed to compare the effects of protein type (animal serum albumin *vs.* human serum albumin) on the corrosion performance of the selected metallic biomaterials.

2. Experimental

The actual chemical composition (in wt.%) of AISI 316L (Goodfellow Cambridge Limited), Ti–6Al–4V alloy (Goodfellow Cambridge Limited) and Co–28Cr–6Mo (ATI Allvac Limited) is provided by the suppliers in Table 1. Plates of the 316L, Co–28Cr–6Mo, and Ti–6Al–4V alloys with a dimension of 20 × 40 × 1 mm^3 were provided. A one-millimeter-diameter hole was drilled at the top center of each plate.

In brief, all the samples were mechanically polished to 6 μm and ultrasonically cleaned consecutively in solutions of soap, deionized water, and ethanol each for 5 min. They were then dried in hot air and their weight was recorded prior to immersion into the solution's containers. The containers were cleaned according to ASTM standard D5245-92 [22] and filled with 200 mL of the PBS–HSA solutions. A plate was fully immersed in the solution for 8, 14, and 22 weeks. The experiments were conducted in the PBS solutions having protein concentrations of 0, 0.2, 0.4, 2, and 4 g L^{-1}. The chemical composition of the PBS solution is (in g L^{-1}) 8 NaCl, 0.2 KCl, 1.15 Na_2HPO_4, and 0.2 KH_2PO_4 which was indicated in ASTM standards F2129 [23]. The HSA was provided by Sigma-Aldrich. It has 585 amino acids [24,25]. The amino acids' sequence of HSA has 17 disulfide bridges, one free sulfhydryl (SH^-), and a single tryptophan ($C_{11}H_{12}N_2O_2$) [24,25]. The primary structure of HSA contains three homologous domains (named I, II, and III) and each domain has two separate helical subdomains (named A and B) [26]. The only tryptophan of HSA is located in subdomain IIA, which is a hydrophobic fold [27].

The pH of the PBS–HSA solutions was about 7.4. The experiments were conducted in aerated environments and maintained at 37 °C. Each immersion test was repeated three times for a certain HSA concentration to find mean values of weight loss and ion release as well as their standard deviation. At the end of each immersion period, the plates were removed from the solution and they were rinsed by deionized water and dried out. The samples were weighed and stored for X-ray photoelectron spectroscopy (XPS) analysis. The oxide surface compositions of specimens were defined by a Leybold MAX200 XPS spectrometer, using a monochromatic Mg K_α X-ray source and pass energies of 192 and 48 eV for survey and narrow scans, respectively. The XPS samples were immersed in the PBS solutions with 0, 0.2, and 4 g L^{-1} HSA for 22 weeks. The amount of ion released into the PBS and HSA solutions were detected by VARIAN 725-ES induced coupled plasma-optical emission spectroscopy (ICP-OES) which had been calibrated by multi-element ICP-AES & MS standard (SCP SCIENCE, 100 μg mL^{-1}). The protein content of all the solutions was digested before ICP-OES analysis. The details of the static immersion experiments, such as protein digestion and ICP-OES calibration, can be found in our previous work [15].

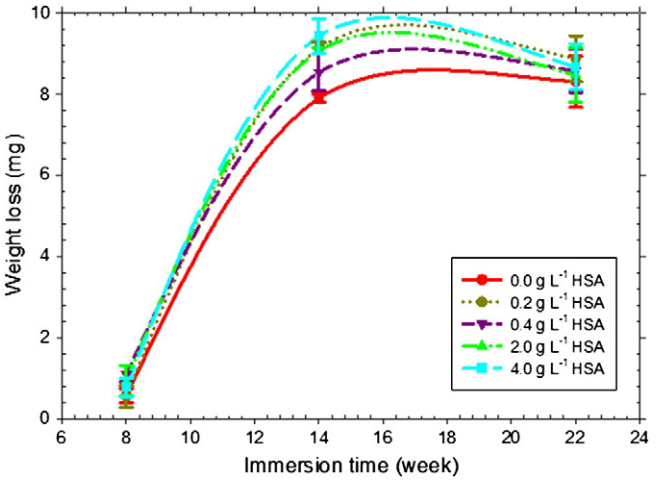

Fig. 1. Weight changes of the 316L after 8, 14, and 22 weeks of immersion in the PBS solutions with various HSA concentrations (0 to 4 g L^{-1}) at 37 °C.

3. Result and discussion

3.1. Weight loss

The weight loss of the AISI 316L, Co–28Cr–6Mo, and Ti–6Al–4V alloys immersed in the PBS solutions containing various HSA concentrations over 8, 14, and 22-weeks is shown in Figs. 1–3. The error bars indicate the standard deviation of the weight loss of the three measurements. The immersed samples lost weight significantly within 8 to 14 weeks. Hanawa [6] stated that the surface of metallic biomaterials undergoes a repeating process of partial dissolution and precipitation in aqueous solutions. Depending on the rates of partial dissolution and precipitation, either ion releases or oxide layer grows [6]. After 14 weeks of incubation, the rate of precipitation is faster than dissolution. Therefore, the weight of the samples slightly increased over 14 to 22 weeks of immersion indicating the growth of the oxide layer and formation of a deposition layer. Koike and Fujii [28] also reported the weight gain of pure Ti when it was immersed in physiological saline solution due to Ti dissolution and the formation of TiO_2. The deposition of calcium and phosphorous was demonstrated on all the samples after 22 weeks of immersion by XPS analysis which will be discussed later in Section 3.3. Further study of

Table 1
Chemical composition of the 316L, Co–28Cr–6Mo, and Ti–6Al–4V alloys (wt.%).

	Fe	Co	Cr	Ni	Mo	Mn	C	S	P	Si	N	Ti	Al	V	O
316L	Bal.		18.1	12	2.4	1.8	0.03	0.02	0.02	0.75					
Co–28Cr–6Mo	0.25	Bal.	28	0.2	6	0.5	0.06			0.5	0.2				
Ti–6Al–4V	0.15						0.04				0.035	Bal.	5.9	3.9	0.11