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Development of Co-based bulk metallic glasses as potential biomaterials

Zeyan Zhou [a], Qin Wei [b], Qiang Li [a,*], Bingliang Jiang [b,*], You Chen [b], Yanfei Sun [a]

[a] School of Physics Science and Technology, Xinjiang University, Urumqi, Xinjiang 830046, People's Republic of China
[b] First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830046, People's Republic of China

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ABSTRACT

A new series of $Co_{80-x-y}Cr_xMo_yP_{14}B_6$ (x = 5 y = 5; x = 5 y = 10; x = 10 y = 10, all values in at.%) bulk metallic glasses (BMGs) with a maximum diameter of 1.5 mm has been developed for using them as potential bio-implant materials by a combination of fluxing treatment and J-quenching technique. The performance of the present Co-based BMGs in biomedical implant applications was investigated as compared to the CoCrMo biomedical alloy (ASTM F75) and 316L stainless steel (316L SS). The corrosion behavior of the samples was investigated in both Hank's solution (pH = 7.4) and artificial saliva solution (pH = 6.3) at 37 °C employing electrochemical measurements. The results indicate that the Co-based BMGs exhibit much higher corrosion resistance in the simulated body solutions than that of 316L SS. Compared with the corrosion resistance of ASTM F75, that of $Co_{70}Cr_5Mo_5P_{14}B_6$ and $Co_{65}Cr_5Mo_{10}P_{14}B_6$ BMGs is found to be lower and that of $Co_{60}Cr_{10}Mo_{10}P_{14}B_6$ BMG is higher. The concentrations of Co, Cr, and Mo ions released into the simulated body solutions from our Co-based BMGs after potentiodynamic polarization are significantly lower than that released from ASTM F75. The biocompatibility of the specimens was evaluated using an *in vitro* test of NIH3T3 cell culture in the specimen extraction media for 1, 3, 5, and 7 days, revealing the non-cytotoxicity of the Co-based BMGs towards NIH3T3 cells. Moreover, examinations on the cell adhesion and growth on the surface of the specimens indicate that the Co-based BMGs exhibit better cell viability compared to ASTM F75 and 316L SS biomedical alloys.

1. Introduction

Metallic biomaterials have the longest history among various biomaterials, and they have been extensively used in different parts of the human body such as in artificial valves in the heart, stents in blood vessels, and replacement implants in shoulders, knees, hips, elbows, ears, and orodental structures [1–3]. Despite the large number of metals and alloys produced in the industry, the specific requirements such as the corrosion resistance and the biocompatibility restrict the use of available commercial metallic materials as biomaterials. The commercial metallic biomaterials can be classified into the following four groups: stainless steels, Co-based alloys, Ti-based alloys, and other miscellaneous materials (*e.g.* NiTi shape memory alloy and alloys of Mg and Ta) [4]. Among these metallic biomaterials, Co-based alloys exhibit good biocompatibility, excellent mechanical properties, and a high corrosion resistance. Hence, they have been widely used as artificial joints in biomedical implants [5–6]. However, the use of Co-based alloy implants is limited owing to their degradation due to the corrosion environment in the body, which reduces their long-term stability and leads to releasing of toxic ions and wear particles into the body [7]. Therefore, further improvements in the corrosion and wear resistance and mechanical properties of the Co-based alloy biomaterials are crucial for their applications in biomedical implants.

With the development of novel biomedicine materials, bulk metallic glasses (BMGs) have attracted significant attention owing to their several desired properties in biomedical implant applications, including excellent biocompatibility, corrosion resistance and wear resistance, high strength, low Young's modulus, and simple processing capabilities compared to the crystal alloy counterparts [8]. Thus, Ti- [9–11], Zr- [12–14] and Fe-based [15–17] BMGs have been developed for biomedical implant applications during the past two decades, and they exhibit enhanced performances as biomedical implant biomaterials compared to several conventional metallic biomaterials. Co-based alloys constitute an important member in the family of metallic biomaterials; however, the applications of Co-based BMGs as biomaterials implants have not yet been investigated.

In this work, a new series of $Co_{80-x-y}Cr_xMo_yP_{14}B_6$ (x = 5 y = 5; x = 5 y = 10; x = 10 y = 10, all values in at.%) BMGs is developed for biomedical implant applications by means of a combination of fluxing treatment and J-quenching techniques [18]. The corrosion performance, ionic releasing, and the biocompatibility of the Co-based BMGs are investigated and compared with those of CoCrMo biomedical alloys (ASTM F75) and 316L stainless steel (316L SS).

* Corresponding authors.
E-mail addresses: qli@xju.edu.cn (Q. Li), gaohan410@126.com (B. Jiang).

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2. Materials and methods

2.1. Material preparation and characterization

$Co_{80-x-y}Cr_xMo_yP_{14}B_6$ (x = 5 y = 5; x = 5 y = 10; x = 10 y = 10, all values in at.%) master alloy ingots were prepared by torch-melting a mixture of high-pure Co powder (99.9 mass%), Cr powder (99.9 mass%), Mo powder (99.9 mass%), Co_2P powder (99.9 mass%), and B piece (99.9 mass%) under high-purity argon atmosphere. The master alloy ingots were then fluxed in a fluxing agent composed of B_2O_3 and CaO with a mass ratio of 3:1 at a temperature of about 1500 K for 4–5 h under vacuum corresponding to a residual pressure of ~50 Pa. After fluxing, the specimens were cooled down to ambient temperature followed by subjecting them to J-quenching, the details of which can be found elsewhere [19] to obtain alloy rods with a diameter of 1.0 mm and length of several centimeters. The glassy nature of the as-cast specimens was examined using X-ray diffraction (XRD, Bruker D2 PHASER) with Cu Kα radiation (30 kV and 30 mA) at room temperature. The thermal behavior of the as-cast specimens was examined by differential scanning calorimetry (DSC, NETZSCH DSC 404C F1) with a heating rate of 0.33 K/s under Ar atmosphere.

2.2. Electrochemical measurements

Electrochemical measurements of the specimens of Co-based BMGs, 316L stainless steel (316L SS), and CoCrMo biomedical alloy (ASTM F75, purchased from Jinyehongtai Metals Co. Ltd., Beijing, China), were examined using a CS315 electrochemical workstation. Electrochemical polarization was carried out in a three-electrode cell using a platinum auxiliary electrode, a saturated calomel reference electrode, and a test specimen as the working electrode in 200 mL electrolytes in air. The whole cell was maintained at 37 °C throughout the test. Hank's solution (pH 7.4) and artificial saliva solution (pH 6.3) were used as electrolytes in the present study, which were purchased from Biohao Biotechnology Co. Ltd. (Beijing, China). Prior to the test, the work surface of the specimens was mechanically polished with 2400-grit wet sand paper washed with distilled water, and dried in air. Potentiodynamic polarization curves of the specimens were recorded from −1.0 V to 1.5 V with a scan rate of 0.2 mV/s after immersing the specimens in electrolytes for 1 h for obtaining a steady open-circuit potential. The corrosion parameters, namely, the corrosion potential (E_{corr}), corrosion current density (I_{corr}), pitting potential (E_{pit}), and corrosion rate (CR) were determined from the potentiodynamic polarization curves to evaluate the corrosion resistance of the specimens.

2.3. Scanning electron microscopy (SEM) observations

The surface morphology of the specimens after cell adhesion was examined by SEM at 5 kV in secondary electron mode.

2.4. Inductively coupled plasma atomic emission spectrometry (ICP-AES) measurements

A full spectrum direct reading ICP-AES was employed to measure the concentrations of ions dissolved from the specimens into the Hank's solution and artificial saliva solution after potentiodynamic polarization. An average of three measurements was taken for each sample.

2.5. Cell culture

Mouse embryonic fibroblast cell (NIH3T3 cell) lines were cultured in 10 mL RPMI 1640 medium (purchased from GIBCO laboratories, Grand Island, NY USA) with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humid atmosphere of 5% CO_2. Cells were washed and incubated in a fresh medium for 24 h followed by changing to a new medium. After 72 h, the bottle was full of cells. Subsequently, 0.25% trypsin solution was added to shed the cells followed by the addition of a growth medium (90 mL 1640 + 10 mL FBS + 100 μL P/S) to stop the reaction. Cells were counted as needed.

2.6. Cytotoxicity test

The Co-based BMGs and 316L SS rods with a diameter of 1 mm and ASTM F75 square bar with a cross-sectional area of 2 mm × 2 mm were cut into thin wafers of 0.8 mm thickness. One side of the wafers was polished to a mirror finish using 2400-grit wet sand paper until the final thickness of the wafers was around 0.5 mm. The wafers were washed successively with acetone, absolute ethyl alcohol, and deionized water by ultrasonication for 20 min and were dried in air. The wafers were packed and sterilized in a high-pressure steam sterilization pot. Five samples of each specimen were plated with the mirror side up to obtain each of three 96-well cell culture plates, and the experiments were performed on a super clean bench in a bioclean room. Cells were incubated in 96-well cell culture plates with 2500 cells per well. After incubating the cells in a humid atmosphere with 5% CO_2 at 37 °C for 1, 3, 5, and 7 days, 10 μL of Cell Counting Kit-8 (CCK-8) was added to each well avoiding blister followed by further incubation with CCK-8 at 37 °C for 1.5 h. The samples were then collected for spectrophotometrical absorbance measurements using a microplate reader at 450 nm.

Four replicates (both the samples and controls) were used for all tests. The cell viability ratio (R_{cv}) of each sample was calculated using the equation: R_{cv} = (cell viability in experimental extract)/(cell viability in negative control).

2.7. Cell adhesion

The Co-based BMGs with a diameter of 1 mm, 316L SS rods with a diameter of 1 mm, and ASTM F75 square bar with a cross-sectional area of 2 mm × 2 mm were used for the cell adhesion test. The test specimens were prepared in the same way as in the cytotoxicity test. 1 mL cell suspension was seeded onto the polished side of the wafers with a cell density of 2500 cells per well in 24-well plates. After 5 days of culture in a humid atmosphere with 5% CO_2 at 37 °C, the cell-seeded discs were washed with D-Hank's three times before fixing with 3% glutaraldehyde for 1.5 h and dehydrated using tertiary butanol and absolute ethyl alcohol in proportions of 30%, 50%, 70%, 80% and 90% for 10 min each time at room temperature (25 °C), respectively. After rinsing twice with 100% tertiary butanol, the 24-well plates were placed in fridge at a temperature below 4 °C for 30 min followed by placing in a vacuum coater machine to remove the frost and vacuum coating in order to analyze the surface morphology of the cells using SEM.

3. Results and discussion

3.1. XRD analysis and GFA

Fig. 1 shows XRD patterns of the as-cast $Co_{80-x-y}Cr_xMo_yP_{14}B_6$ (x = 5 y = 5; x = 5 y = 10; x = 10 y = 10, all values in at.%) glassy alloy rods of the maximum diameter (D_{max}). The XRD patterns exhibit broad diffuse peaks at a diffraction angle (2θ) of about 43°, and Bragg peaks corresponding to crystalline phases are not observed, indicating the fully glassy nature of the specimens. As $Co_{80-x-y}Cr_xMo_yP_{14}B_6$ BMGs do not contain any harmful elements (such as Ni, Al, Cu, *etc.*) and rare earth elements, they could be used as potential biomedical implant materials. The diameter of the specimens used in the subsequent tests is 1.0 mm, which is less than D_{max} of the Co-based alloys, in order to avoid partial crystallization. For convenience, $Co_{70}Cr_5Mo_5P_{14}B_6$, $Co_{65}Cr_5Mo_{10}P_{14}B_6$, and $Co_{60}Cr_{10}Mo_{10}P_{14}B_6$ BMGs are hereafter denoted as Cr_5Mo_5, Cr_5Mo_{10}, and $Cr_{10}Mo_{10}$, respectively.