Surface Modification of CoCr alloy Using Phosphoric and Phosphonoacetic Acids: Protein and Platelet Interactions

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Introduction: Cobalt-Chromium (CoCr) alloy is extensively used for a variety of cardiovascular medical devices such as stents, stent grafts, and heart valves. Although the alloy has excellent mechanical properties, the blood compatibility is always an issue as with most other cardiovascular biomaterials. The formation of thrombi (blood clots) on cardiovascular medical devices can lead to fatal complications.[1] Hence, there is a need to improve the surface properties of cardiovascular biomaterials such as CoCr alloy for inhibiting thrombi formation. In this research, the CoCr alloy was surface modified using varying concentrations of phosphoric acid (PA) and phosphonoacetic acid (PAA) to obtain surfaces with gradients of surface wettability, chemistry, and roughness. The interaction of blood plasma proteins such as albumin and fibrinogen as well as the adhesion, activation, and aggregation of blood platelets with the various surfaces generated were investigated.

Methods CoCr alloy specimens (1 cm × 1 cm) were mechanically polished and chemically cleaned as previously described.[2] The cleaned specimens were immersed in five different concentrations (1, 25, 50, 75, and 100 mM) of PA and PAA in deionized water (di-H_2O) for 24 hours. The specimens were then transferred to an oven without rinsing and heated in air at 120 °C for 19 hours. After that, the specimens were cleaned by sonication in di-H_2O for 1 min followed by N_2 gas drying. The control CoCr, PA, and PAA coated CoCr surfaces were characterized using contact angle, FTIR, and AFM for studying the surface wettability, chemistry, and roughness, respectively. For protein adsorption studies, the solutions of albumin and fibrinogen were prepared at two different concentrations (120 and 200 µg/mL) in 0.9% sodium chloride. The protein solutions were then microdrop deposited on control, PA, and PAA coated CoCr surfaces at 37 ºC for 15 min. The amount of proteins adsorbed on the various surfaces was determined by bicinchoninic acid (BCA) protein assay. For platelet interaction studies, the blood was collected from healthy donors and the platelet rich plasma (PRP) was isolated as per the standard procedure.[3] The platelets were retrieved from the PRP and resuspended in Tyrode's solution. Then, the CoCr specimens were immersed in the platelet suspension and incubated at 37 ºC for 1 hr. After that, the platelet suspension was removed and used for activation and aggregation studies while the platelets adsorbed on the specimens were used for the adhesion study. The adhesion of platelets was measured by quantifying the total amount of protein released by disrupting the adhered platelets by sodium dodecyl sulfate buffer. The protein released from the platelets was quantified by the BCA kit. The platelet activation was quantified by flow cytometry using an antibody against P-selectin. The aggregation of platelets was determined by analyzing the changes in turbidity (opacity) of platelet suspension collected before and after immersion of alloy specimens.

Results: The contact angle of control CoCr was 59 ± 3º. The contact angles of CoCr coated using 1, 25, 50, 75, and 100 mM conc. of PA are 47 ± 8, 44 ± 14, 27 ± 7, 27 ± 8, and 30 ± 14, respectively. The contact angles of CoCr coated using 1, 25, 50, 75, and 100 mM conc. of PAA are 41 ± 5, 35 ± 8, 16 ± 9, 5 ± 1, and 5 ± 1, respectively. FTIR suggested the formation of P–O–metal covalent bonds between PA and CoCr, and PAA and CoCr at 1117 and 1133 cm^{-1}, respectively. Also, FTIR suggested the formation of different bonding configurations (mono-, bi-, and tri-dentate) depending on the conc. of PA and PAA used. AFM showed the formation of homogeneous PA and PAA coatings on CoCr irrespective of the conc. of coating solutions used. The RMS roughness of control CoCr was 11 ± 2 nm. The RMS roughness of CoCr coated using 1, 25, 50, 75, and 100 mM conc. of PA are 17 ± 5, 13 ± 1, 5 ± 1, 3 ± 1, and 12 ± 3, respectively. The RMS roughness of CoCr coated using 1, 25, 50, 75, and 100 mM conc. of PAA are 16 ± 3, 12 ± 2, 3 ± 1, 3 ± 0.3, and 16 ± 2, respectively. The protein adsorption studies showed the amount of albumin or fibrinogen adsorbed on 25, 50, and 75 mM PA-CoCr was less when compared to that of control CoCr. However, more proteins were adsorbed on 100 mM PA-CoCr when compared to control CoCr. For PAA coating, a greater amount of albumin or fibrinogen was adsorbed on 25, 50, 75, and 100 mM PAA-CoCr especially when high conc. of protein solution was used. All PA coated CoCr showed reduced platelet adhesion and activation when compared to control CoCr (Fig 1). Also, 75 and 100 mM PA-CoCr showed reduced platelet aggregation. For PAA coated CoCr, no significant difference in platelet adhesion and activation was observed between PAA coated CoCr and control CoCr.

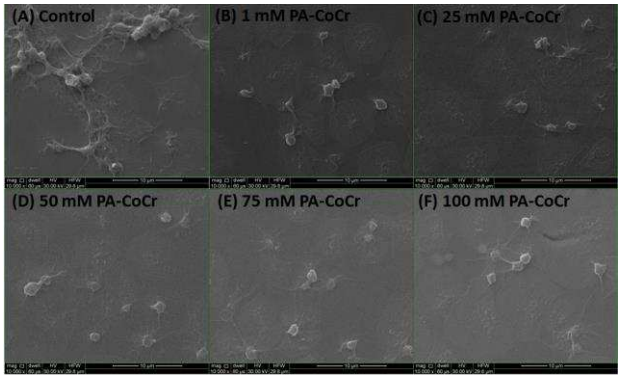

Fig 1. SEM images of platelets adhered on control CoCr alloy and PA coated CoCr alloy.

Conclusions: This study demonstrated that CoCr alloy can be surface modified using PA for potentially reducing the formation of blood clots and improving the blood compatibility of the alloy.

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References: (1) Biomaterials 2007, 28, 1689-1710; (2) Langmuir 2014, 30, 6237-6249; (3) JBMR 1998, 41, 304-311.