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Short communication

Study of the tribocorrosion behaviors of albumin on a cobalt-based alloy using scanning Kelvin probe force microscopy and atomic force microscopy

Yu Yan *, Hongjuan Yang, Yanjing Su, Lijie Qiao

Corrosion and Protection Center, Key Laboratory for Environmental Fracture (MOE), University of Science and Technology Beijing, Beijing 100083, China

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ABSTRACT

Once biomaterials are implanted in patients, the first reaction that occurs on the surface is the adsorption of biomolecules such as proteins, amino acids, etc. For load-bearing surfaces, some adsorbed proteins can be removed by relative motion (tribology) and some adsorbed proteins can be denatured due to tribochemical reactions. Although the effect of proteins on the corrosion behaviors of metals has been studied, the local reaction induced by the protein adsorption under tribological contact at the micro level is still unknown. The adsorption of bovine serum albumin (BSA) on CoCrMo alloy surfaces and the tribocorrosion behaviors were studied by AFM and SKPFM. The results showed that adsorbed BSA molecules could decrease the work function and promote the corrosion process for CoCrMo alloys. In the wear track, the albumin denatured, and changed the surface potential as time progressed.

1. Introduction

For biosensors, drug delivery systems, artificial tissues, and so on, the adsorption of proteins has been an area of focused research [1–4]. Protein adsorption on a solid surface is a complex process combining hydrophobic, electrostatic, and hydrogen-bonding interactions. For bovine serum albumin (BSA), its isoelectric point is at pH 4.7 [5,6]. If the pH value is higher than 4.7, albumin molecules carry negative net charges. For artificial joints, the pH of the surrounding fluid is about 7.4. The effect of proteins on corrosion behaviors of biomaterials has been studied widely. It has shown for Co, Cu, and Ni metals, the corrosion rates would increase by proteins. However, Ag, Ti, Cr, and Mo showed unaffected by proteins [7].

Tribocorrosion is a material degradation process resulting from the interaction of mechanical tribology and electrochemical corrosion [8–10]. For load-bearing surfaces of joint implants, such as hip implants and knee implants, they are surrounded by biological fluids, which are enriched with proteins and amino acids. Among those proteins, albumin is one of the most abundant. Adsorbed proteins can be removed and changed through the tribocorrosion process. The folding state of proteins is very difficult to maintain under the high shear rate of the tribology force. The accelerated release of metallic ions can also affect the status of proteins. It has been shown that proteins can be denatured under the tribocorrosion process [11]. Organometallic formation has been found on the surface of retrieved hip implants and samples from hip simulator tests [12,13]. However, how the tribocorrosion process affects the adsorption of proteins at the micro level is still unknown.

Atomic force microscopy (AFM) and scanning Kelvin probe force microscopy (SKPFM) can be used to obtain the morphology and the corresponding surface potential at the same time [14–17]. The surface potential is related to the work function of the surface. SKPFM can provide insightful information on the interaction between proteins and metal substrates at the micro level. In this study, the main objective is to understand the mechanisms of proteins in tribocorrosion reactions using SKPFM and AFM.

2. Experimental methods and materials

2.1. Material and sample preparation

Cobalt–chromium–molybdenum (CoCrMo) alloys are the most commonly used hip implant materials. The forged CoCrMo alloy used in this study contained 63% Co, 28% Cr, 5% Mo, and 0.2% C (wt.%) (a small amount of Ni, Si, etc.). The surface of the specimen was wet ground with SiC paper up to 2000 grit, then fine polished with diamond paste. All specimens can achieve a surface roughness R_a value of about 10 nm, which is consistent with the surface finish of most commercial hip replacement components.

2.2. Electrochemical tests

The simulated body fluid employed for carrying out the electrochemical experiments was phosphate-buffered solution (PBS) with BSA. Every liter of the PBS solution contains 0.8 g NaCl, 0.2 g KCl,

* Corresponding author.
E-mail address: yanyu@ustb.edu.cn (Y. Yan).

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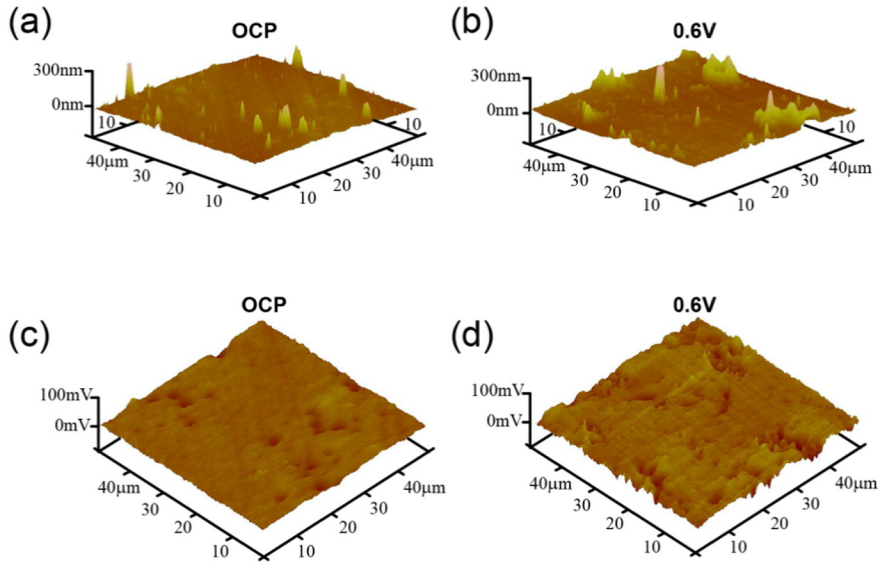

Fig. 1. Topographic images of CoCrMo alloy sample surface in 1% BSA containing PBS solution at OCP (a) and at +0.6 V vs. Ag/AgCl (b); surface potential images at OCP (c) and at +0.6 V vs. Ag/AgCl (d).

1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4. Then a certain amount of BSA was added to the PBS solutions, resulting in a 10 g/L solution concentration of BSA for the electrochemical experiments. The pH of PBS with BSA was adjusted to be at 7.4, which is according to the normal synovial fluid.

The tribocorrosion tests were carried out with a UMT-II reciprocating wear tester. The friction force was measured during all tests at 100 readings per second. The test parameters were set as follows: experiment temperature was controlled at 37 ± 1 °C (normal body temperature) to mimic the normal body temperature; the average speed of sliding was set at 30 mm/s, which is close to the normal walking cycles; the frequency of sliding was 1 Hz and the applied load was 0.8 N. The counterpart used in this study was Si_3N_4 balls (5 mm in diameter). The sliding distance per stroke was 15 mm.

The electrochemical experiments were carried out with three-electrode cell, which consists of the specimen as the working electrode (WE) (20 mm in diameter), a platinum wire as the counter electrode (CE), and a silver/silver chloride electrode as the reference electrode (RE).

2.3. AFM and SKPFM measurements

AFM and SKPFM measurements were carried out using a dimension Nanoscope V (Veeco Instruments Inc.). All measurements were conducted under the tapping mode. AFM was used to obtain the topographic images and SKPFM was employed for the surface potential measurements at the same area as the AFM scan. The probes used in measurements were Pt-Ir-coated silicon tips. The Pt-Ir coating is about 25 nm thick for an optimum combination of durability, conductivity, and resolution. The lift mode was used to recode a second signal in addition to the surface topography. SKPFM is calibrated before testing with a standard specimen. The spatial resolution is determined by the size of the tip. In this study, the resolution was 3 nm. In the experiments, three different regions were scanned for every sample.

2.4. Fluorescence labelling

Fluorescence labelling of BSA was carried out using fluorescein isothiocyanate (FITC). BSA was dissolved in a sodium bicarbonate buffer (0.1 M, pH 7.4) and the FITC dissolved in deionized water to the concentration of 1 mg/ml. The conjugation reaction was carried out by adding the FITC solution to the protein solution followed by an incubation process for 12 h at 4 °C in a dark environment. Fluorescence images were obtained using an IX71Fluoresce Microscope (Olympus). Labelled BSA can emit a green light under the Fluoresce Microscope.

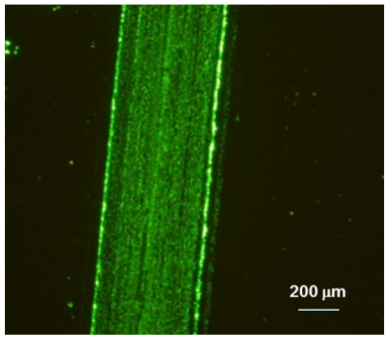

Fig. 2. Fluoresce microscope image of labeled BSA with FITC on CoCrMo sample surface after wear test in PBS with 1% BSA for 30 min at 37 °C.

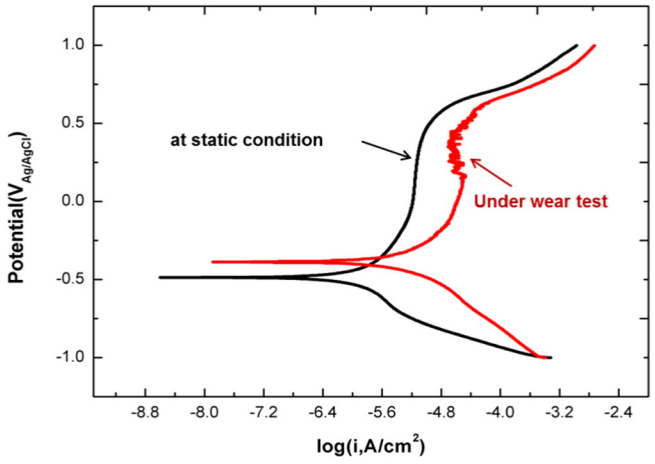

Fig. 3. Potentiodynamic curves for CoCrMo samples at static condition and under wear test in BSA containing PBS solution at 37 °C.