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Peptide-based biocoatings for corrosion protection of stainless steel biomaterial in a chloride solution

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ABSTRACT

In this work, PEGylated D-amino acid K122-4 peptide (D-K122-4-PEG), derived from the type IV pilin of *Pseudomonas aeruginosa*, coated on 304 stainless steel was investigated for its corrosion resistant properties in a sodium chloride solution by various electrochemical measurements, surface characterization and molecular dynamics simulation. As a comparison, stainless steel electrodes coated with non-PEGylated D-amino acid retroinverso peptide (RI-K122-4) and D-amino acid K122-4 peptide (D-K122-4) were used as control variables during electrochemical tests. It was found that the D-K122-4-PEG coating is able to protect the stainless steel from corrosion in the solution. The RI-K122-4 coating shows corrosion resistant property and should be investigated further, while the D-K122-4 peptide coating, in contrast, shows little to no effect on corrosion. The morphological characterizations support the corrosion resistance of D-K122-4-PEG on stainless steel. The adsorption of D-K122-4 molecules occurs preferentially on Fe_2O_3, rather than Cr_2O_3, present on the stainless steel surface.

1. Introduction

Metallic alloys, such as stainless steels, cobalt, titanium-based alloys, etc., have been commonly used as biomaterials for medical applications, including cranial plates, orthopedic fracture plates, spinal rods, endovascular stents, prosthetic joints and dental implants [1]. The metals are also used in integrated circuits for implantable electronic devices such as cardiac pacemakers and neuroprostheses such as cochlear stimulators [2]. Statistics show that, between 2012 and 2013, there were more than 30,000 primary hip replacements in Canada, the majority of which had complete or partial metallic bearing surfaces [3]. Given the huge burden of metallic implants used in medicine today, significant focus has been placed on developing materials that provide maximal biocompatibility and durability while maintaining the desired properties.

Metallic biomedical implants are under constant corrosion attack due to the chemistry and pH of physiologic fluids [1,4–6]. Cations and anions, as well as negatively charged proteins are abundant in extracellular fluids and cause corrosion and degradation of the biomedical device [1,6–9]. Corrosion of metallic biomaterials results in not only the device failure, but also undesirable host responses. This problem is especially serious given the long lifespan expected for devices such as joint prostheses, endovascular stents or cardiac pacemakers. Corrosion releases metallic ions into local and systemic environments, causing adverse effects in patients. Metallic ions or debris can activate macrophages or osteoblasts, resulting in localized tissue reactions, chronic inflammation and granuloma formation [7,10]. Moreover, the release of metal ions from prosthetic devices into circulation is associated with multiple systemic toxicities and allergy in humans [11,12]. Thus, strategies to inhibit corrosion of metallic biomaterials used in medical devices are critical to improve the longevity and safety of implants in humans.

In previous work, a series of peptides (K122-4) derived from the receptor-binding domain of the type IV pilin of *Pseudomonas aeruginosa* bacteria were identified, and the peptides effectively bind via a semi-covalent interaction to a variety of metallic materials including stainless steels [13,14]. It was found that these peptides demonstrate highly adhesive properties on the steels, and alter their surface characteristics by increasing the electron work function [13]. Furthermore, peptides synthesized using D-amino acids to create enantiomeric and retroinverso forms were found to be extremely durable and protease-resistant when bound to surfaces such as stainless steels [15,16]. The physical characteristics of the K122-4 peptides suggest that they may serve as effective coatings to improve the corrosion resistance of stainless steels and other metals in biological fluids.

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In this work, the corrosion resistance of a D-amino acid K122-4 peptide conjugated to a hydrophobic polyethylene glycol moiety (D-K122-4-PEG) coated on 304 stainless steel was investigated by various electrochemical measurements, surface characterization and molecular dynamics simulations. For comparison, the steel specimens were also coated with D-K122-4 peptide and non-PEGylated D-amino acid retroinverso peptide (RI-K122-4), respectively. The corrosion current density and corrosion potential of the coated steel, as well as the efficiencies of enhanced corrosion resistance by various biocoatings were determined from potentiodynamic polarization curve and electrochemical impedance measurements. The morphological observations were conducted to confirm the corrosion testing results obtained on the steel electrodes coated with D-K122-4-PEG. Moreover, the molecular dynamics simulation was conducted to model the preferential adsorption of peptide molecules and their orientation on the steel surface.

2. Materials and methods

2.1. Peptide synthesis

D-amino acid enantiomeric peptides ACTSNADNKYLPKTCQT-amide corresponding to AA128-144 of the PilA receptor binding domain of *Pseudomonas aeruginosa* strain K122-4 were synthesized with a N-terminal tetra-glycine linker by solid phase peptides, and purified by a reversed-phase high-performance liquid chromatography (HPLC). A disulfide bridge was generated by air oxidation, and the peptides were biotinylated at the N-terminus. These peptides were referred to as D-K122-4. Peptides were reconstituted in phosphate buffered saline (PBS) with a pH 7.4. The oxidized D-K122-4 peptide was coupled to polydisperse polyethylene glycol (PEG, molecular weight of ~1000) via a tri-glycine linker at the N-terminus to generate D-K122-4-PEG, which was then purified by a reversed phase HPLC to yield a product with over 95% purity determined by HPLC analysis. The polydispersed D-K122-4-PEG was synthesized by Ambiopharm Inc. (North Augusta, SC). PEGylated peptides were reconstituted in dimethyl sulfoxide (DMSO).

The retroinverso (RI) D-amino acid peptide was synthesized as TQCTKPLYKNDANSTCA-amide with an N-terminal tetra-glycine linker and biotin, and was referred to as RI-K122-4.

2.2. Preparation of steel specimens

Specimens used in this work were cut from a plate of 304 stainless steel, with a surface area of 1.0 cm^2. The specimens were ground up to grit 1200 SiC emery paper, polished with 1 μm diamond paste, and cleaned using deionized water and ethanol. The specimens were then coated with one of the following peptides, i.e., D-K122-4-PEG (1 μg/mL, 5 μg/mL, 10 μg/mL, 40 μg/mL and 100 μg/mL), D-K122-4 (10 μg/mL), or RI-K122-4 (10 μg/mL), in coating solutions for 2 h. The D-K122-4-PEG was diluted in 100% methanol, and the D-K122-4 and RI-K122-4 were diluted in deionized water.

2.3. Electrochemical corrosion measurements

Electrochemical corrosion measurements were performed using a Solartron 1280C electrochemical system on a three-electrode system, where the coated stainless steel specimen was used as working electrode (WE), a saturated calomel electrode (SCE) as reference electrode (RE), and a carbon rod as counter electrode (CE). The test solution was 3.5 wt.% NaCl solution. The WE was immersed in the solution for at least 1 h prior to electrochemical testing in order to reach a quasi-stationary open-circuit potential (OCP) value of the electrode. It was noted that corrosion of stainless steel in 0.9 wt.% NaCl solution (which resembles physiologic fluid chemistry) proceeded at a slow rate creating impractical experimental conditions. Since this work attempted to investigate the prepared biocoatings' resistance to corrosion in chloride environments present in bodily fluids, the testing was conducted in an environment which was more aggressive (or more corrosive) than physiologic fluids in order to accelerate the experimental testing. Although the corrosion process was accelerated, the results remained directly applicable to the expected corrosion dynamics and the coatings' behavior in physiologic chloride solutions.

After a steady value of OCP was reached, electrochemical impedance spectroscopy (EIS) was measured with a frequency range of 0.05 Hz to 2×10^4 Hz and an AC disturbance amplitude of 10 mV. The obtained Nyquist diagram and Bode plots were fitted with appropriate electrochemical equivalent circuits to determine the electrochemical impedance parameters that are directly relevant to the corrosion and coating properties of the WE.

Immediately after the EIS measurement, the potentiodynamic polarization curve was measured on the WE at a potential scanning rate of 0.1 mV/s. Corrosion parameters, including corrosion potential (E_{corr}), corrosion current density (i_{corr}), and anodic and cathodic Tafel slopes (β_a and β_c), were determined by fitting the anodic and cathodic polarization curves with a CView software. The efficiency of the enhanced resistance by the biocoatings for steel corrosion, *E%*, was determined by:

$$E\% = \frac{i^0_{corr} - i_{corr}}{i^0_{corr}} \times 100\% \quad (1)$$

where i^0_{corr} and i_{corr} were corrosion current densities of non-coated and coated steel electrodes, respectively.

To ensure the reproducibility of the testing results, each test was repeated at least three times.

2.4. Surface characterization

To characterize the protective performance of the biocoatings on corrosion of the steel electrode in chloride solution, an accelerating corrosion testing was conducted in 3.5 wt.% NaCl solution with pH 1.0 (adjusted by 0.1 M HCl solution) for 24 h. The morphologies of the coated steel electrode before and after corrosion testing were characterized by a Keysight 5500 atomic force microscope (AFM). Silicon nitride probes with average spring constants of 0.02–0.77 N/m were used while the AFM was set as "contact mode". The scanning rate was 1 Hz and the imaging was conducted over frames of 50 μm × 50 μm and 10 μm × 10 μm. Further tests were conducted with a scanning electron microscope (SEM) in order to image the surface morphology of the steel electrode at a larger scale after corrosion testing.

2.5. Molecular dynamics simulation

The molecular dynamics simulation was conducted to model the adsorption of peptide molecules on the surface of Fe_2O_3 and Cr_2O_3, the key oxides formed on the surface of a stainless steel to resist corrosion. The atomic and molecular orientations and the adsorption energy were determined.

3. Results

3.1. Polarization curve measurements

Fig. 1 shows the potentiodynamic polarization curves measured on steel electrodes uncoated and coated with D-K122-4-PEG at various concentrations in 3.5 wt.% NaCl solution. It is seen that, as the D-K122-4-PEG concentration increases, the corrosion potential is shifted less negatively, with the exception of 1 μg/mL of D-K122-4-PEG. At individual potential, the anodic current density decreases with the increasing D-K122-4-PEG concentration, indicating the increasing corrosion resistance of the coated steel. Moreover, passivity is obvious on both uncoated and coating stainless steel electrodes, and the passive current density becomes smaller with the increase of the D-K122-4-PEG concentration.