Materials Science and Engineering C 71 (2017) 200–205

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Effects of simulated inflammation on the corrosion of 316L stainless steel

Emily K. Brooks [a], Richard P. Brooks [a], Mark T. Ehrensberger [a,b,*]

[a] Department of Biomedical Engineering, State University of New York at Buffalo, United States
[b] Department of Orthopaedics, State University of New York at Buffalo, United States

ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form 28 September 2016
Accepted 7 October 2016
Available online 11 October 2016

Keywords:
Corrosion
Stainless steel
Inflammation
Orthopaedics
316L

ABSTRACT

Stainless steel alloys, including 316L, find use in orthopaedics, commonly as fracture fixation devices. Invasive procedures involved in the placement of these devices will provoke a local inflammatory response that produces hydrogen peroxide (H_2O_2) and an acidic environment surrounding the implant. This study assessed the influence of a simulated inflammatory response on the corrosion of 316L stainless steel. Samples were immersed in an electrolyte representing either normal or inflammatory physiological conditions. After 24 h of exposure, electrochemical impedance spectroscopy (EIS) and inductively coupled plasma mass spectroscopy (ICPMS) were used to evaluate differences in corrosion behavior and ion release induced by the inflammatory conditions. Scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDX) were used to evaluate surface morphology and corrosion products formed on the sample surface. Inflammatory conditions, involving the presence of H_2O_2 and an acidic pH, significantly alter the corrosion processes of 316L stainless steel, promoting aggressive and localized corrosion. It is demonstrated that particular consideration should be given to 316L stainless steel implants with crevice susceptible areas (ex. screw-head/plate interface), as those areas may have an increased probability of rapid and aggressive corrosion when exposed to inflammatory conditions.

1. Introduction

Stainless steel materials are used for medical applications due to their high strength and corrosion resistance, ease of formability, and low cost [1]. A chromium rich oxide layer nucleates on the surface to provide the materials with their resistance to corrosion [2]. Because of these favorable properties stainless steels, namely 316L, have become popular choices for orthopaedic implants, dental implants, and stents. However, the most frequent use of stainless steel implants is for fracture fixation of bone. In this case, a plate is fixed to the bone with screws in order to provide mechanical stability and facilitate healing following fracture [3].

Although these materials are considered corrosion resistant, they can be susceptible to pitting and crevice corrosion depending on the environment. It has been estimated that 10–20% of all stainless steel implants need to be prematurely removed, and while corrosion was identified as the primary cause for nearly a quarter of these premature removals, nearly all instances of implant removal showed evidence of some degree of implant corrosion [4]. Of note, multicomponent designs, such as those often employed for fracture fixation were more vulnerable to corrosion attack, with deep crevices created at the junctions of the components. Areas between the screw head and plate have been identified as the most common site of corrosion on stainless steel implants [3]. These observations have led some to hypothesize that corrosion is in fact the predominant initial cause for most failures in stainless steel implants [5]. On the contrary, many studies have displayed acceptable *in vitro* corrosion performance of stainless steels [6,7], resulting in a seeming disconnect between *in vitro* corrosion testing and *in vivo* implant performance.

Exact *in vivo* conditions an implant will encounter are complex and difficult to both predict and replicate. The studies undertaken in this work are an attempt to characterize how a simulated inflammatory response may contribute to the increased corrosion susceptibility of 316L stainless steel implants. After the implantation of an orthopaedic device, an inflammatory reaction is initiated both in response to the invasive procedure and the presence of the device. This reaction results in the production of reactive oxygen species by macrophages, neutrophils, and other inflammatory response cells [8], which enzymes in the body quickly destroy by catalyzing their conversion to hydrogen peroxide (H_2O_2) [9]. In addition to the local increased concentration of H_2O_2, neutrophils produce lactic acid leading to an acidic pH in the immediate implant environment. Previous work has shown that a simulated inflammatory response is able to affect the electrochemical properties of titanium and its alloys [10,11], as well as next generation magnesium alloy biomaterials [12].

The purpose of this study is to evaluate if the aggressive conditions of a simulated inflammatory response alters the electrochemical properties of 316L stainless steel. It is quite possible this harsh environment may be, at least in part, responsible for the accelerated corrosion of 316L implants resulting in premature implant retrieval. Samples of

* Corresponding author at: Department of Biomedical Engineering, State University of New York at Buffalo, 3435 Main Street, 445 Biomedical Research Building, Buffalo, NY 14214, United States.
E-mail address: mte@buffalo.edu (M.T. Ehrensberger).

http://dx.doi.org/10.1016/j.msec.2016.10.012
0928-4931/

316L were evaluated in solutions simulating the normal physiological environment or an inflammatory environment. Solution properties including changes in pH and H_2O_2 concentration were monitored over the 24 h incubation period. After 24 h of immersion in the test electrolytes, electrochemical impedance spectroscopy (EIS) was performed to assess the faradaic and non-faradaic properties of the metal-solution interface. Inductively coupled plasma mass spectroscopy (ICPMS) was then performed on aliquots of the test solution to quantify the concentration of metal ions released into the test electrolyte. Scanning electron microscopy (SEM) in combination with energy dispersive X-ray spectroscopy (EDX) were employed to examine the resulting surface morphology and composition.

2. Materials and methods

2.1. Sample and corrosion cell preparation

Samples of 316L stainless steel (McMaster-Carr) were machined into discs 3 cm in diameter by 0.75 cm in thickness. The metal samples were sequentially wet sanded to a 600 grit finish, ultrasonically cleansed in deionized water for 10 min, sterilized under ultraviolet (UV) light for 30 min and were subsequently mounted in a custom 3-electrode corrosion cell [13]. The 316L stainless steel samples served as the working electrode while a graphite rod served as the counter electrode, and a chlorided silver wire (Ag/AgCl) acted as the reference electrode. The reference electrode was placed into a reference well filled with phosphate buffered saline (PBS, Mediatech) and electrically connected *via* an agar KCl salt bridge positioned close to the working electrode surface. All voltages reported in this study are with respect to the Ag/AgCl reference electrode.

The corrosion cell exposed approximately 3.8 cm^2 of the working electrode to 8 mL of test electrolyte. The test electrolytes consisted of PBS at an initial pH = 7.2 to simulate normal physiological conditions; and a 150 mM H_2O_2 solution in PBS titrated to pH = 5.0 with HCl (J.T. Baker) used to simulate inflammatory conditions [10,12,14]. The corrosion cell was placed into a humidified 37 °C, 5% CO_2 incubator and appropriate connections were made to a potentiostat (Ref 600, Gamry Instruments).

2.2. Electrochemical tests

EIS was performed on four independent samples of each metal after 24 h of incubation at each experimental condition. EIS was conducted by imposing a 10 mV sinusoidal oscillation about OCP over a frequency range of 10 KHz to 5 mHz. A complex-non-linear-least-squares method was used to fit the impedance spectra to a modified Randles circuit (EIS 300 software, Gamry Instruments). For normal conditions, the circuit model contained a solution resistor (R_s) in series with a parallel combination of a constant phase element (CPE) and a polarization resistor (R_p). Components of the CPE include its magnitude (Y_O), and the CPE exponent (alpha, α). The values obtained for the CPE parameters were applied to calculate the capacitance of the interface utilizing the following equation: $C = Y_0^{\frac{1}{\alpha}}(R_s^{-1} + R_p^{-1})^{(\alpha-1)/\alpha}$. This equation was determined by Huang et al. and verified by Brug et al. [15–18]. To better represent the results obtained under inflammatory conditions, a Warburg admittance (W_d) parameter was added to the circuit in series with R_p.

2.3. Electrolyte properties

The pH of the test electrolyte was measured at the start of the experiments and after 24 h of incubation using a pH meter (pH 11 Series, Oakton Instruments) calibrated immediately preceding each use. The H_2O_2 concentration of the test electrolyte was determined before and after the 24 hour incubation period using a colorimetric VACUette ampoule test (K-5510C, CHEMetrics). At the end of the incubation period, aliquots of the test electrolytes were analyzed by ICPMS to identify the concentration of metal ions in solution. ICPMS analysis was performed with a Perkin Elmer Sciex model ELAN DRC-II. Optima grade nitric acid was used to prepare the calibration curve and standards. Three independent samples of solution (2 mL) for each test condition were analyzed in triplicate for the ICPMS analysis.

2.4. Surface characterization

At the end of the 24 hour incubation period, samples from both normal and inflammatory conditions were extracted from their chambers, rinsed with dI H_2O, allowed to air dry and examined with a SEM (Hitachi SU70). Representative regions of the samples were imaged at 500 x magnification in secondary electron mode for assessment of the surface topography. Subsequently, these same regions were imaged in backscattered electron mode, and energy dispersive x-ray spectroscopy (EDX) was conducted on both the entire field of view and separately on areas of interest to analyze differences in surface composition.

2.5. Statistical analysis

A minimum of four independent samples were examined for each experimental condition. Student's *t*-test were performed with statistical software (IBM SPSS for Macintosh V22) to compare the pH, H_2O_2 concentration, OCP, EIS outcomes, and the log transformed ICPMS results across experimental conditions. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. pH and H_2O_2 concentration

The pH and concentration of H_2O_2 present in the test electrolyte was measured at the start and conclusion of the 24 h incubation period for both experimental conditions. Although the pH of the inflammatory conditions increased over time, it remained significantly lower than that of the normal conditions after 24 h ($N = 6.73$, $I = 5.94$, $p < 0.001$). The majority of H_2O_2 in the inflammatory solution was consumed during the test period (Final $[H_2O_2] = 1420$ ppm).

3.2. OCP

Trends in the open circuit potential (OCP) over the incubation time are displayed in Fig. 1. At initial time points the inflammatory conditions shifted the OCP to more electro-positive potentials when compared to the normal conditions. Under normal conditions, the OCP gradually becomes more electro-positive over time. Conversely, the OCP under

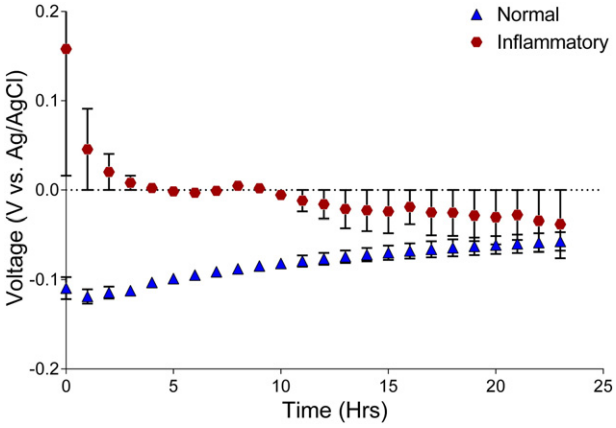

Fig. 1. The OCP over the 24 h immersion period for 316 L exposed to either normal or inflammatory conditions. Data was extracted at one hour intervals. Each point represents the mean ± 1 standard deviation for three independent samples.