In vitro biocorrosion of Titanium by macrophage cells

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Statement of Purpose:

Metallic alloys constitute a large percentage of biomaterials used in implants. While implant alloys have been selected in part due to their low corrosion, the alloys will release metal ions, which may cause adverse reactions. Many questions remain regarding the mechanisms and processes of implant alloy corrosion in vivo. The study of the corrosion of biomaterials in the presence of cells and proteins is essential to understanding in vivo performance and for improving longevity and durability of implant alloys. The aim of this study was to evaluate the effects of macrophage cells on the corrosion properties of an implant alloy since macrophage cells are central to healing and inflammatory reactions.

Methods:

Titanium squares (2.5cm x 2.5cm) were wet polished to 1000 grit with SiC paper, and cleaned ultrasonically in acetone, ethanol, and then passivated in 30% nitric acid according to ASTM standard F86 to simulate clinical conditions. Three repetitive experiments were conducted as follows: a Ti sample was placed into specialized corrosion chamber [1] with 3 ml cell culture media (DMEM +10% FBS +ab/am) in an incubator at 37°C. Open circuit potentials and linear polarization curves were measured using a potentiostat (Princeton Applied Research, Oak Ridge, TN) every six hours for a total of nine days. On the third day, TIB mouse macrophage cells (TIB-71) were seeded onto the Ti surface at $1x10^5$ cells/cm^2. On the sixth day, the cells were stimulated to release reactive oxygen species, ROS (NO, H_2O_2) with the addition of 5ug/ml LPS and 0.05ug/ml IFN-γ. A Griess Reagent Kit (Molecular Probes, Eugene, OR) was used to monitor the nitric oxide concentration in the media every 24 hours after cell activation. A Cell Titer 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) was used to determine cell viability on the ninth day.

Results/Discussion:

The open circuit potential shows only a slight increase over the first three days of testing in media (figure 1). At the time of cell addition, however, there is a transient drop, followed by a continuing slow rise in the open circuit potential. A less dramatic transient drop is observed at the time of cell activation. The increase in E_{corr} at the end is variable between runs, and is likely associated with ROS released after stimulation. ROS can lead to increases is alloy surface oxides which would lead to an increase in corrosion potentials.

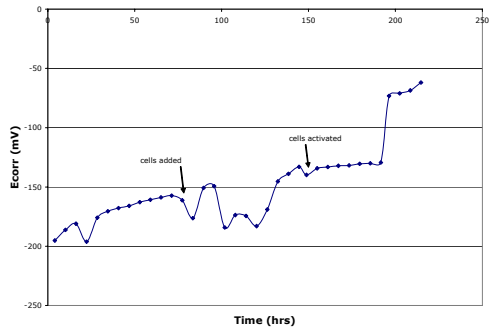

Figure 1. Representation graph of open circuit potential

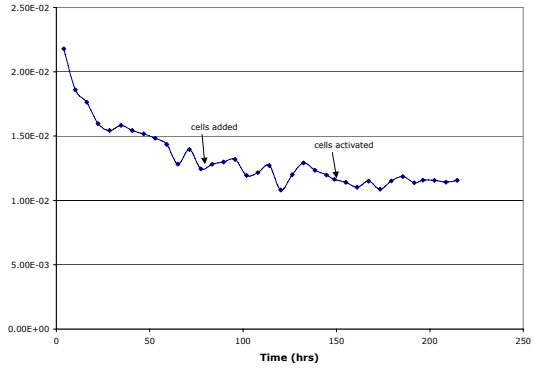

Figure 2. Corrosion current

The corrosion current shows a sharp decrease over the first three days, followed by a more gradual decrease after the addition of cells (figure 2). This significant decrease in corrosion current over the course of the experiment, has been seen in similar studies on other alloys [1], and suggests that the corrosion rate decreases as a result of the presence and activation of cells on the surface. The release of ROS from the cells enhances the oxidation of the sample, and an increased oxide layer thickness results in a lower corrosion rate.

NO levels showed a 17% average increase in the days after activation, verifying that reactive chemical species were being released and were therefore available to affect the oxide layer growth. Cell viability tests showed that 75% of the cells, on average, were still viable on day nine. These data support the hypothesis that cells and cellular products are important to implant alloy corrosion processes in vivo

Conclusions:

This study has shown that the corrosion of Titanium is affected by the presence of macrophage cells and their release of ROS. Increases in alloy corrosion potentials and decreases in corrosion rates are associated with the addition of the macrophage cells and the release of ROS. These changes are currently attributed to additional oxidation of the alloy surface oxide layer.

References:

1. Lin H, Bumgardner J, J Orthop Res 2004; 22(4): 1231-36.