Effects of Macrophage Cells Stimulated to Release Reactive Oxygen Species on Corrosion of Titanium

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Statement of Purpose: Titanium and its alloys are commonly used for dental, craniofacial, and some orthopedic implants due to their high strength and corrosion resistance. However, these metals inevitably corrode and release metal ions to local and systemic tissues, which may cause adverse reactions. The mechanisms of implant corrosion *in vivo* are not well understood. The study of biomaterial corrosion under simulated physiological conditions is essential to understand *in vivo* implant performance and improve longevity and durability of implant alloys. Since macrophage cells are central to wound healing and inflammatory reactions, the aim of this study was to evaluate the effects of macrophage cells on the corrosion properties of titanium.

Methods: Commercially pure titanium squares (2.5 cm x 2.5 cm x 0.1 cm) were wet polished to 1000 grit with SiC paper, cleaned ultrasonically in acetone and ethanol, and passivated in 30% nitric acid, according to ASTM standard F86 to simulate clinical conditions. A Ti sample was placed into a custom electrochemical corrosion cell [1]. Open circuit potentials and linear polarization curves were measured with a potentiostat (Princeton Applied Research, Oak Ridge, TN) every six hours for a total of six days. Mouse macrophage cells (ATCC TIB-71) were seeded at $1x10^5$ cells/cm^2 in 3 ml cell culture medium (DMEM +10% FBS +1% ab/am) on the Ti surface. The corrosion chamber was placed in a 37°C incubator. After four hours for cell attachment, the cells were stimulated with 0.05ug/ml IFN-γ (Millipore) and 5ug/ml LPS (Sigma, MO) to release reactive oxygen species (ROS: NO, H_2O_2), simulating the inflammatory response associated with wound healing. On the third day, the cell culture medium was replaced with fresh medium without IFN-γ and LPS. 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. The experiment was repeated three times. To serve as a control, the same experiments were conducted without the addition of cells.

Results: The corrosion current was determined from linear polarization curves, which were conducted every six hours throughout the six-day experiment. The charge transfer was found by integration of the area under the corrosion current curve. In comparison to the condition with no cells (medium only), the charge transfer for the activated cells resulted in a lower value for days 1-3, days 4-6, as well as the total charge transfer (table 1). There is a trend for lower charge transfer with cells on the titanium surface, which agrees with previous studies conducted on other implant alloys [1-2]. There is an increase in charge transfer for cells that have been stimulated. The cells were stimulated on days 1-3 to produce ROS via the addition of LPS/IFN-γ exhibit a lower charge transfer in comparison to the next three days when the medium was replaced with fresh and did not include the activating factors. The large standard deviations obtained for the medium only condition are likely due to the small sample size and may decrease with an increased number of runs.

Table 1. Charge transfer (μC)

Condition:	Days 1-3	Days 4-6	Total
Medium only	39.7 ± 62.7	806 ± 1390	846 ± 1451.6
Cells activated w/LPS/IFN-γ	1.04 ± 0.2	2.2 ± 0.2	2.5 ± 1.3

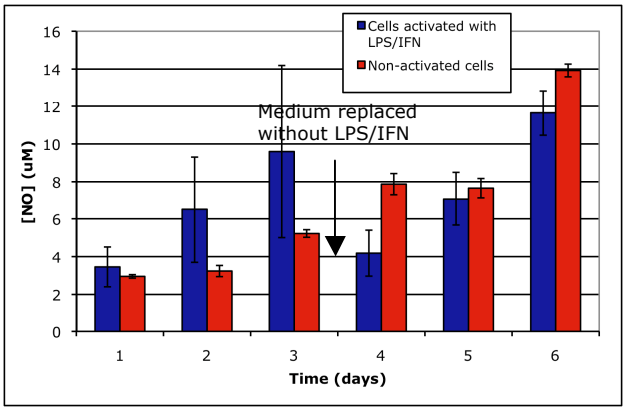

Figure 1. Nitric oxide production

The nitric oxide concentration in the supernatant was monitored every 24 hours after activation of cells (figure 1) as a measure of ROS production. As a control, NO production of non-activated macrophage cells was also monitored. For the activated cells, days 1-3 showed an increase in NO production with a higher concentration over the control. There was a decrease in NO on day 4, which was after the culture medium was replaced without the activation factors. Days 4-6 also showed an increase in NO over the time period; however, the NO production was at or below the control concentration. The increase in NO production exhibited by the control non-activated cells is likely due to the growing number of cells over the 6-day time period.

A study of titanium ion release into the culture medium via atomic absorption spectroscopy is currently in progress in order to correlate ion release with charge transfer and ROS production data.

Conclusions: These data have demonstrated that the corrosion properties of titanium may be affected by the presence and activation state of cells. There is a trend for lower total charge transfer for cells that have been activated than for medium alone.

References:

1. Lin H, Bumgardner J. J Orthop Res. 2004;22(4):1231-1236.
2. Parker SH, et al. ASTM STP 1438. 2003.

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