Electrochemical induced cell death of monocyte inflammatory cells on CoCrMo alloy *in vitro*

Mike Wiegand, Gregory Kubacki, Jeremy Gilbert.
Syracuse Biomaterials Institute, Syracuse University, NY 13244.

Statement of Purpose: Cobalt-chromium-molybdenum alloy (CoCrMo, ASTM F-*1537*) has been in use as a biomaterial for over 80 years due to its ability to resist wear and corrosion, and its high level of inertness[1]. Gilbert et al. reported evidence of direct inflammatory cell-induced corrosion on retrieved CoCrMo implant surfaces[2]. It has previously been reported that pre-osteoblast cells (MC3T3-E1) cultured on CoCrMo alloy surfaces biased with either cathodic (<-400 mV) or anodic (>+500 mV) voltages begin to lose adhesion and viability after 24 hours[3]. It is our hypothesis that inflammatory cells (U937 monocytes) cultured on CoCrMo will exhibit similar viability response to corrosion as that of MC3T3 cells, when polarized over time. Monocyte U937 cells were cultured on CoCrMo metal surfaces *in vitro* to assess cell behavior in response to applied voltages. Furthermore, cell viability was characterized by real-time visualization as well as fluorescent imaging.

Methods: CoCrMo alloy discs (ASTM F-*1537*) with an exposed surface area of 3.8 cm^2 were polished to a mirror-finish and UV sterilized. U937 (ATCC, VA) monocyte inflammatory cells and MC3T3-E1 subclone 4 (ATCC, VA) pre-osteoblasts were grown in Gibco's® RPMI 1640 medium (ThermoFisher, MA) and 10% fetal bovine serum (FBS) (ThermoFisher, MA) in a T25 flask (Corning, NY) until confluent. 200,000 cells were seeded at a volume of 50 µL. Phorbol 12-myristate 13-acetate (PMA) (Sigma, MO) was added to the 50 µL cell volume at a concentration of 100 ng/mL for better cell attachment and adhesion to the metallic surface. After 45 minutes in the hood under adequate air flow, the disc and cell culture media were added to the electrochemical chamber. The samples were incubated and tested at fixed potentials (-1000 to +1000 mV (vs. Ag/AgCl) in 250 mV increments) for 24 hours using a potientiostat. Live/dead viability/cytotoxicity kit for mammalian cells (L3224, Invitrogen, OR) was used to confirm cell death. Scanning electron microscopy (SEM; Jeol JSM-5600, Tokyo, Japan) was used to image cells and debris after live dead imaging (Axiovert 40CFL, Zeiss, Denmark).

Results: Previous work using MC3T3 cells indicated a loss in cell viability after 24 hours between -300 and -400 mV[3]. Real-time imaging showed U937 cells to be alive and spread out after 8 hours at -750 mV with no evidence of cell death. Fluorescent imaging confirmed cell viability after 8 hours in the time lapse set up. Live dead staining revealed that all MC3T3 cells were dead at -1000 and -750 mV after 24 hours while U937 cells demonstrated increased viability under the same cathodic conditions. At -1000 mV U937 cells had a viability of 25% and at -750 mV, U937 cells had a viability of 65%. Under anodic conditions, U937 cells performed similarly to MC3T3 cells[3]. U937 cells were 92% viable at +250 mV and at or above +500 mV, all cells were dead for both MC3T3 and U937 samples cultured on CoCrMo for 24 hours. SEM imaging revealed the cells to be well attached to the surface as viable cells. Images taken at +500 mV displayed balled up cells indicating apoptosis or necrosis.

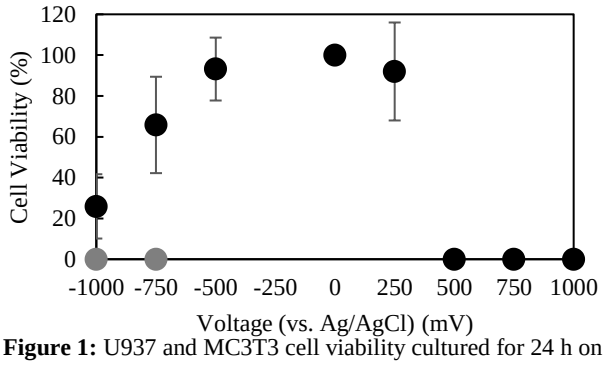

Figure 1: U937 and MC3T3 cell viability cultured for 24 h on the surface of CoCrMo disks at different voltages. U937 cells are shown with black circles and MC3T3 cells are shown with grey circles

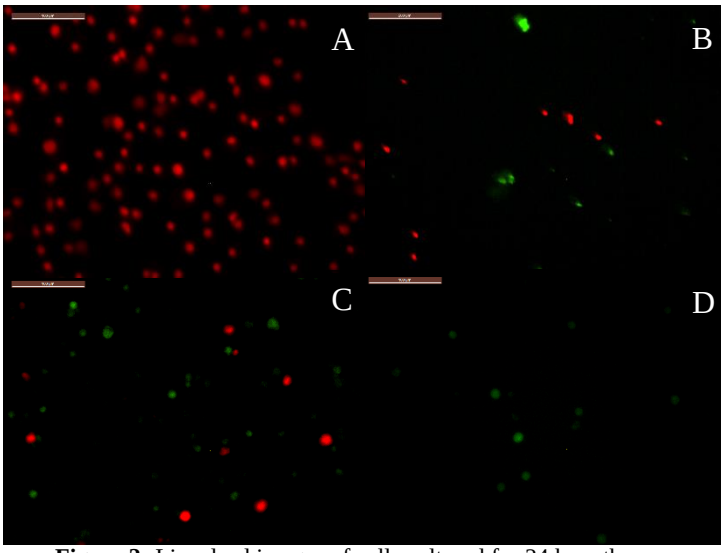

Figure 2: Live dead images of cells cultured for 24 h on the surface of CoCrMo at (A) -750 mV (MC3T3), (B) -1000 mV (U937), (C) -750 mV (U937) and (D) -500 mV (U937)

Discussion: Inflammatory cells can survive more cathodic electrochemical conditions than MC3T3 cells indicating that U937 cells have a greater ability to handle reductive stress conditions.

Conclusions: U937 inflammatory cells display a large potential range of viability under cathodic conditions than MC3T3 osteoblasts on CoCrMo alloy *in vitro*. It is possible that the mechanism responsible for this sustained viability in inflammatory cells plays a role in other tissue reactions. Further work to compliment this study will include 24 hour time-lapse imaging of U937 cells plated on CoCrMo discs. This will give insight into the different cellular mechanisms during prolonged electric stimulation.

References:

1. Sivan S. Biomater. 2013:101:1489-97, 2. Gilbert JL. J Biomed Mater Res A. 2015:103:211-233. 3. Haeri M. Biomater. 2012:33:6295-6304