Osteoblast Behavior and Electrochemical Impedance at Electrically Polarized Titanium Surfaces

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Statement of Purpose: It has been documented that the open circuit potential of fretting modular hip replacements can shift to as much as -1V[1] and that these shifts affect cell behavior by reducing the [O_2] profile adjacent to the implant[2]. The impedance properties of titanium have also been shown to vary as a function of voltage[3]. Therefore, this study seeks to address the relationship between the electrochemical impedance and osteoblast behavior at the interface of electrically polarized titanium.

Methods: Grade 2 commercially pure titanium samples (0.32 cm^2) were sequentially wet sanded to a 600 grit finish and mounted in a custom designed, electrochemically controlled cell culture chamber. Subsequently, about 5000 MC3T3 pre-osteoblasts were seeded on the Ti surface and allowed to attach for 15mins. Next, culture media (AMEM+ 10%FBS+1%Pen-Strep+ 1%L-glut) was added to give a working volume of 15mL and the chamber was placed in a 37°C and 5% CO_2 incubator. The electrical potential of the titanium sample was controlled by connecting it as the working electrode to a potentiostat (EG&G 263). A carbon rod and chlorided silver wire were placed into the culture media and served as the counter and reference electrodes, respectively.

Experiments were conducted for 24 hours at 0 mV, -300 mV, and -1000 mV. At specified points in time (0, 0.5, 2, 18, and 24 hours) a modified step polarization impedance spectroscopy[3] method was used to monitor the polarization resistance (Rp) and capacitance of the metal-cell interface. After 24 hours, the cells were fixed in 4% formaldehyde in PBS, dehydrated in steps to 100% ethanol, freeze dried, and then gold coated and imaged with a scanning electron microscope (JEOL 5600) in backscattered imaging mode.

Results/Discussion: Figure 1 displays typical cell morphology results at 0 mV(A), -300 mV(B), and -1000 mV(C). Cells at 0 mV are well spread with multiple attachment sites. Also notice that many intracellular components are distinguishable at 0 mV. Cells at -300 mV are smaller, less spread, and appear plump with smearing around the edges. Cells cultured at -1000 mV appear to be in distress. They are small is size, with matrix-like material spilling out of the membrane, and have what appears to be apoptotic bodies migrating away from where the nucleus once was.

Graph 1 indicates Rp values over time are highest at 0 mV and lowest at -1000 mV. Graph 2 shows that capacitance over time is lowest at 0 mV and highest at -1000 mV. The currents at 0 mV remained anodic and reduced over 24 hrs to 5 nA/cm^2, while currents for -1000 mV remained cathodic and increased over time to 62 uA/cm^2. Impedance data to date at -300 mV indicates both Rp and capacitance are between those at 0 and -1000 mV.

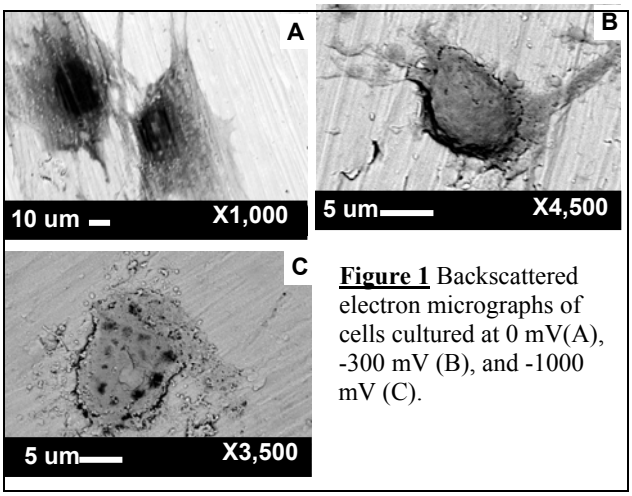
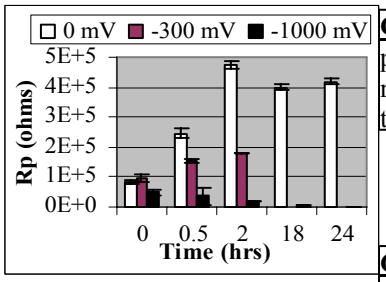
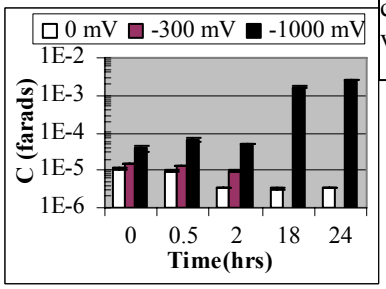


Figure 1 Backscattered electron micrographs of cells cultured at 0 mV(A), -300 mV (B), and -1000 mV (C).



Graph 1 Plot of polarization resistance versus time



Graph 2 Plot of capacitance versus time

Combined, these results indicate that limiting charge transfer (high Rp) and reducing surface charge effects (low capacitance) may be biologically advantageous in terms of initial cell attachment and spreading on titanium.

Conclusions: This preliminary data points out it is important to elucidate and understand the influence that both faradaic and non-faradaic electrochemical processes have on the bone surrounding orthopedic implants. Perhaps electrically biasing implants may facilitate optimizing these processes to improve osseointegration. Studies are underway which expand the number of voltages tested and include more quantitative morphological and biochemical analysis.

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References: (1.Goldberg JR. JBMR, 2003. 64B(2):78-93. 2.Gilbert JL. JBMR, 1998. 42(2):321-30. 3.Gilbert JL. JBMR, 1998. 40(2):233-43.)