Cell Behavior on Electrochemically Controlled Ti-6Al-4V Surfaces using Active Metal (Mg) Coupling

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Statement of Purpose: Over the past decades electrochemically active implant surfaces have been used to accelerate bone growth in the case of non-union fractures[1]. Moreover, recent studies[2,3] have shown that reduction reactions on the surface of metallic biomaterials can modulate cell behavior and that these effects are both voltage and time dependent. Based on this phenomenon, it is hypothesized that by coupling active metals such as magnesium to metallic biomaterials such as titanium, cathodic electrochemical control of biological interaction with the titanium implant surface is possible. Magnesium was chosen as the active metal for this study because of its excellent ability to biodegrade and for its osteoconducive properties. Coupling magnesium to titanium will generate a cathodic potential on the titanium surface. This study investigated in-vitro electrochemical behavior of Mg-Ti couples and their subsequent effects on cells. The goals of this study were to determine if biological control of cells on the surface could be developed using coupled metal constructs.

Methods: Ti-6Al-4V discs were wet polished to 600 grit, ultrasonicated in 70% ethanol. It was washed with 70% ethyl alcohol and UV sterilized for 30 minutes. The discs were placed into a custom made electrochemical cell culture chamber [3] with electrical contacts to the Ti-6Al-4V disc to be as a working electrode, with graphite counter and chlorided silver wire as reference electrodes. For Mg-Ti-6Al-4V couple experiments, AZ61wire was galvanically coupled to the titanium disc. Electrochemical behavior of the Mg-Ti-6Al-4V couple was monitored galvanostatically for all experiments. It was determined that the average drop in the electropotential was around -1500 mV (vs. Ag/AgCl). In order to access the invitro cell behavior, 10,000 MC3T3 E1 preosteoblasts (ATCC, CRL 2593) were plated on uncoupled titanium surface at open circuit potential (OCP) as controls. The cells were grown in Alpha Minimal Essential Media (AMEM, Cellgro, VA) supplemented with 10% fetal bovine serum (FBS) (Gibco, NY), 1% Penicillin-streptomycin Glutamine (PSG) (Gibco, NY). Cells were also plated on titanium surface held at -1500 mV for 2, 4, and 6 hours in order to assess and compare their behavior to cells on coupled samples. For cells on titanium sample coupled with magnesium, the cells were first plated on the titanium samples and kept at OCP for 1 hour. After 1 hour, the magnesium wire was coupled to the titanium sample. At 2, 4 and 6 hours, the sample surfaces were gently rinsed in phosphate buffered solution (PBS) and fixed with 4% formaldehyde. The samples were then dehydrated in gradients of ethanol (50, 70, 90% in PBS and 100%).The samples were then sputtered with gold and cell morphology was assessed using scanning electron microscope (SEM) (JEOL 5600, Japan). Cell area was calculated by fitting an ellipse over the cell using ImageJ software (NIH). 2way anova was performed with α=0.05 (n=3 for all groups, 141 cells randomly selected from each sample).

Results

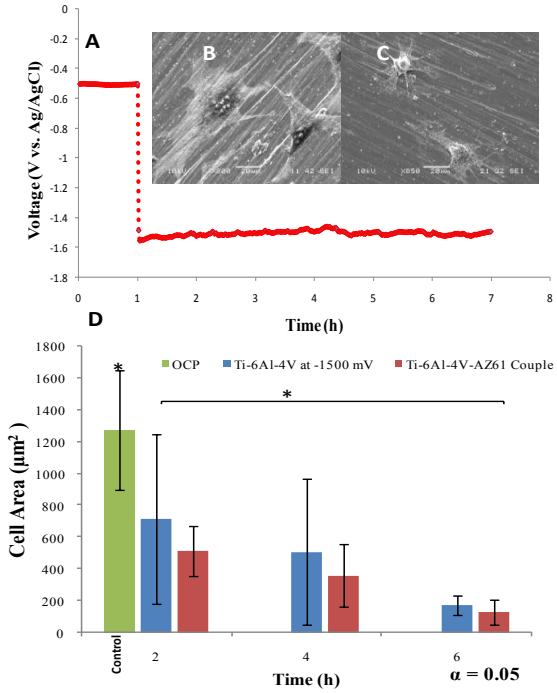

Figure 1. A. The graph shows the voltages experienced by cells when plated on the Mg-Ti-6Al-4V couple. Note: The graph clearly shows the drastic drop in potential at 1 h when Mg was coupled to the titanium surface, **B.** MC3T3 cells on control shows the cells are well spread out, **C.** cells balled up at the end of 6h on the Mg-Ti-6Al-4V couple. **D.** Graph shows that the cell morphology is adversely affected on both titanium surface held at -1500 mV and Mg- Ti-6Al-4V couple.

Figure 1A shows the cathodic voltages experienced by the cells when magnesium was coupled to titanium. The cells went from being well spread out at OCP (Fig. 1 B) to a balled up morphology, which implies that the cells are undergoing cell death, possibly by apoptosis. The graph (Fig. 1D) shows projected cell area at OCP was significantly larger than cells on both titanium (@-1500 mV) and Mg- Ti-6Al-4V surfaces.

Discussions and Conclusions: This study demonstrates that by coupling an active metal (Mg) to titanium surfaces, electrochemically modulated cell behavior is possible. This concept will ultimately be used to design new implants with a capability to actively control bacterial infections and inflammation on the implant surface and even enhance bone regeneration remotely from the implant surface. We also intend to develop new strategies to target and suppress tumors and cancerous cells based on this technique.

References: (1) Brighton, C. et al A multicenter study of the treatment of non-union with constant direct current. J Bone Joint Surg Am. 1981 January 1;63(1):2-13 (2) Kalbacova et al, Biomaterials 28 (2007) 3263–3272 (3) Ehrensberger et al., 2010 JBMR-A(93A: 1500–1509);
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