In Vitro Modeling of Cathodic Voltage-Controlled Electrical Stimulation at the Bone-Titanium Interface

Eric P. McDermott[1], Mark T. Ehrensberger[1]
[1]*University at Buffalo, Buffalo NY*

Introduction: Previous in-vivo work has shown that applying cathodic voltage-controlled electrical stimulation (CVCES) of -1.8V for 1 hour to titanium (Ti) implants is an effective treatment for the eradication of bacterial biofilms and does not produce histologically deleterious effects on the bone tissue adjacent to the stimulated Ti implant [1,2]. However, previous in-vitro studies showed that cathodic polarization of Ti substrates in the range of -400mV to -1V can reduce viability of freshly seeded pre-osteoblasts [3,4]. This in-vitro study characterized how the larger magnitudes of CVCES used for antimicrobial treatments can affect cellular behavior on the stimulated metal. The tests evaluated CVCES on both a confluent monolayer of pre-osteoblasts (48-hour incubation) and also a mature, mineralized layer of differentiated osteoblasts (21-day osteogenic incubation).

Methods: Pre-osteoblast cells (MC3T3-E1) were seeded onto commercially pure titanium (cpTi) coupons, within a custom three-electrode electrochemical chamber as previously described [3]. The seeded cells were either allowed to incubate for 48 hours to achieve a cellular monolayer, or 21 days to achieve a mature extracellular matrix (ECM) using osteogenic medium. Three CVCES magnitudes (-1.0V, -1.5V, & -1.8V vs. Ag/AgCl) were tested for a 1-hour duration using a potentiostat (Gamry, Ref600). Open circuit potential (OCP) acted as the control for this study. Following stimulation, all electrodes were disconnected, and a live/dead assay or fixation followed by scanning electron microscopy (SEM) were performed to analyze each cellular layer. Wide-field fluorescence was used for the imaging of the live/dead assay of the 48-hr monolayer, and confocal microscopy was used for the 21-day mature ECM. For SEM imaging, backscatter electron (BSE) and secondary electron (SE) micrographs were acquired. Electrical impedance spectroscopy (EIS) was also used to assess the cellular layer as it grew over the 21-day period.

Results: Representative BSE images of the 48-hr monolayer experiments can be seen for the OCP control and each tested CVCES magnitude (*Fig. 1*). As the cathodic magnitude was increased, a retraction of the cells from the cpTi surface was observed. Viability results showed a complete reduction of average %viability at the -1.8V magnitude (*Fig. 2*). Representative SE micrographs of the 21-day mature ECM experiments showed retention of the ECM at the -1.0V and -1.5V magnitudes (*Fig. 1*). At the -1.8V magnitude, large disruption to the ECM was observed. Viability results confirmed this with the -1.8V magnitude showing complete reduction in average %viability (*Fig. 2*). EIS monitoring of the layer over 21 days showed a significant increase in polarization resistance compared to the 48-hr monolayer.

Conclusion: This study found that a 21-day mature ECM was more resistive to damage at the -1.5V magnitude when compared to the 48-hour monolayer. This may indicate that the mineralized extracellular matrix provides some protection to the cells. Interestingly, the results of -1.8V for 1 hour showing ECM disruption and reduced cell viability are in contrast to the prior in-vivo histological results for this CVCES treatment. More recent unpublished in-vivo studies have confirmed that osseointegration of Ti implants is maintained following CVCES of -1.8V for 1 hour. Therefore, there is a disconnect between the in-vitro and in-vivo effects CVCES has on bone cells/tissue. Further work is needed to better understand these differences in order to develop more complex and relevant in-vitro models of the bone-implant interface.

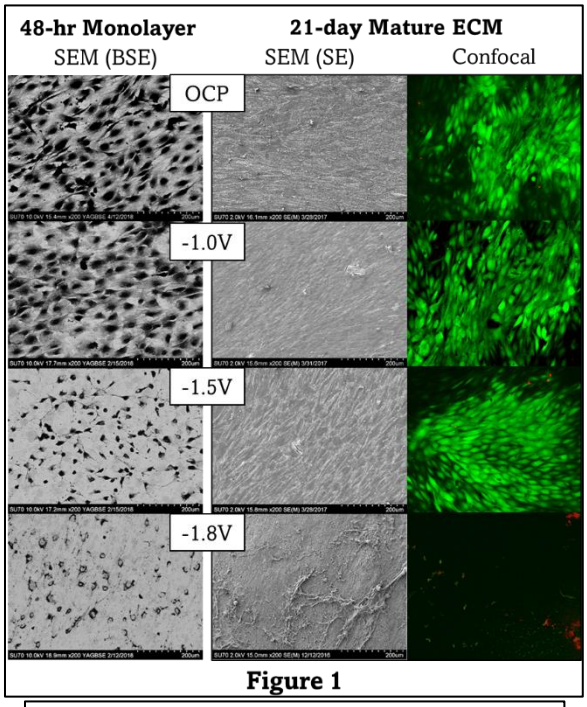

Figure 1

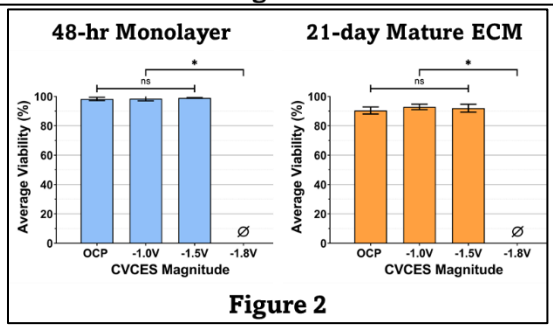

Figure 2

References: 1. Ehrensberger, Biomaterials, 2015. 41(0)97-105. **2.**Nodzo, Clin Ortho Rel Res, 2016. 474(7): 1668-75. **3.**Ehrensberger, J Biom Mat Res A, 2010. 93(4):1500-9. **4.**Sivan, J Biom Mat Res B, 2013. 101B:1489–1497.