Cellular Behavior on Anodized vs. Non-anodized Titanium Under Cathodic Polarization

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Statement of Purpose: Metallic biomaterials upon implantation inside body attain a voltage known as open circuit potential (OCP), which depends on the chemical and physical properties of the biomaterial and the biological milieu. Excursions from this voltage to more cathodic voltages could happen in vivo due to fretting corrosion, presence of oxidants, etc. Few studies have addressed how these voltage shifts affect cellular behavior next to an implant. Results from *in vitro* cell culture studies by Gilbert et al.[1] and Ehrensberger et al.[2] on cathodically polarised Ti samples showed that large cathodic voltages lead to cell death. High cathodic voltages also proved lethal to cells in similar studies done on Ti_6Al_4V[3] and CoCrMo [4] biomedical alloys. However, the underlying phenomena causing cell death at large enough cathodic voltages are still largely unknown, and no real attempt has been made to elucidate the mechanisms involved in this process. It is hypothesised that charge transfer through the surface oxide layer of the implant and redox reactions are possible causes of cell death.The goal of this abstract is to study the effect of surface oxide thickness on cellular morphology and viability of pre-osteoblast cells on polarized cpTi surfaces. Results of this study help to understand the effect of polarization resistance and charge transfer on the observed cellular behavior at polarized surfaces. This also could give hints as how to make cathodically polarized surfaces more biocompatible.

Methods:

*Sample preparation***:** cpTi samples were polished up to 600 grit, sonicated in DI, washed with 70% ethyl alcohol and UV sterilized. Anodization was done in 0.1 M PBS for 3 min at 10 V. Manual scratches were made on some samples by a razor blade to create areas of high and low oxide thickness/polarization resistance.

Electrochemical Impedance Spectroscopy (EIS): Impedance of anodized and non-anodized samples were measured at different frequencies with a 10 mV input at the polarization voltage.

Cell culture: MC3T3-E1 pre-osteoblast cells were cultured using a culture media composed of 89% AMEM, 10% FBS, 1% Penicillin Streptomycin L-glutamine. 10,000 Cells per surface were placed on each sample and kept at 37˚C, 5% CO_2 and 95 % humidity.

Effect of voltage: anodized and non-anodized samples were potentiostatically held at -400 mV for 18h and -600 mV for 4h. A minimum of 3 samples per condition was studied.

SEM analysis: cells were fixed using 4% formaldehyde for 15 minutes, and dehydrated using alcohol gradients in PBS (50, 75, 90 and 100 %).

Viability assay: LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (SKU# L-3224) (Invitrogen) was used. Statistical analysis of cell viability was performed using Student's t-test with $P < 0.01$ being significant.

Results and Discussion: Fluorescence microscopy images of the cells on anodized and non-anodized samples are shown in Fig. 1. It is clearly seen that while cells grown on anodized samples remained live (green) after 18 hours at -400 mV, the non-anodized surface rendered most of the cells non-viable (red). That being said, the morphology of the cells on the anodized polarized samples is different from the cells grown at OCP, which appears to be more spread. Similar results were obtained for polarization voltage of -600 mV after 4 hours. Moreover, it was found that at very low current densities cells behave like cells grown at OCP regardless of the voltage. Polarization resistance of the anodized samples from EIS measurements appear to be higher than non-anodized samples, which is consistent with the lower currents measured for anodized samples. Moreover, a more capacitative nature of the non-anodized samples vs. more resistive for anodized samples was evident from their corresponding phase angles and α (dispersion factor of Constant Phase Element) values of the EIS data (0.9 and 0.7 respectively). SEM images of scratched anodized samples do not show any preferential growth or spreading on the grooves or the ridges at OCP. Furthermore, cells near areas with a high scratch density on polarized samples looked balled up and non-viable in or out of scratches.

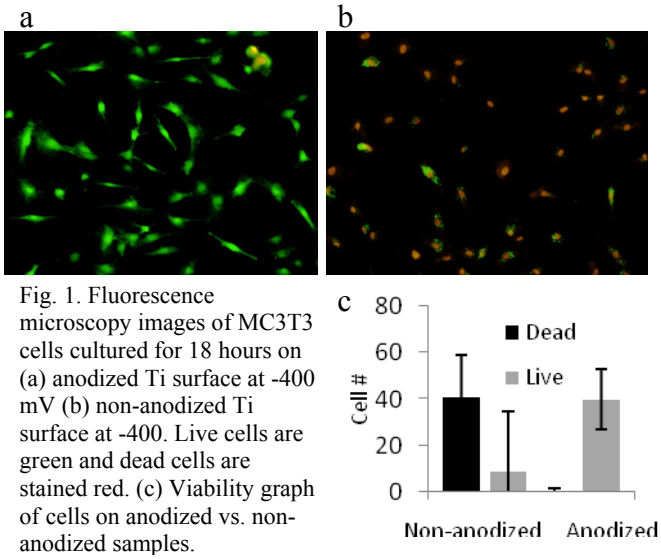

Fig. 1. Fluorescence microscopy images of MC3T3 cells cultured for 18 hours on (a) anodized Ti surface at -400 mV (b) non-anodized Ti surface at -400. Live cells are green and dead cells are stained red. (c) Viability graph of cells on anodized vs. non-anodized samples.

Conclusions: Thicker oxide layers of anodized Ti show better cellular biocompatibility compared with non-anodized samples under polarization at the same voltage and time interval. There seems to be a current threshold below which the voltage, on its own, does not affect viability of the cells.

References:

1. Gilbert et al., JBMR. 1998:42: 321
2. Ehrensberger and Gilbert, Trans ORS, 2009
3. Sivan et al., SFB annual meeting abstracts, 2009
4. Haeri et al., MPMD Conference proceedings, 2009