Time-lapse study of change in cellular adhesion on a cathodically polarized CoCrMo alloy

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Statement of Purpose: The voltage of metallic biomaterials change upon implantation in the body and shifts from its resting potential towards anodic or cathodic voltages. The effect of such voltage shifts on the cellular behavior has not been fully investigated. Gilbert et al. [1] and Ehrensberger et al. [2] showed that large cathodic voltages lead to cell death on cathodically polarised Ti samples. Similar results have been reported for Ti_6Al_4V [3] and CoCrMo [4] biomedical alloys at high cathodic voltages. However, the mechanisms involved in this process are still largely unknown and details of the interaction between polarized surfaces and cells are yet to be studied. The goal of this abstract is to study how cathodic voltages influence the adhesion of cells to their metallic substrate by monitoring the dynamics of changes to numbers and distributions of the focal adhesion protein vinculin. Results of this study will help to understand how the loss of focal adhesion sites correlates with the observed cell morphology and ultimately cell death at high cathodic voltages.

Methods:

*Sample preparation***:** CoCrMo samples (F-1537) were mirror polished to 0.3 um alumina finish, sonicated in DI, washed with 70% ethyl alcohol and UV sterilized.

Cell culture: MC3T3-E1 pre-osteoblast cells (p9) were cultured using a culture media composed of 90% AMEM, 10% FBS. 40,000 Cells per surface were placed on each sample and kept at 37˚C, 5% CO_2 and 95 % humidity for 24 hour before transfecting the cells with GFP-vinculin.

Transfection: A GFP-vinculin plasmid was transfected into the cells using a transfection agent (Optifect, Invitrogen) according to the protocol provided by the manufacturer 24 hours before imaging.

Fluorescence microscopy and image analysis: Samples with transfected cells on top were mounted on a custom-made electrochemical cell filled with culture media and imaged using an upright Axiovert 200M Carl Zeiss, Inc microscope. Images of samples at OCP and samples potentiostatically held at -1000 mV (Ag/AgCl) were captured within time intervals of 1-2 min for 1 hour and movies were made. Images were analyzed using Image J NIH software, and the change in average focal contact area of cells imaged at OCP was compared with the corresponding area of cells exposed to the cathodic voltage. Kymographs, which give graphical representation of spatial position of vinculin over time, were produced in both cases. A line is drawn at the site of interest and the movement of vinculin is indicated by movement of the pixels along the line.

*Live/Dead assay and Immunostaining: C*ells cultured at -700 mV(Ag/AgCl) for 6 hours were immunostained with antibodies to reveal the actin cytoskeleton and vinculin focal adhesion points. Also, viability of cells cultured at -400 mV(Ag/AgCl) after 24 hr was done using LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (SKU# L-3224) (Invitrogen).

Statistical analysis: Student's t-test was performed to compare the focal contact area of cells at OCP and the cathodic voltage, and the fraction of live to dead cells on these two surfaces with P-value of 0.05 indicating significance.

Results and Discussion: The actin cytoskeleton and vinculin adhesion molecules can clearly be distinguished in images of cells at OCP (Fig. 1a). However, there is almost no sign of vinculin in cells cultured at -700 mV for 6 hours (Fig. 1b). These cells were completely balled up in what looked like bundles of actin wrapped around the cell nucleus. Typical kymographs of cells at OCP and at -1000 mV (Ag/AgCl) in Fig. 2 show a higher motility of the focal adhesion sites after voltage is applied to the metallic substrate. This happens in line with the overall cell area reduction that is observed for the entire cell. The image analysis did not show a significant change in number of focal adhesion structures in half an hour time period at either OCP or for the polarized condition. The focal contact area fraction rate change, however, was 2% at OCP vs. 6% on the polarized surface. This suggests that the initial stages of voltage effect on cells might mainly involve an accelerated reorganization of the focal adhesion molecules, without a major loss of adhesion and cell death, which happens later on. (93±14% of cells were remained alive at OCP vs. no live cells at -400mV after 24 hr)

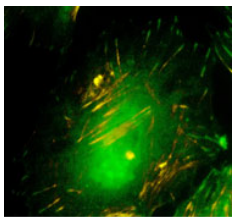
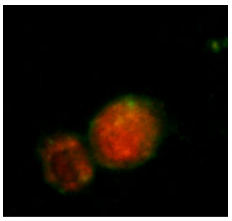
a  b 

Fig. 1. Fluorescence microscopy images of MC3T3 cells cultured for 6 hours on CoCrMo surface at OCP (left) and -700mV (right). Immunostained vinculin and actin cytoskeleton are green and red respectively.

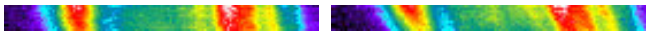


Fig. 2: A typical kymograph of the edge of a cell before (left) and after application of voltage (right). The distance and time progress from left to right and top to bottom respectively. Larger shifts of pixels towards right indicate higher velocity of movement.

Conclusions: The cathodic voltages increase the motility of the focal adhesion sites and the total rate of cell shrinkage, which ultimately lead to total loss of adhesion and cell death. The lethal effect of voltage seems to be a function of time and has no significant effect on the number of adhesion sites in times as short as half an hour at -1000 mV (Ag/AgCl).

References: 1. Gilbert et al., JBMR, 42: 321: 1998
2. Ehrensberger et al., JBMR, 93:1500: 2010
3. Sivan et al., SFB annual meeting abstracts, 2009
4. Haeri et al., MPMD conference proceedings, 2009