Nitinol Corrosion Ions Inhibit α-actin Expression and Decrease Aspect Ratio of Rat Vascular Smooth Muscle Cells in vitro

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Statement of Purpose:

Ni-Ti alloy (Nitinol) has become an increasingly popular biomaterial for use in vascular stenting due to its shape memory properties. However, its high nickel (Ni) content has been a concern due to possible cell toxicity and alterations in cellular activity. In an effort to understand these interactions between cells and nickel-rich alloys, there have been many in vitro studies evaluating the biocompatibility of Nitinol. Most of these studies focused on observations such as cellular proliferation, apoptosis, and DNA synthesis. More subtle changes such as altered protein expression are often ignored. This study aimed at developing a clinically relevant in vitro model to evaluate the response of smooth muscle cells (SMCs) to varying low levels of Ni ions released and present in vascular tissue following the implantation of an endovascular stent.

Methods:

Wire specimens: Nitinol wires (ASTM F2063, Small Parts) 4.4 cm long and 0.127 mm in diameter were ultrasonically cleaned and EtO sterilized. Two wires were then placed in 15 ml conical tubes with 10 ml DMEM (Mediatech, Inc., Herndon, VA, USA) and allowed to corrode for various time periods. At the predetermined time point, the media was removed and transferred to another sterile 15ml conical tube.

Metal Ion Quantification: Metal ions present in the conditioned media were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES) (Ultima 2, Horiba JY, Longjimeau, France). Measurements were taken using a modified protocol developed previously [1].

Smooth Muscle Cell Culture: Aortic smooth muscle cells isolated from female Sprague-Dawley Rats 6-10 weeks of age and maintained with DMEM (10% FBS, 1% Antibiotic-Antimycotic). Cells between passages 4-8 were seeded at a density of 2 x 10^4 cells/well in a 24 well plate incubated for 48 hours to allow the cells to attach. Following the 48 hr attachment period, the culture media was removed from each well and replaced with 1ml of either control DMEM or corrosion conditioned media samples. The cells were subjected to the test media solutions for a duration of 48 hrs. The media was then removed, and cells were fixed in a 2% paraformaldehyde solution. The cytoskeletal F-actin and cell nuclei were stained using Rhodamine-Phalloidin (Invitrogen, R415) and by 4',6-diamidino-2-phenylindole (DAPI) (Molecular Probes, D-1306, Eugene, OR) respectively. The cells were then imaged using fluorescent microscopy (Nikon Inc., Diaphot 30). ImagePro Plus image analysis software (Media Cybernetics, Inc., Version 5.1, Silver Spring, MD) was then used to measure the major and minor axes of the cells. The aspect ratio was then calculated as the ratio of the major to minor axis lengths.

Expression of α-actin: Cells were immunostained using a primary mouse anti-α-actin antibody with Alexa Fluor 488 tagged donkey anti-mouse secondary antibody. Cells were DAPI stained and imaged (Nikon Inc., Diaphot 300, Melville, NY) and densitometry performed. Data were evaluated using ANOVA statistical analysis paired with Tukey analysis using SigmaStat statistical analysis software (Systat Software, Inc., San Jose, CA) with $p<0.05$ indicating a significant difference.

Results:

Ni. ion Concentrations: Average Ni. ion concentrations of 0.05, 0.06, 0.08, and 0.15 ppm were measured for corrosion time points of 2, 10, 18, and 31 days, respectively.

Cell morphology**:** A significant difference in aspect ratio in each test group was measured as compared to the standard DMEM control ($P=<0.001$). A decreasing trend for the aspect ratio in response to increasing nickel ion concentration was observed., ~~H~~however, no significant difference was observed between the different experimental groups in response to increasing ionic concentrations. At the highest Ni concentration tested, the mean aspect ratio was decreased by approximately 25% compared to the control.

Expression of α-actin: Interestingly, the most significant difference observed in cell response was with respect to the expression of α-actin ($P=<0.001$). A significant difference in fluorescence compared to the control was observed at all but the lowest concentration of Ni ions tested in this study. Fluorescence intensity decreased proportionally to increasing Ni. concentration ~~correlation~~ ($R^2= 0.86$). At the highest concentration tested, a 26% decrease in fluorescence intensity was observed as compared to the control.

Conclusions:

The present study utilized ionic concentrations well below the threshold shown to affect SMC proliferation [2] in an effort to evaluate more subtle effects that such low concentrations have on cell response more likely to carry over into clinical observations. Our results have shown an altered cell morphology as well as a decreased smooth muscle cell α-actin expression in response to low nickel concentrations in vitro. It is reasonable to predict that the trends of altered cell morphology and α-actin expression would continue with increasing Ni. ion concentrations. These observations shed some light on one possible contributing factor to the development of in-stent restenosis.

Acknowledgments:

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References:

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[2] Shih, C. J Biomed Mat Res. 2000. (Vol 52. p. 395-403).