The Effect of Metallic Nanoparticles on Vascular Smooth Muscle Cells

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Statement of Purpose: Nanoscience has shown great potential toward biomedical engineering such as the effects on cellular function as well as change in cell morphology [1-3]. Nanoparticles (NP) are currently being used for specific applications of drug delivery, carbon nanotubes, and cancer detecting agents among many others [2]. However, there is still concern that NP may not be considered safe therapeutic agents [1,3]. So, the goal of our study is to look at the structure and mechanics of vascular smooth muscle cells (VSMCs) after treatment with coated NP.

Methods: VSMCs were cultured in T75 flasks until confluent and used between passage 6 and passage 8. Cells were seeded onto plasma cleaned collagen coated coverslips [4]. NP were capped with pluronic F-127, pluronic F-68, carobxymethyl cellulose (CMC), and citrate. Different concentrations of pluronic F-68 were used. Controls were made using the coating solution with no NP present. NP solutions and control solutions were placed on VSMCs and AFM was performed on days 2 - 5 post treatment. Cytotoxicity was measured using an MTS assay. Mechanical testing of live samples were taken using Atomic Force Microscopy (AFM). Cells were indented to a depth of ~1 µm at 1 µm/s using a 5 µm borosilicate spherical probe (0.12 N/m nominal cantilever spring constant). Elastic modulus was estimated from the curves using the Hertz linear elastic model fit to the first 250 nm of indentation. In addition, stress-relaxation tests were performed using the same tip. Cells were indented to 1 µm quickly (10 µm/s) and held under nearly constant deformation for 60s. Following testing, cells were fixed in paraformaldehyde and permeabilized for imaging. Fluorescence microscopy was done on the fixed cells on days 2 – 5 post nanoparticle treatment. Cells were stained with DAPI for nuclei, phalloidin for actin, and rhodamine for microtubules.

Results: Cells treated with pluronic F-127 capped nanoparticles died within 48 hours of treatment while the control solution treated cells did not. There was no measurable cytotoxicity using MTS assay for the citrate and CMC nanoparticle treated cells. In confocal imaging, cells treated with coated NP of F-68 and CMC were thicker than the control solution. However, the citrate control solution treated cells were thicker than the citrate coated NP treated cells. The 1% pluronic F-68 control solution created an abnormal morphology at day 5. Cells were enlarged, more elongated, and had more actin. Cells treated with 0.2% wt pluronic F68 particles had changes in morphology (Fig. 1) although these were not as pronounced.

AFM testing showed the 1% Pluronic F-68 NP treated cells had significantly higher elastic moduli than the cells in control F-68 solution (Fig. 2). The cells in control F-68 solution had moduli that were the same as cells in normal vascular smooth muscle cell media with no additive. In addition, pluronic treated cells didn't relax as much as the controls during stress relaxation experiments (Fig. 2b). This indicates that the changes in morphology observed in optical imaging correspond to stiffer and more elastic cells.

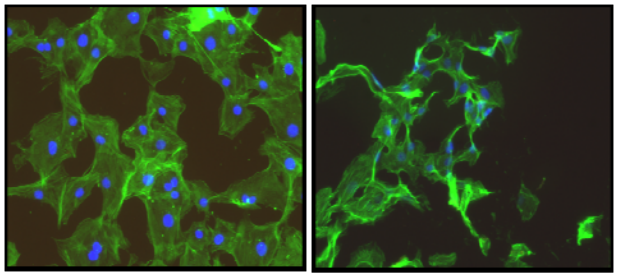

Figure 1. 0.2% wt pluronic F-68 on Au NP's (left) and 0.2% wt pluronic F-68 control solution (right)

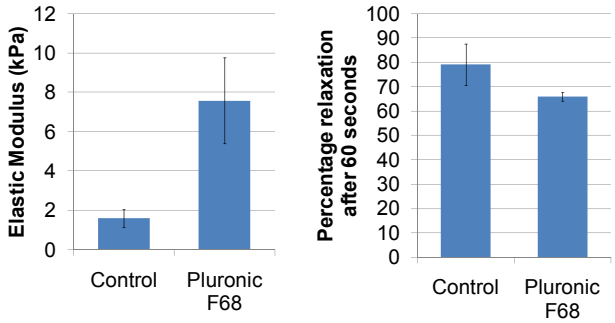

Figure 2. Cell modulus of control cells and Pluronic F-68 treated cells (left) and percent relaxation of control cells and Pluronic F-68 treated cells after 60 second hold

Conclusions: VSMCs were found to have a lower elastic modulus in the control solutions and the cells relaxed more during a 60 second hold. The cells treated with NP had a higher elastic modulus and relaxed less during a 60 second hold. This indicates that while the nanoparticles were not cytotoxic, they do have significant effect on cell phenotype. In particular, the 1% Pluronic F-68 coated nanoparticles caused a high degree of cell stiffening and a decrease in viscous (nonelastic) behavior. Treatment of VSMCs with coated NP was found to cause mechanical and morphological changes and these changes could be related to concentration of NP. The exact mechanism by which nanoparticles effect cell mechanical properties and structure is not known and studies are underway to elucidate how nanoparticles can affect cell function.

References: [1]Gwinn+ Environ. Health Persp. 2006, [2] Emerich+ Biomol. Eng., 2006; [3]Kagan+ Nanomed. Nanotech. Biol. Med. 2005; [4]Hemmer+. *J. Eng. Med.* 2008

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