Vascular Smooth Muscle Cell Mechanics in Response to Gold Nanoparticles

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Statement of Purpose: Nanotechnology has shown great potential toward biomedical engineering for applications in bioimaging to tissue engineering. Nanoparticles (NP) are currently being used for specific applications like drug delivery to cancer tumors [1-3]. However, while NPs have shown great promise in these areas, longer term biological effects of nanomaterials are not well-understood. Furthermore, some nanomaterials have been shown to induce cancer. Therefore, there is a great need for more thorough investigations on nanomaterials and cell interactions to help elucidate how nanoparticles can affect cell function.

Methods: VSMCs were cultured in T75 flasks until confluent and used between passage 5 and passage 8. Cells were seeded onto plasma cleaned collagen coated coverslips [4]. NPs were capped with pluronic F127, pluronic F68, carobxymethyl cellulose (CMC), and citrate. Different concentrations of pluronic F68 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 was done 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 NP treatment. Cells were stained with DAPI for nuclei and phalloidin for actin.

Results: Initial experiments were done on high passage (>10) VSMCs. Cells treated with pluronic F127 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 NP treated cells. In confocal imaging, cells treated with coated NP of F68 and CMC were thicker than the control solution. However, the citrate control solution treated cells were thicker than the citrate coated NP treated cells. Cells treated with 0.2% wt pluronic F68 particles had changes in morphology.

AFM testing showed the 1% pluronic F68 NP treated cells had significantly higher elastic moduli than the cells in control F68 solution. The cells in control F68 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 experiment. Recent citrate experiments done on low passage (between 5 and 8) VSMCs have shown that both the control solutions and NPs cause significant decrease in elastic modulus (Fig 1). This indicates that the changes in morphology observed in optical imaging correspond to stiffer and more elastic cells. Fluorescence staining showed synthetic VSMC phenotype (Fig 2a) with the control solution treated cells while NP treated cells showed a shift to the contractile phenotype (Fig 2b).

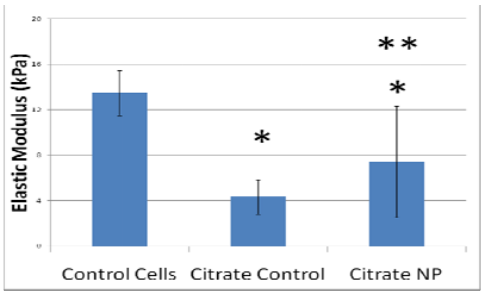

Figure 1. Cell modulus of citrate treated and control cells $p<.05$

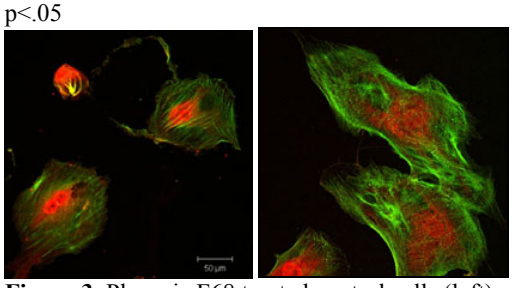

Figure 3. Pluronic F68 treated control cells (left) and pluronic F68 NP treated cells (right)

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 F68 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. This property shift is occurs in both high and low passage VSMCs. The exact mechanism by which nanoparticles affect 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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