Characterization of Porcine Vascular Tissue and Gold Nanoparticles as a Vascular Graft Material

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Statement of Purpose: The purpose of this study was to characterize a xenograft with various modifications. The central hypothesis was that crosslinking the basic components of the xenograft and adding gold nanoparticles to the surface would strengthen the graft, increase cell proliferation and in-growth, and decrease inflammation. Cardiovascular disease is the leading cause of mortality in the United States. Currently the best way to treat peripheral artery disease is to use autologous vessels, however these are not available in approximately 10-30% of patients for various reasons such as co-morbidities, previous bypass surgeries, and the increased cost and morbidity associated with harvesting the vessels(1, 2). More natural grafts are being explored, such as using xenograft scaffolds with the host DNA removed, which leaves the stable non-immunogenic extracellular matrix (ECM). These function well as vessels, but can be weaker and less uniform than synthetic vessels. Surface modifications using nanoparticles are being utilized to create a better graft. Gold nanoparticles have been shown to increase cell in growth in certain tissues and also have antioxidant and anti-inflammatory properties (3).

Methods: Porcine aortas were collected from freshly euthanized animals and underwent a decellularization process using the non ionic detergent Triton X-100. 100 nanometer gold nanoparticles at a concentration 4 times the stock solution (AuNP-4x) were crosslinked to the aortic scaffolds using a solution of acetone and phosphate buffered saline (PBS) with 2 mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and 5mM N-Hydroxysulfosuccinimide (NHS) that causes an amide bond and links collagen molecules. Tissue was sterilized using peracetic acid. Three experimental groups were used – decellularized, crosslinked, and AuNP-4x. Mechanical testing was performed using an Instron tensile testor to calculate the tensile strength of each experimental group as compared to natural tissue. There were two subsets of each group, the x-axis, or the circumferential part of the vessel, and the y axis, or the longitudinal part of the vessel. Hematoxylin and eosin staining were used to evaluate the tissue subjectively for any remaining cellular debris via light microscopy. Scanning electron microscopy (SEM) was used to look at the microstructure of the graft and Energy Dispersive Spectroscopy (EDS) was used identify the nanoparticles. A Cell Proliferation Reagent WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) Assay for biocompatibility was performed using human umbilical endothelial cells that were cultured on the grafts for three days.

Results: Mechanical testing showed no significant differences in tensile strength at yield or the modulus of elasticity between groups and compared to the native tissue. Light microscopy showed no evidence of cellular debris (Figure1). SEM showed no cellular debris or collagen damage. Using a backscatter detector nanoparticles were observed and identified as gold using EDS. The WST assay showed no significant differences in biocompatibility between groups. For mechanical testing and the WST assay Graph Pad Prism 4 was used to perform an Analysis of Variance with a Tukeys Test as the post-hoc. Significance was set at $P<0.05$.

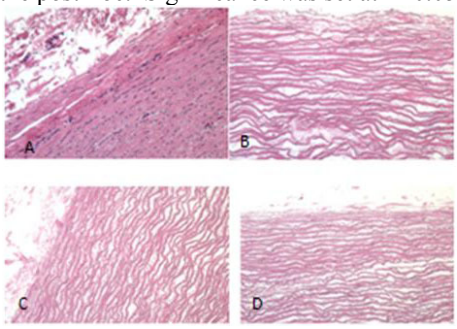

Figure 1: Light microscopy of H&E stained tissue at 40x
a) Natural Tissue
b) Decellularized
c) Crosslinked
d) 20 nm AuNP 4x

Conclusions: The lack of significant differences between experimental groups and the native tissue with regard to the tensile strength at yield and the elastic modulus shows that the decellularization and crosslinking processes did not damage the tissue. Therefore the graft can be considered as strong as a native vessel. The lack of cellular debris seen with light microscopy indicates a successful decellularization and removal of immunogenic material from the extracellular matrix. The SEM results show that the decellularization and crosslinking of the tissue does not damage the structure of the extracellular matrix. The EDS results prove that the nanoparticles are being attached to the grafts. Since the nanoparticles are a major aspect of this material ensuring their attachment after sterilization is very important. The WST assay showed that the crosslinking and addition of AuNP-4x does not cause cytotoxicity. The endothelial cells grew as well on the crosslinked and decellularized grafts as they did on the grafts with gold nanoparticles. When implanted into the body the nanoparticles should not cause an adverse reaction with regards to cell growth. Future experiments include longer WST studies; providing the cells more time to grow to determine if there are significant differences between groups. In addition further cell culture assays will be performed to examine the rate of cell proliferation and the effects of AuNP on the production of reactive oxygen species. These assays will further characterize the material. Overall the current data shows no signs that the decellularization, crosslinking, or addition of AuNPs caused mechanical, structural, or biocompatibility changes in the tissue.

References:

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