In Vitro and *In Vivo* Investigation of Nanostructured Vascular Patches

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Statement of Purpose: The goal of the study was to investigate the effectiveness of a novel vascular patch material that could be utilized in the treatment of stenosis in the carotid or femoral vessels. Acellular porcine blood vessels were merged with gold nanoparticles (AuNPs) for the development of blood vessel patches. It was hypothesized that the AuNP-vascular patch would promote cellularity and maintain strength, while avoiding the critical problems such as ruptures, poor flow dynamics, and calcification.

Synthetic materials, such as polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE) are utilized as vascular patches. These materials possess good mechanical properties and acceptable *in vivo* durability, but they are unable to promote endothelial cell migration and remodeling while leading to chronic phase inflammation [1]. Additionally, thrombus formation and calcium deposition may occur [2]. Alternatively biological patches, such as bovine pericardium, can be utilized. However, there is a high incidence of patch calcification and restenosis [3]. In particular the use of glutaraldehyde crosslinkers has been shown to promote these processes.

The use of decellularized arterial blood vessels conjugated with AuNPs is advantageous since an *arterial* matrix will be replacing part of a disease *artery*. The utilization of conjugated AuNPs offers additional advantages of cellular in-growth, reduced inflammation, antimicrobial effects, and long-term patency [4].

Methods: Porcine aortas and carotids were harvested and decellularized following euthanasia of swine after a laboratory exercise at the University of Missouri School of Medicine. They were immersed in distilled water for 24 hours at 4°C to rupture cell membranes. Next, the vessels were treated with 0.025% trypsin EDTA diluted in Dulbecco's phosphate buffered saline for 24 hours at 37°C. The tissue was then put into a solution of 1% Triton x-100 and 0.1% ammonium hydroxide in distilled water for 72 hours at 4°C to remove nuclear components and lyse cell membranes and cytoplasmic proteins. The tissue was then put into a solution of Eagle's Minimum Essential Medium to deactivate any remaining trypsin for 24 hours at 37°C. Once decellularized, the tissue was conjugated to 100 nm diameter AuNPs (Ted Pella, 4x stock solution) using a crosslinking solution 50:50 (v/v) of acetone and PBS with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). The AuNPs were functionalized with 2-mercaptoethylamine. The specimens were sterilized using an aqueous solution of 0.1%(v/v) peracetic acid with 1.0M NaCl for 30 minutes. Mechanical testing, *in vitro* cell culture studies as well as a 3 week and 9 week *in vivo* study were performed.

Results: Mechanical testing: Uniaxial tensile testing was performed using a mechanical testing system (Instron TA.XT2). Natural porcine aorta and tissue that was decellularized, crosslinked, crosslinked with AuNPs, sterile and non sterile were compared to one another. The tensile stress at maximum load, the modulus of elasticity, and the percent tensile strain at maximum load were determined with standard error of the mean. There were no significant differences between the native tissue and the experimental groups. Biocompatibility Testing: Cell proliferation Reagent WST-1 (Roche Diagnostics) was used to compare the biocompatibility of the tissue groups. The WST-1 assay showed no significant differences ($P>0.05$) between any of the groups when the percent viability of the experimental groups were compared to the decellularized tissue. *In vivo* studies: Domestic swine underwent carotid endarterectomy and patch angioplasty for a three week and nine week study using the experimental patches (crosslink and AuNP) and commercial control (bovine pericardium; Synovis). The experimental patches were sutured to the right carotid while a control patch was sutured into the left. Patency, hemodynamics (via ultrasound), and histology of the explants were performed. Ultrasound images showed long term patency of the carotid artery for all groups. Fig. 1a is the AuNP-vascular patch after implantatation and Fig. 1b is the inner lumen of the vessel after explantation showing Evan's Blue dye staining, indicating new endothelial cell growth (b).

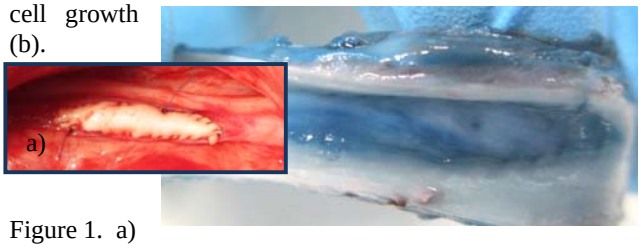

Figure 1. a) AuNP-patch after implantation and b) patch after harvesting.

Conclusions: The mechanical testing indicated that the decellularization process did not decrease the strength of the vessel, and the AuNP crosslinking process did not cause stiffening, therefore the vessel maintained its native structure. The WST-1 assay indicated that the crosslinking process and the addition of gold nanoparticles did not adversely affect cell growth. The *in vivo* patches maintained good patency and hemodynamics for the 3 and 9 week time points. At 3 weeks, regenerating endothelial cell growth was noted on the experimental patches. Histology showed normal inflammatory and healing response in all the experimental and control groups. At 9 weeks, the experimental groups showed better integration with the host tissue grossly, particularly the AuNP carotid patch.

References: 1. (Quarti A. Interact Cardiovasc Thorac Surg. 2011; 13:569-572) 2. Awad I. Stroke. 1989; 20:417-422.) 3. Li X. Ann Vasc Surg. 2011; 25:561-568.) 4. (Deeken CR. J Biomed Mater Res B. 2011;96:351-359.)