Fabrication of tissue-engineered blood vessel using electrospinning

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Statement of Purpose: Substantial efforts are being invested to develop blood vessel substitutes suitable for patients with cardiovascular diseases[1]. With the development of the biotechnology and biomaterials, tissue-engineered blood vessels may offer an option in replacing or repairing diseased blood vessels. Recently, electrospinning has been recognized as an efficient processing method to manufacture nanoscaled fibrous vessel conduits[2]. Besides mimicking the architecture of natural tissue matrix, electrospinning may provide improved mechanical strength and enhanced biological properties to the fabricated matrices mainly due to higher surface area to volume ratio in ultra-fine fibers. The present study aimed to create a biomimetic, small-diameter vascular graft using electrospinning technology and to evaluate its function *in vitro* and *in vivo*.

Methods: In this study, vascular graft scaffolds have been fabricated by electrospinning polymer blends of collagen type I from calf skin, elastin, and poly (D,L-lactide-*co*-glycolide) (lactide:glycolide ratio 50:50 Mw 110,000). The solutes were mixed in 1,1,1,3,3,3-hexafluoro-2-propanol (99+%) (HFIP) at a total solution concentration of 15w/v% (150mg/mL). Scaffolds were crosslinked afterwards for increased stability and strength using glutaraldehyde.. The structure of the scaffold was characterized by environmental scanning electron microscopy (E-SEM). Collagen and elastin distributions were assessed by immuno-histochemistry and Masson's trichrome. The mechanical properties of the scaffold were evaluated by compliance testing, axial and circumferential segment testing and burst pressure testing. The mitochondrial metabolic (MTT) activity assay was performed to test the biocompatibility of the vascular scaffold *in vitro*. The luminal surface of the scaffold was seeded with bovine endothelial cells (5×10^5 /mL) for the first day and the outer surface was seeded with smooth muscle cells (5×10^5 /mL) for another 3 days. Cell attachment and maturation was observed using E-SEM. Distribution of endothelial and smooth muscle cells on the scaffold was analyzed by H&E and immunostaining using anti-Alpha Smooth Muscle Actin and anti-CD-31. Elastin distribution within the scaffolds was assessed by Movat staining. To evaluate the tissue compatibility and long-term systemic toxicity in mice, 20 week-old, CD-1 male mice were implanted subcutaneously with electrospun vascular scaffolds and sacrificed 1, 2, 3 and 4 weeks after implantation (n = 4 per time point). White blood cells (WBC) were counted using an automated hematology analyzer. Kidney, lung, liver and spleen were processed for histology, sectioned at 3–5 mm. The sections were stained with H&E for morphological examination.

Results / Discussion: We have improved the mechanical properties (compliance, strength) of the vascular scaffolds by adding high MW PLGA to the mixture. We were able to produce thick, strong scaffolds, while maintaining the collagen and elastin ratio constant. SEM of the scaffolds showed fiber diameters of 0.72±0.35 μm and a random orientation of fibers. Collagen type I stained positively on the electrospun scaffolds, demonstrating a uniform distribution. Elastin distribution was uniform throughout the scaffold wall.

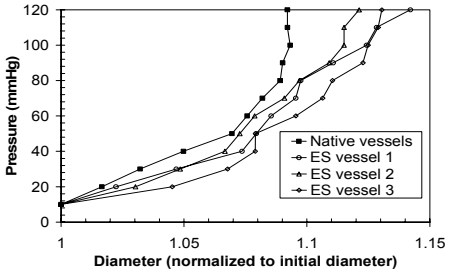

Figure 1.Compliance curves for native and electrospun (n=3) vessels. Native vessel is bovine iliac

Figure 1 shows the electrospun scaffolds have a compliance curve similar to that of a native vessel. The results demonstrate that these scaffolds possess adequate physical strength and elasticity to be developed as substitutes for native vessels. An average of 82% of smooth muscle cells and 72% of endothelial cells survived on the scaffold as analyzed by MTT assay. SEM micrographs reveal a confluent layer of endothelial cells on the inner surface after 4 days and smooth muscle cells on the outer surface of the scaffold after 3 days. H&E staining showed endothelial cells and smooth muscles on the two sides of the scaffold. Double-immunostaining of endothelial and smooth muscle cells indicated that the electrospun scaffold can support long-term cell growth, proliferation and maturation. For the implantation of scaffold in mice, no behavioral changes or visible changes of physical impairment were observed during the post-operative examinations. Complete blood count of the implanted animals was within normal limits during the entire study period.

Conclusions: This study shows that electrospun scaffolds exhibit structure and mechanical behavior similar to native vessels. The results also indicate a favorable interaction between this synthetic nanofibrous scaffold with two different types of vascular wall cells. Electrospun vascular scaffolds possess components and distribution similar to normal vessels. Collectively, this study demonstrates the promise of electrospinning as an effective fabrication process for cardiovascular grafts.

References:

1. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. *Science*. 1986;231:397-400.

2. Li WJ, Laurencin CT, Caterson EJ, Tuan RS, Ko FK. Electrospun nanofibrous structure: a novel scaffold for tissue engineering. J Biomed Mater Res 2001; 60:613-21.