Electrospun Bilayered Vascular Scaffolds for Engineering Small Diameter Blood Vessels

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Statement of Purpose: The demand for small diameter (<5 mm) vascular substitutes for coronary and peripheral revascularization procedures has been increasing steadily, while key challenges associated with good functional outcome of vascular grafting remain unsolved. The principle of vascular tissue engineering, employing cells seeded on a biodegradable tubular scaffold, has been demonstrated in animal models. In our previous study we developed engineered vessels utilizing a novel bilayered vascular scaffold which provides different pore sizes to enhance the cellular interactions of endothelial cells (ECs) on the lumen and the infiltration of smooth muscle cells (SMCs) into the outer layer (Figure 1). This study presents the development of an engineered small diameter blood vessel composed of the bilayered vascular scaffold with an endothelialized lumen and confluent, external SMC layer and the demonstration of the clinical feasibility using this system in sheep arterial interposition model.

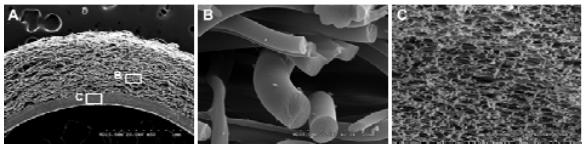

Figure 1. SEM images of electrospun bilayered vascular scaffold: (A) entire structure, (B) outer layer, and (C) inner layer of the scaffold.

Methods: Bilayered tubular scaffolds were electrospun using collagen type I mixed with poly(ε-caprolactone) (PCL) in a 1:1 ratio and dissolved in HFP at 5% (w/v). The inner layer was fabricated using nanofibers of 500 nm in diameter and the outer layer was fabricated using microfibers of 5 μm in diameter (Figure 1). Scaffolds were crosslinked in gluteraldehyde vapor for 6-8 h and then EO sterilized. Autologous SMCs and endothelical progenitor cells (EPCs) were isolated from a femoral artery biopsy and column filtration using CD133 antibody from female sheep aged 3-6 months, respectively. SMCs were seeded on the exterior surface at a density of 10^8 cells/ml. Scaffolds were placed in a bioreactor system. EPCs were seeded at 2×10^6 cells/ml using a combination of static and dynamic cell seeding. Dual cell seeded scaffolds were placed into 37°C incubators and the construct maturation was achieved by means of medium flow through the scaffold and progressive increase of amplitude every 24 h over the course of 10 days. Preconditioned vascular scaffolds were implanted as an arterial interposition in the left carotid artery of the sheep. *In vivo* evaluation was performed by carotid Doppler ultrasound, and CT angiography.

Results: We showed that the electrospun bilayered vascular scaffolds fabricated by hybridizing PCL and collagen possess adequate biomechanical properties that resist high degrees of pressurized flow over the long-term. In addition, these scaffolds showed adequate biocompatibility that can support cell adhesion and growth, and displayed comparable compliance matching native vessel. After 10 day-preconditioning, SMC seeding resulted in a robust, confluent multi-cellular layer on the outer surface of scaffold and uniform coverage of EC was achieved when the cells were seeded multiple times with the rotation of vascular scaffolds. Figure 2 shows histological evaluation of bioengineered blood vessel at 1 and 6 months implantation. The results indicate that dual cell-seeded vascular scaffolds maintained the patency over 6-month period in the sheep arterial interposition model.

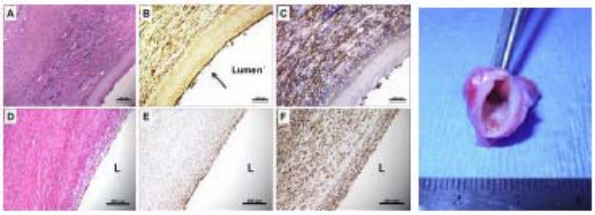

Figure 2. Histological evaluation of bioengineered vascular grafts at (A-C) 1 month and (D-F) 6 months of implantation: (A,D) H&E, (B, E) Von Willebrand Factor (vWF), (C,F) α-smooth muscle action (α-SMA). (G) Gross appearance of retrieved bioengineered vascular graft at 6 months of implantation

Conclusions: These results show that our system is capable of generating fully cellularized small diameter blood vessels combined with bilayered scaffolds and vascular cells. Dual cell-seeded PCL/collagen electrospun grafts maintained a high degree of patency and structural integrity without eliciting a histologic inflammatory response over the course of 6-month period in a sheep arterial interposition model. It is demonstrated that electrospun scaffolds in conjunction with vascular cells may be a clinically applicable alternative to traditional prosthetic vascular graft material.

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References:

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