Bilayered Scaffold for Engineering Fully Cellularized 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 [1,2]. 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 fabricated a vascular composite scaffold by electrospinning poly(ε-caprolactone) (PCL) and collagen. These composite scaffolds are designed to provide sufficient biomechanical properties and are configured to accommodate vascular endothelial cells (EC) and smooth muscle cells (SMC) for the use of vascular tissue engineering [3]. However, the small pore size of typical electrospun scaffolds may limit the penetration of SMC. In this study we developed engineered vessels utilizing a novel bilayered vascular scaffold which provides different pore sizes to enhance the cellular interactions of EC with the lumen and the infiltration of SMC into the outer layer.

Methods: Vascular composite scaffolds were fabricated by electrospinning with a polymer blend of PCL and type I collagen with the ratio of 1:1 in weight. The PCL/collagen solution was delivered through a blunt tip at a constant flow rate of 3 mL/hr by using a syringe pump. The mandrel was a stainless steel rod. The distance between the syringe tip and the mandrel was 10 cm and the rotating rate was 1000 rpm. For bilayered vascular scaffolds, primary electrospinning provided a small fiber diameter (<500 nm) for EC adhesion and secondary electrospinning provided a large fiber diameter (1 - 5 μm) for SMC infiltration. The bilayered scaffolds were examined by evaluating the EC adhesion and SMC infiltration and mechanical properties of the scaffold with different fiber diameters.

Results: Controlled variables of electrospinning include the solution concentration, flow rate, electric field strength, distance between tip and collector, needle tip design, and collector composition and geometry. These parameters can control the fiber morphology, diameter, and alignment. Figure 1 shows that the fiber diameter can be controlled using various parameters, as indicated. It is that the increase of fiber diameter of the vascular scaffold increases the pore area of the vascular scaffold.

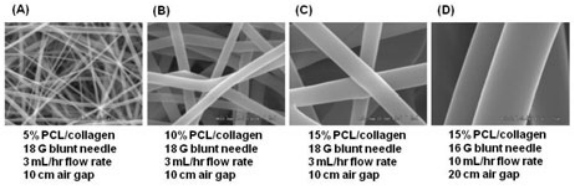

Figure 1. SEM images of electrospun scaffolds; (A) 500 nm, (B) 1.0 μm, (C) 2.5 μm, and (D) 5.0 μm (×10K). Fiber diameters can be controlled by various parameters, including solution concentration, size of needle, flow rate, and air gap.

Figure 2 shows that the cytoskeletal organization and focal adhesion of EC onto the electrospun scaffold with different fiber diameters. EC on nano-scaled fibers (0.5 μm) showed a better-developed cytoskeletal organization and focal adhesion compared to the others.

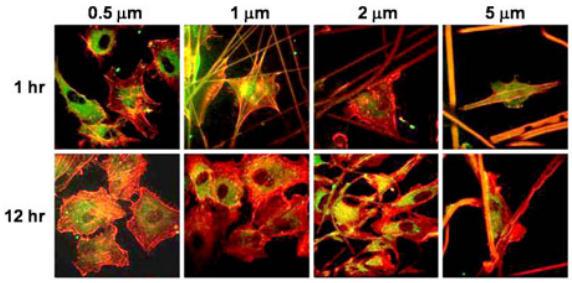

Figure 2. Cytoskeletal organization and focal adhesion of EC.

On the contrary, increasing fiber diameters of the scaffold increased SMC infiltration into the scaffold. Based on these results, we fabricated a bilayered vascular scaffold with different fiber diameters (Figure 3).

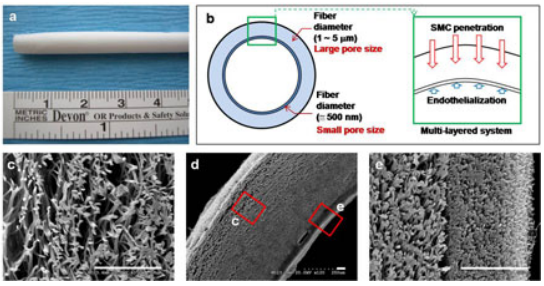

Figure 3. Bilayered vascular scaffolding system: (a) gross appearance, (b) illustration of bilayered scaffold with different fiber diameters, SEM images: cross-sectional (c) outer layer, (d) entire, and (e) interface between outer and inner layers of the vascular scaffolds. Scale bar indicates 50 μm.

Conclusions: We have developed a bilayered scaffold that can allow EC adhesion on the lumen and SMC infiltration into the outer layer. The scaffolds are biocompatible, biodegradable, easily fabricated and are able to support cell adhesion and infiltration. This study suggests that bilayered scaffolds facilitate endothelialization and smooth muscle maturation, which may result in improved vessel function and patency.

References:

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3. Lee SJ, et al. Development of a composite vascular scaffolding system that withstands physiological vascular conditions. Biomaterials 2008;29:2891