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Electrospun vascular scaffold for cellularized small diameter blood vessels: A preclinical large animal study

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ABSTRACT

The strategy of vascular tissue engineering is to create a vascular substitute by combining autologous vascular cells with a tubular-shaped biodegradable scaffold. We have previously developed a novel electrospun bilayered vascular scaffold that provides proper biological and biomechanical properties as well as structural configuration. In this study, we investigated the clinical feasibility of a cellularized vascular scaffold in a preclinical large animal model. We fabricated the cellularized vascular construct with autologous endothelial progenitor cell (EPC)-derived endothelial cells (ECs) and smooth muscle cells (SMCs) followed by a pulsatile bioreactor preconditioning. This fully cellularized vascular construct was tested in a sheep carotid arterial interposition model. After preconditioning, confluent and mature EC and SMC layers in the scaffold were achieved. The cellularized constructs sustained the structural integrity with a high degree of graft patency without eliciting an inflammatory response over the course of the 6-month period in sheep. Moreover, the matured EC coverage on the lumen and a thick smooth muscle layer were formed at 6 months after transplantation. We demonstrated that electrospun bilayered vascular scaffolds in conjunction with autologous vascular cells may be a clinically applicable alternative to traditional prosthetic vascular graft substitutes.

Statement of Significance

This study demonstrates the utility of tissue engineering to provide platform technologies for rehabilitation of patients recovering from severe, devastating cardiovascular diseases. The long-term goal is to provide alternatives to vascular grafting using bioengineered blood vessels derived from an autologous cell source with a functionalized vascular scaffold. This novel bilayered vascular construct for engineering blood vessels is designed to offer "off-the-shelf" availability for clinical translation.

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1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in the world. Approximately 17.3 million people died from CVD in 2008, and this number is estimated to reach to 23.3 million by 2030 [1]. In the United States alone, more than 570,000 coronary artery bypass grafts (CABG) are performed annually. Hence, significant improvement in the technology of vascular grafts is necessary to meet the clinical demand [2,3]. Segments of autologous vessels remain the standard for many revascularization procedures; however, autografts are limited in supply and dimensions, while allografts and xenografts are constrained by a high degree of the immunogenic responses [4–6]. Synthetic grafts have been used for over half a century. Expanded polytetrafluoroethylene (ePTFE) and Dacron (polyethylene terephthalate fiber) have been widely employed as large diameter (>5 mm) bypass conduits. Such synthetic grafts; however, have been shown to be unsatisfactory for replacing small-diameter (<5 mm) blood vessels due to the high frequency of thrombosis, stenosis, occlusion, and infection [7–10].

Tissue engineering strategy provides an attractive cell-based approach to vascular grafting. The basic tactic is to create an engineered vessel by combining autologous cells with a natural, synthetic, or hybrid tubular scaffold under appropriate culture conditions [11,12]. The tubular vascular scaffold should be able to withstand the tension and shear force and possess sufficient retention strength to endure suturing [13]. With these

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considerations in mind, we previously developed a nanofibrous vascular scaffold consisted of poly(ε-caprolactone) (PCL) and type I collagen using an electrospinning technique [14]. These electrospun PCL/collagen scaffolds showed excellent biomechanical properties and maintained their structural integrity under a continuous perfusion pulsatile bioreactor for up to 4 weeks *in vitro*. Further, after implantation *in vivo* in a rabbit aortoiliac bypass model, these scaffolds maintained their structural integrity with minimum host response [15]. However, the nano-scale pore size of electrospun scaffolds showed limited cellular infiltration. As the scaffold degrades, cells must remodel and replace the scaffold with their natural extracellular matrix (ECM) proteins. Therefore, we hypothesized that a fully cellularized vascular scaffold is required for vascular tissue maturation which can result in long-term patency. To accurately accommodate the vascular cells, we have further developed a novel bilayered scaffolding system with a small pore structure on the surface of the lumen to accelerate endothelialization, but a much larger pore structure in the bulk of the remaining vascular scaffold to enhance smooth muscle cell (SMC) infiltration [16]. Our previous outcomes suggested that bilayered vascular scaffolds could promote endothelialization and smooth muscle maturation for improved vascular tissue functions.

For accelerating the clinical translation, a clinically relevant large animal study is required. We have previously established a sheep carotid arterial interposition model for vascular tissue engineering [17]. Sheep have been used as a model for cardiovascular disease due to their anatomical and physiological similarities to humans [18]. Even though electrospinning technology offers a scaffolding system for vascular tissue engineering applications, no one has accomplished this in a preclinical large animal study due to limitations of cellularity and biomechanical properties of the electrospun scaffolds. We hypothesized that an efficient and stable coverage of endothelial cells (ECs) on the luminal surface and a thick smooth muscle layer of the vascular constructs could be used to replace damaged vessels. In this study, we investigated the ultimate feasibility of using this cellularized vascular scaffolding system for clinical translation.

2. Materials and methods

2.1. Vascular scaffold fabrication and characterizations

Bilayered tubular scaffolds were fabricated by electrospinning a polymer blend of poly(ε-caprolactone) (PCL) and type I collagen [16]. The scaffolds were electrospun using the polymer blend (1:1 weight ratio) of PCL (1.77 dL/g, Absorbable Polymers, Pelham, AL) and type I collagen (Elastin Products Co., Owensville, MO) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma-Aldrich, Co., St. Louis, MO). Briefly, 3 mL of 5% w/v PCL/collagen blend solution was electrospun through an 18-gauge blunt tip at 3 mL/h flow rate and 10 cm distance to form the inner fibrous structure. Subsequently, 750 μL of 18% w/v PCL/collagen blend solution was electrospun through a 16-gauge blunt tip at 10 mL/h flow rate and 15 cm distance to form the outer fibrous structure. The collecting stainless steel rod mandrel had a diameter of 4.75 mm, and the rotation rate was approx. 1000 rpm. After the electrospinning procedure, the PCL/collagen scaffolds were crosslinked using vapor of a 2.5% glutaraldehyde solution at room temperature for 8–10 h.

The fibrous structure of the bilayered scaffolds was observed under scanning electron microscopy (SEM; Hitachi Co. Ltd., Japan). The scaffolds were sputter-coated with gold (Hummer™ 6.2, Anatech Ltd, Denver, NC) to a thickness of 10–15 nm. SEM images were acquired using the SEM operating at an accelerating voltage of 25 kV with a 15-cm working distance. The images were analyzed with UTHSCSA ImageTool 3.0 (provided by the University of Texas Health Sciences Center at San Antonio, TX) to detect fiber diameters and pore areas of the scaffolds. Tensile properties of the scaffolds were measured using a uniaxial load test machine (Instron Co., Issaquah, WA) equipped with a maximum 1 kN load cell at a crosshead speed of 0.5 mm/s.

2.2. Primary vascular cell isolation and culture

To obtain ECs, circulating endothelial progenitor cells (EPCs) were isolated from Female Dorper Cross sheep (aged 3–4 months, Mocksville, NC) peripheral blood using a CD133-expressing cell purification system [19]. Captured cells were released from CD133-antibody with 0.5% trypsin treatment, and then plated on fibronectin-coated culture plates and cultured in EGM2 medium (Lonza, Walkersville, MD) until the appearance of endothelial colonies. The cells were continually cultured in the EGM2 medium and expanded for 3–5 passages. EPC-derived ECs were characterized with EC-specific markers, including von Willebrand factor (vWF, 1:200, DAKO, Copenhagen, Denmark), vascular endothelial cadherin (VE-cadherin, 1:50, Santa Cruz Biotechnology, Dalla, TX), and vascular endothelial growth factor receptor 2 (VEGFR-2 or Flk1, 1:50, Santa Cruz Biotechnology). To evaluate EC's functions, nitric oxide (NO) production and platelet adhesion were performed. For NO detection, supernatant at 1 and 2 days of EC culture was harvested and analyzed using an NO assay kit (abcam, Cambridge, MA) as directed by manufacturer's instruction. For the platelet adhesion test, sheep peripheral blood was collected, and blood platelets were isolated by centrifugation using Ficoll-Pague (Sigma-Aldrich). The isolated platelets were placed on the EC-cultured plates and incubated. After 30 min, non-adhered platelets were removed from EC-covered plates, and immunostaining for platelet IIb/IIIa complex (1:50, Santa Cruz Biotechnology) was performed to detect the platelet adhesion. Primary ECs obtained from a femoral artery biopsy served as a control.

Autologous SMCs were obtained from femoral artery biopsy. Briefly, vessel segments were washed with 1% antibiotics and antimycotics in phosphate-buffered saline (PBS). The adventitial layer was removed with a scalpel and minced into 1 mm^2, and then plated in culture plates. SMCs cultured in SmBM-2 medium supplemented with SmGM-2 single quotes (Lonza). These cells were subsequently cultured and expanded for 3–5 passages and immunostained with α-smooth muscle actin (α-SMA, 1:500, Santa Cruz Biotechnology).

2.3. Cell seeding and pulsatile bioreactor preconditioning

Electrospun PCL/collagen scaffolds were sterilized with ethylene oxide (EO). Before cell seeding, the scaffolds were hydrated using Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich Co.) for 3 h. Each end of the scaffold was fixed with syringe caps, and the scaffold was placed in a 100-mm tissue culture dish. Both ECs and SMCs were seeded onto the scaffolds, sequentially. Firstly, 1×10^8 cells/mL of SMC suspension were seeded on the outer surface of the scaffold and incubated at 37 °C, 5% CO_2 for 2 h. This procedure was repeated with three 90° rotation of the scaffold. After 1 day in culture, the SMC-seeded scaffolds were mounted in the pulsatile bioreactor device to provide constant rotation. Secondly, 2×10^6 cells/mL of ECs were seeded into the lumen of the scaffold and allowed cell attachment and homogeneous cell seeding while constant rotating at 37 °C in a CO_2 incubator for 24 h. The gravity was used to settle both cells on the scaffold. Following cell seeding, the vascular constructs were connected to a computer-operated gear pump (Ismatec MCP-Z Process, Glattbrugg, Switzerland) controlled by an Ismatec ProEdit program (V1.1.00). The program transitioned from steady to pulsatile flow for simulating arterial hemodynamic conditions (shear stress of

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