Materials Science & Engineering C 82 (2018) 121–129

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Fabrication and preliminary study of a biomimetic tri-layer tubular graft based on fibers and fiber yarns for vascular tissue engineering

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ARTICLE INFO

Keywords:
Tri-layer tubular graft
Fibers
Fiber yarns
Vascular tissue engineering

ABSTRACT

Designing a biomimetic and functional tissue-engineered vascular graft has been urgently needed for repairing and regenerating defected vascular tissues. Utilizing a multi-layered vascular scaffold is commonly considered an effective way, because multi-layered scaffolds can easily simulate the structure and function of natural blood vessels. Herein, we developed a novel tri-layer tubular graft consisted of Poly(L-lactide-*co*-caprolactone)/collagen (PLCL/COL) fibers and Poly(lactide-*co*-glycolide)/silk fibroin (PLGA/SF) yarns *via* a three-step electrospinning method. The tri-layer vascular graft consisted of PLCL/COL aligned fibers in inner layer, PLGA/SF yarns in middle layer, and PLCL/COL random fibers in outer layer. Each layer possessed tensile mechanical strength and elongation, and the entire tubular structure provided tensile and compressive supports. Furthermore, the human umbilical vein endothelial cells (HUVECs) and smooth muscle cells (SMCs) proliferated well on the materials. Fluorescence staining images demonstrated that the axially aligned PLCL/COL fibers prearranged endothelium morphology in lumen and the circumferential oriented PLGA/SF yarns regulated SMCs organization along the single yarns. The outside PLCL/COL random fibers performed as the fixed layer to hold the entire tubular structure. The *in vivo* results showed that the tri-layer vascular graft supported cell infiltration, scaffold biodegradation and abundant collagen production after subcutaneous implantation for 10 weeks, revealing the optimal biocompatibility and tissue regenerative capability of the tri-layer graft. Therefore, the specially designed tri-layer vascular graft will be beneficial to vascular reconstruction.

1. Introduction

Cardiovascular diseases are considered to be the leading cause of death globally [1,2]. Although the native vein and artery sections remain the best way to repair defected blood vessels *via* peripheral or coronary bypass procedures, their availability is still limited when the autologous blood vessels are occluded or diseased, or the size is not matched with the defected site [3]. Hence, it is urgently needed to develop clinically approved vascular prostheses as alternatives due to the morbidity and mortality caused by vascular diseases and disorders. Nowadays, some commercial artificial blood vessels such as Dacron or e-PTFE grafts have been commonly used for vascular repair [3]. However, the artificial grafts occurred failure for long-term patency and especially for the small-diameter vascular application, because the small-diameter vascular graft increased the danger of thrombosis and occlusion [4]. In the recent years, tissue-engineered scaffolds based on nanofibers have been developed and employed in different biomedical applications [5–12]. Based on the strategies of tissue engineering, large amounts of tissue-engineered vascular scaffolds with good biocompatibility, controllable mechanical properties, and manageable biodegradability have been designed [13–18]. Tissue-engineered vascular grafts can be easily manufactured because of the stability and

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http://dx.doi.org/10.1016/j.msec.2017.08.072
Received 18 June 2017; Received in revised form 13 August 2017; Accepted 16 August 2017
Available online 17 August 2017
0928-4931/

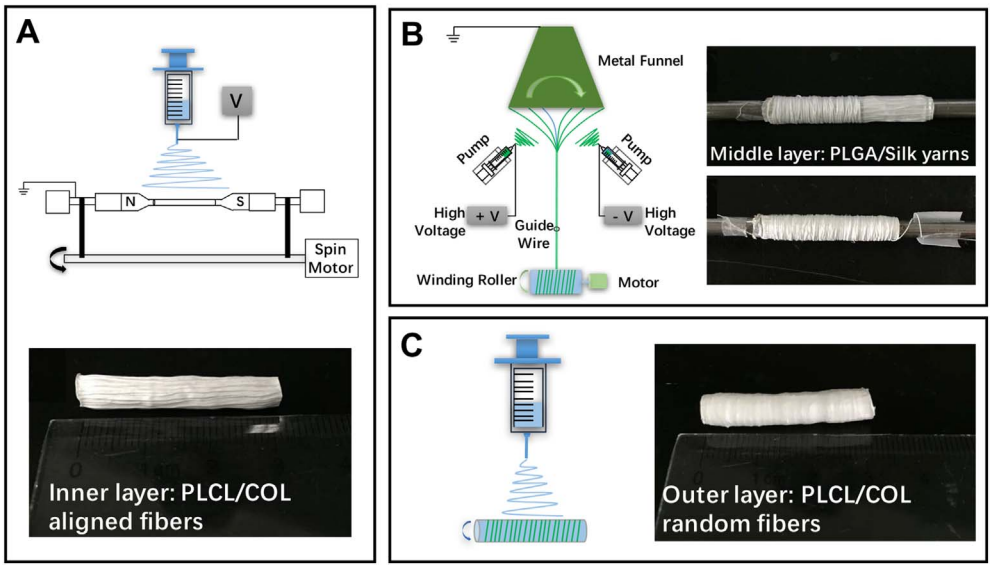

Fig. 1. The schematic diagram showing the fabrication of tri-layer tubular graft: (A) Fabrication of PLCL/COL axially aligned fibers (inner layer) *via* the general electrospinning method with a customized rotating collector; (B) Fabrication of the PLGA/SF yarns by a customized electrospinning equipment. The PLGA/SF yarns served as the middle layer by twining on the inner layer in circumferential orientation; (C) Fabrication of a thin layer of PLCL/COL random fibers (outer layer) on the prepared complex (inner and middle layers) to fix the whole layers.

maneuverability of the preparation technologies such as electrospinning, phase separation, freeze drying, and three-dimensional printing, *etc.* [19–22].

To biomimetic the structure and function of native blood vessels, designing multi-layered vascular scaffolds is an effective way. Electrospinning has been widely used, because it is easier to blend or mix various materials and build non-delamination layers to develop multi-layered scaffolds [23–30]. McClure et al. fabricated a three-layered electrospun matrix to mimic native arterial architecture using polycaprolactone, elastin, and collagen [27]. The results indicated that the graft had sufficient tensile strength, dynamic compliance, suture retention, and burst strength by altering layer properties. Then, they further developed a tri-layered vascular graft composed of polycaprolactone, elastin, collagen, and silk [28]. The results revealed the optimization of graft properties and concluded that the multi-layered graft architecturally mimicked the native vascular wall and mechanically matched the gold standard of vessel replacement, saphenous vein [28]. Zhang and Han et al. designed multi-layered small-diameter vascular scaffolds which had dual-loading of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) [23,24]. The grafts synergistically regulated the growth of vascular endothelia cells in lumen and smooth muscle cells on the exterior through the controlling of VEGF/PDGF releasing. Valence et al. compared two bilayered vascular grafts with different porosity and evaluated their potential for surgical applicability and tissue regeneration [25]. The results demonstrated that the graft with a low-porosity layer on the lumen and a high-porosity layer on the adventitial side reduced blood leakage, promoted cell invasion from the surroundings, and did not affect the endothelialization rate. Therefore, it is of foremost importance to design a multi-layered microarchitecture for biodegradable vascular prostheses.

The endothelium in the native blood vessel is a simple but well-organized monolayer, and the orientation of endothelia cells can regulate biological signaling events including intracellular protein expression, cytoskeleton construction, and cell-to-cell interactions [31–34]. Correspondingly, the smooth muscle cells are spindle-shaped and aligned their long axis perpendicular to the blood vessel length, which plays an important role in maintaining elasticity, mechanical strength, and vasoactive responsiveness of blood vessels [35–38]. To simulate the tri-layer structure of the native blood vessel and the function of vascular lumen and media, a novel tri-layered vascular graft was designed and fabricated through a three-step electrospinning method in this study. The graft consisted of axially aligned Poly(L-lactide-*co*-caprolactone)/collagen (PLCL/COL) fibers in lumen, circumferentially oriented Poly(lactide-*co*-glycolide)/silk fibroin (PLGA/SF) yarns in media, and random PLCL/COL fibers in adventitia. Then, the mechanical properties, endothelia cells and smooth muscle cells proliferation and morphology, and *in vivo* evaluation of subcutaneous implantation in mice were assessed to determine the potential application of the tri-layer vascular graft for vascular tissue engineering.

2. Materials and methods

2.1. Materials

Poly(L-lactide-*co*-caprolactone) (PLCL, LA:CL = 50:50; Mn: 450,000) was purchased from Jinan Daigang Biomaterial Co., Ltd. (Jinan, China). Porcine-derived type I collagen (Mn: 100,000) was supplied by ChengDu Kele Bio-tech Co., Ltd. (Chengdu, China). 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was acquired from Shanghai Fine Chemicals Co., Ltd. (Shanghai, China). Poly(lactide-*co*-glycolide) (PLGA, LA:GA = 82:18; IV(dl/g): 1.9) was supplied by Jinan Daigang Biomaterial Co., Ltd. (Jinan, China). *Bombyx mori* silkworm cocoons were supplied by Jiaxing Silk Co. Ltd. (China) and the regenerated silk fibroin (SF) was prepared as previously reported [39]. Glutaraldehyde aqueous solutions (GA, 25%) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Human Umbilical Vein Endothelial Cells (HUVECs) and smooth muscle cells (SMCs) were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). All the cell culture reagents were provided by Gibco Life Technologies Co., (USA) unless specified.

2.2. Fabrication

PLCL/collagen (PLCL/COL) blends were dissolved in HFIP with a weight ratio of 3:1 and at a concentration of 10%. PLGA/Silk (PLGA/SF) composites were dissolved in HFIP with a weight ratio of 3:1 and at a concentration of 15%. The tri-layer tubular graft was fabricated *via* three steps. Firstly, the axially aligned PLCL/COL fibers in inner layer were fabricated by placing a collector in a magnetic field generated from N-poles to S-poles (Fig. 1A). A custom-made Teflon conduit mold served as the collector (the diameter was 4 mm, the length was 3 cm, and the rotating speed was 50 rpm). A high voltage of 12 kV, a flow rate of 1.0 mL/h, and a collect distance of 12 cm were applied, and the axially aligned PLCL/COL fibers were collected because of the axial magnetic environment in the electrospinning process. The PLGA/SF yarns were pre-prepared *via* an electrospinning equipment with double-nozzle system (TFS-700, Beijing Technova Technology Co., Ltd., China)