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Circumferentially aligned fibers guided functional neoartery regeneration *in vivo*

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ABSTRACT

An ideal vascular graft should have the ability to guide the regeneration of neovessels with structure and function similar to those of the native blood vessels. Regeneration of vascular smooth muscle cells (VSMCs) with circumferential orientation within the grafts is crucial for functional vascular reconstruction *in vivo*. To date, designing and fabricating a vascular graft with well-defined geometric cues to facilitate simultaneously VSMCs infiltration and their circumferential alignment remains a great challenge and scarcely reported *in vivo*. Thus, we have designed a bi-layered vascular graft, of which the internal layer is composed of circumferentially aligned microfibers prepared by wet-spinning and an external layer composed of random nanofibers prepared by electrospinning. While the internal circumferentially aligned microfibers provide topographic guidance for *in vivo* regeneration of circumferentially aligned VSMCs, the external random nanofibers can offer enhanced mechanical property and prevent bleeding during and after graft implantation. VSMCs infiltration and alignment within the scaffold was then evaluated *in vitro* and *in vivo*. Our results demonstrated that the circumferentially oriented VSMCs and longitudinally aligned ECs were successfully regenerated *in vivo* after the bi-layered vascular grafts were implanted in rat abdominal aorta. No formation of thrombosis or intimal hyperplasia was observed up to 3 month post implantation. Further, the regenerated neoartery exhibited contraction and relaxation property in response to vasoactive agents. This new strategy may bring cell-free small diameter vascular grafts closer to clinical application.

1. Introduction

The small diameter vascular grafts (SDVGs) are in considerable need for clinical replacement of damaged and blocked blood vessels [1]. The native blood vessels consist of tunica intima, media and adventitia from inner to outer with distinct patterns. The tunica intima is an antithrombogenic monolayer with longitudinally aligned endothelial cells (ECs). The tunica media contains multiple layers of vascular smooth muscle cells (VSMCs) and ECMs (mainly collagens and elastin) that are circumferentially aligned, which plays a crucial role in maintaining the mechanical strength and vasoactive responsiveness of the blood vessels [2]. Therefore, the ideal vascular grafts should have potential ability to regenerate new blood vessels with structure and function similar to those of the native blood vessels [3].

Cell alignment has been widely observed at various scales in native tissues and organs [4], which plays a critical role in maturation and regeneration of functional tissue. As reported, cell alignment could be achieved *in vitro* by means of the guidance role of various geometric cues including nano or micro-sized fibers [5,6] and channels [7,8]. For instance, based on the structural feature of native blood vessel, Jiang et al. developed a stress-induced rolling

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membrane technique to fabricate tubular structures, in which VSMCs and ECs location and orientation could be precisely controlled. Using this approach, tubular grafts with longitudinally aligned endothelial cells and circumferentially aligned VSMCs were successfully obtained [7]. Moreover, Jone et al. prepared the circular microchannel embedded in the curvature surface of tubular structure by micro-fabrication technology, which realized circumferential alignment of human aortic smooth muscle cells (HASMCs) during *in vitro* culture [9]. Recently, Huang et al. prepared a composite tubular scaffold with circumferentially aligned poly (L-lactic acid) (PLLA) nanofibers in the inner surface. Their results indicated that the aligned PLLA fibers could guide VSMCs' orientation *in vitro* [10]. The above studies have demonstrated that VSMCs were aligned and elongated along the direction of geometric cues, and had significant advance in methodology for the design and fabrication of vascular graft which could regulate orientation and function of VSMCs *in vitro*. However, most of these studies were limited to *in vitro* model.

Besides the VSMCs circumferential alignment, infiltration of VSMCs into the interior of the vascular scaffolds is another key factor for the regeneration of small-diameter blood vessels. For this objective, suitable pore size and highly interconnected porous structure of the scaffolds are essentially important [11,12]. Up to date, the vascular scaffolds with different pore structure have been developed by various techniques such as phase separation [13], salt leaching [14], electrospinning [15,16] and so on. For instance, He et al. fabricated a bi-layered elastomeric porous vascular scaffolds by thermally induced phase separation method and the VSMCs could infiltrate into the wall of the grafts *in vivo* [13.] Wu et al. reported that a porous tubular poly (glycerol-sebacate) scaffolds fabricated by salt leaching method could realize sufficient cell infiltration *in vivo* [14]. In our previous study, the electrospun poly (ε-caprolactone) (PCL) vascular grafts with thick fibers and large pore obviously enhanced cell infiltration into the grafts wall compared with the grafts of thin fibers [16]. Despite some encouraging results have been obtained, the regenerated VSMCs in these works arranged randomly due to the lack of well-defined aligned geometric cues. How to design and fabricate a vascular graft with well-defined aligned geometric cues to realize simultaneously VSMCs infiltration and circumferential alignment *in vivo* remains a great challenge [17]. To our knowledge, there are no reports on this issue, particularly *in vivo* [18]. Numerous reports have demonstrated that the host is not only a superior source of cells and but also an all-round "bioreactor" [18], the host self-remodeling capability could promote *in vivo* neoartery regeneration from the cell-free vascular grafts [14,19]. It suggests that the ideal vascular grafts should be well adapted to implantation environment, and coordinate with the host self-remodeling ability to enhance vascular regeneration.

Wet-spinning is a simple and straightforward method for fabricating fibrous scaffolds with fibers in microscale [20]. In wet-spinning process, the polymer solution is extruded into a coagulation bath to precipitate the polymer in the form of a fiber because of solvent diffusion. The polymer fibers are collected onto collectors to form fibrous scaffolds. Wet-spun microfibers have attracted considerable interest as a scaffold matrix to guide and direct the behavior of cells for a variety of applications, such as cartilage, tendon and ligament tissue engineering [21]. However, there are few reports about the *in vivo* application of vascular grafts fabricated using wet-spinning. Additionally, PCL is a non-toxic, biocompatible polyester biomaterial that has been used to fabricate small diameter vascular grafts [22].

Inspired by the results mentioned above, we hypothesize that simultaneous VSMCs infiltration and circumferential alignment could be achieved under the guidance of grafts with circumferentially oriented microfibers, and the regenerated VSMCs could facilitate endothelium formation and its maturation upon implantation in rat abdominal aorta (Fig. 1a). In this study, we designed a bi-layered vascular graft. The internal layer is composed of circumferentially aligned microfiber prepared by wet-spinning, which could provide a topographic guidance for the regeneration of circumferentially aligned VSMCs. The external layer is composed of random nanofibers prepared with electrospining method in order to enhance the mechanical property and prevent bleeding during implantation. Furthermore, we evaluated cell infiltration and alignment in the scaffolds *in vitro*, and assessed the feasibility of the circumferentially oriented regeneration of VSMCs *in vivo* by implantation of the bi-layered grafts into rat abdominal aorta. The goal of this study was to develop a small-diameter vascular graft with the ability to induce the regeneration of circumferentially aligned VSMCs and thus mimic the organization of native blood vessels.

2. Materials and methods

2.1. Materials

Poly (ε-caprolactone) (PCL) pellets (Mn = 70,000–90,000) were purchased from Sigma (USA). Analytical reagents including chloroform, tetrahydrofuran, trifluoroethanol and hexane were

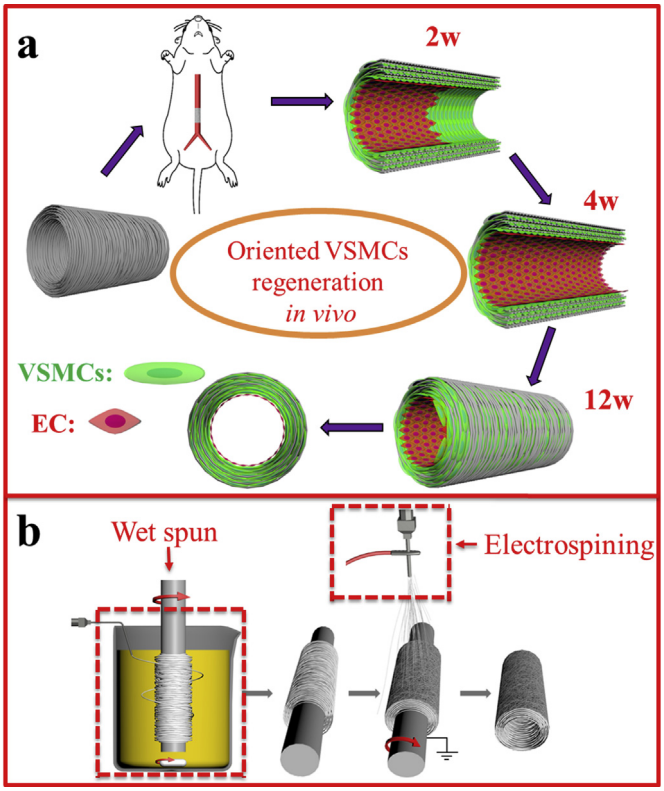

Fig. 1. Schematic illustration showed the hypothesis and fabrication process in the present study. (a) Our hypothesis is that the circumferentially aligned microfibers of the grafts could guide VSMCs regeneration in circumferential orientation within the internal layer of the grafts *in vivo*, and complete endothelialization could be achieved rapidly. VSMC is abbreviation of vascular smooth muscle cell; EC is abbreviation of endothelial cell. (b) The fabrication process of vascular grafts. The internal layer composed of circumferentially oriented fibers which provide a topographic guidance for VSMCs regeneration was firstly prepared by wet-spinning method. Then the external layer composed of randomly oriented nanofibers was prepared by electrospinning method, which could enhance the mechanical property of the grafts and prevent from bleeding in implantation.