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Combining cell sheet technology and electrospun scaffolding for engineered tubular, aligned, and contractile blood vessels

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ABSTRACT

Herein we combine cell sheet technology and electrospun scaffolding to rapidly generate circumferentially aligned tubular constructs of human aortic smooth muscles cells with contractile gene expression for use as tissue engineered blood vessel media. Smooth muscle cells cultured on micropatterned and N-isopropylacrylamide-grafted (pNIPAm) polydimethylsiloxane (PDMS), a small portion of which was covered by aligned electrospun scaffolding, resulted in a single sheet of unidirectionally aligned cells. Upon cooling to room temperature, the scaffold, its adherent cells, and the remaining cell sheet detached and were collected on a mandrel to generating tubular constructs with circumferentially aligned smooth muscle cells which possess contractile gene expression and a single layer of electrospun scaffold as an analogue to a small diameter blood vessel's internal elastic lamina (IEL). This method improves cell sheet handling, results in rapid circumferential alignment of smooth muscle cells which immediately express contractile genes, and introduction of an analogue to small diameter blood vessel IEL.

1. Introduction

The current gold standard in coronary artery bypass surgery is a blood vessel autograft where a vessel explanted from the patient is attached to the diseased artery in order to reroute blood around the obstruction and restore blood flow to the heart. However, this strategy is suboptimal due to second site morbidity, a limited supply of autografts, and loss of patency [1–5]. Similarly, the use of synthetic grafts in coronary artery (and other small diameter arteries) augmentation is clinically unsuccessful due to loss of patency which occurs due to thrombus accumulation on the lumen of the graft [6–8]. To address the current limitations in treating small diameter blood vessel disease, researchers in the medical community have strived to develop a tissue engineered small diameter artery for more than two decades [9–11].

Early approaches towards blood vessel tissue engineering are termed "top down" approaches and are based on seeding cells on porous scaffolds or embedding cells in hydrogels to support the cells and achieve the formation of tubular tissue engineered constructs. However, the archetypal approach of using biodegradable scaffolds to provide initial strength for the newly constructed vessels raises concerns about foreign body reaction, inflammation and infection due to bacterial colonization [12–15]. Furthermore, the blood vessel is a multi-layered and three dimensional tissue, and the successful function of the tissue is based on the complex anatomy of the vessel [16]. Recapitulating the complex structure of the blood vessel through top down tissue engineering techniques and generating clinically successful grafts for bypass surgery have yet to be achieved.

"Bottom up" tissue engineering strategies were later developed. In this approach, individual sections of the tissue are generated and these sections are then brought together to generate a tissue engineered construct which replicates native tissue structure with higher fidelity. Furthermore, bottom up approaches tend to be scaffold free which promises less inflammation and toxicity which may occur due to scaffold degradation [17–21].

A scaffold-free and bottom up approach to human blood vessel tissue engineering – cell sheet tissue engineering – was introduced more than a decade ago by L'Heureux et al. [21]. In this technique non-aligned sheets of smooth muscle cells (SMCs) are cultured and rolled over a mandrel to create the medial layer of the blood vessel, sheets of non-aligned fibroblasts are then rolled outside SMCs to create the outer layer of the blood vessel (tunica adventitia), and

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finally endothelial cells (ECs) are seeded inside the tubular structure and grown to confluence creating a structure resembling inner layer of a blood vessel (tunica intima). The constructs then undergo maturation in a pulsatile bioreactor during which time the fibroblasts and SMCs circumferentially align and generate their own extracellular matrix (ECM) resulting in a fully biological tissue engineered blood vessel with appropriate strength to prevent aneurysm and rupture. Also during this maturation step the SMCs differentiate from synthetic to contractile phenotype which is necessary for the vessel to possess appropriate contractility. Though this research represents a major step forward in blood vessel tissue engineering, it suffers from several major limitations: 1) achieving appropriate circumferential cell alignment and necessary contractile phenotype of smooth muscle cells required a maturation time of up to 3 months limiting this technique's clinical applicability, 2) individual cell sheets were incredibly delicate and can be easily damaged during handling and construction of the tissue engineered blood vessel, and 3) cell senescence may occur during the lengthy maturation of the tissue engineered vessel [22,23].

We hypothesize that by using a combination of bottom up and top down tissue engineering techniques, we can address the above-mentioned limitations of cell sheet tissue engineering and produce a tubular construct of circumferentially aligned smooth muscle cells which shows higher expression of contractile genes and enough mechanical strength to enable facile cell sheet handling within days [24]. A schematic which illustrates our proposed cell culture method is presented in Fig. 1. Human aortic smooth muscle cells (AoSMCs) will be cultured on a thermoresponsive NIPAm-grafted PDMS substrate which is micropatterned with discontinuous walls which fosters cell alignment and contractile gene expression of the smooth muscle cells [25]. A small section of this cell culture surface will be covered with an aligned electrospun scaffold of polycaprolactone (PCL) which also induced alignment of the adherent cells (Fig. 2A and B). Upon cooling, the cells will detach from the NIPAm-grafted surface but not the electrospun scaffold. The scaffold, along with its adherent cells, followed by the unsupported cell sheet will then be wrapped around a mandrel to produce the tissue engineered blood vessel media.

2. Materials and methods

2.1. Electrospun nanofiber scaffold preparation

Polycaprolactone (PCL) (molecular weight 65,000, Sigma Aldrich, USA) was dissolved in trifluoroethanol (TFE) (≥99.0 Fluka, China) to obtain a 10% solution. The polymer solution was loaded in a 2 mL syringe fixed with a 30 gauge needle. The syringe was placed in a syringe pump and polymer solution was expelled from the syringe at a flow rate of 1.0 mL/h (New Era pump systems Inc. USA). The distance between the grounded rotating drum and the needle was 12 cm. High DC voltage was applied to the polymer mixture (GAMMA high voltage research, USA) at a voltage of +10 for the needle and −3 kV for the collector. The electrospun fibers

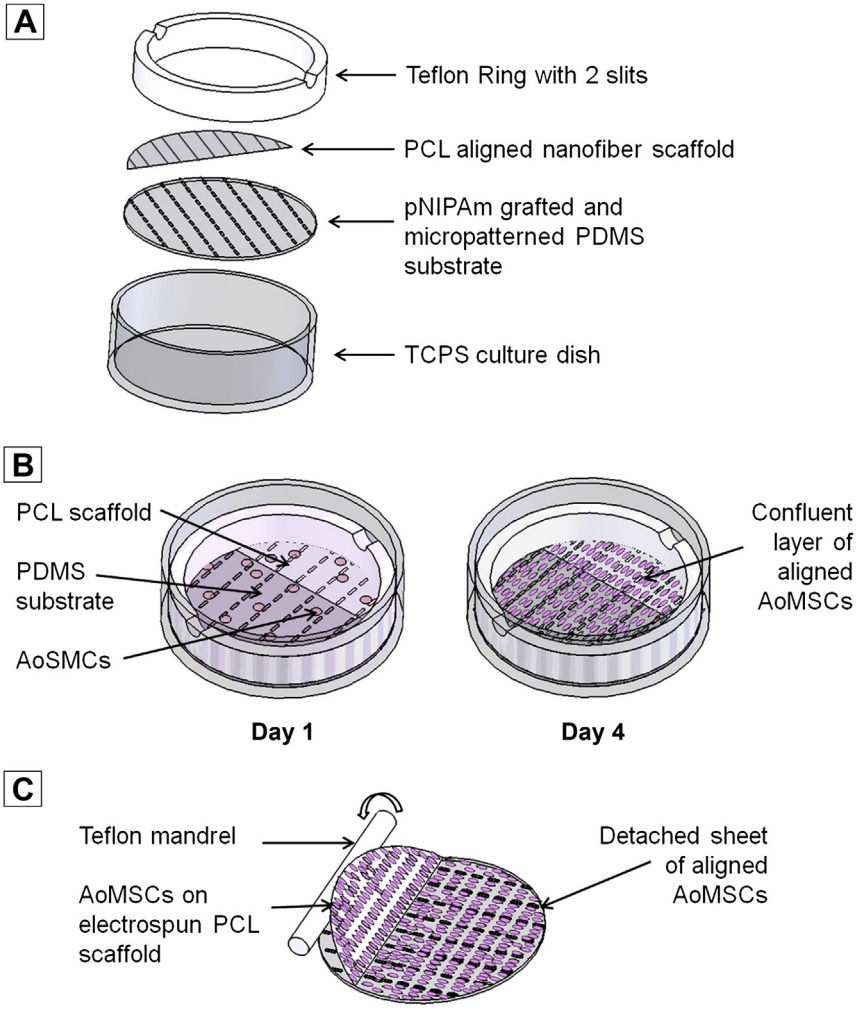

Fig. 1. Schematic of scaffold-assisted cell sheet engineering of vascular media: (A) Assembly of cell culture surface which combines aligned and electrospun PCL scaffolding with thermoresponsive and micropatterned PDMS, (B) alignment of cells during 4 days of culture, and (C) rolling nanofiber scaffold and aligned cell sheet over the mandrel to generate tubular constructs. (For the size of 6 well plates the mandrel diameter is 3 mm.)