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Biomimetic microenvironment complexity to redress the balance between biodegradation and *de novo* matrix synthesis during early phase of vascular tissue engineering

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ABSTRACT

Physiological functionality of a tissue engineered vascular construct depends on the phenotype of smooth muscle cells (SMCs) cultured into the scaffold and mechanical robust of the construct relies on two simultaneous mechanisms including scaffold biodegradation and *de novo* matrix synthesis by SMCs which both can be influenced by scaffold properties and culture condition. Our focus in this study was to provide an appropriate environmental condition within tissue engineering context to meet foregoing requisites for a successful vascular regeneration. To this end, SMCs seeded onto electrospun Tecophilic/gelatin (TP(70)/gel(30)) scaffolds were subjected to orbital shear stress. Given the improvement in mechanical properties of dynamically stimulated cell-seeded constructs after a span of 10 days, effect of fluctuating shear stress on scaffold biodegradation and SMC behavior was investigated. Compared to static condition, SMCs proliferated more rapidly and concomitantly built up greater collagen content in response to dynamic culture, suggesting a reasonable balance between scaffold biodegradation and matrix turnover for maintaining the structural integrity and mechanical support to seeded cells during early phase of vascular tissue engineering. Despite higher proliferation of SMCs under dynamic condition, cells preserved nearly spindle like morphology and contractile protein expression likely thanks to composition of the scaffold.

1. Introduction

To overcome the limitation on the availability of vascular autografts and the disappointing consequence of synthetic vascular grafts, tissue engineering techniques are developed for the fabrication of vascular replacements [1]. Tissue engineering is a promising implication based on exploitation of the regenerative potential of cells using scaffolds that provide cell signaling cues and mimic the native extracellular matrix (ECM). Native blood vessel wall is a complex multi-layered construction composed of different types of specialized cells and proteins [2,3]. The function of endothelial cell (EC) monolayer in the lumen of the blood vessel is preventing the clotting of the blood and infection and inflammation of adjacent tissues [4], while the SMCs in the middle layer (media) have a unique contractile function. SMCs contract and regulate the blood vessel tone-diameter, blood pressure, and blood flow distribution [5,6]. SMCs are also responsible for synthesizing the ECM of the medial layer which plays a key role in mechanical behavior of the native vessel [6–8].

A successful tissue-engineered vascular graft shall avoid thrombogenicity and promote tissue regeneration for full functional recovery [9]. In this regard, two main strategies are generally employed for cell and scaffold- based vascular tissue engineering in vitro; (i) providing the necessary mechanical properties by a rational design of the scaffold and seeding ECs on its surface forming a confluent EC monolayer to inhibit thrombosis, (ii) seeding SMCs into the scaffold composed of hemocompatible materials and controlling various factors to modulate phenotypic behavior of SMCs. The absence of SMCs in the first strategy limits the contractile response of the graft [10] and production of collagen and elastin which might lead to the mechanical loss of the biodegradable graft after its implantation. Therefore, the second method seems to have more potential for fabrication of a successful tissue-engineered vascular graft. Nanofibrous materials with similar architecture, size scale and composition to the native ECM present suitable candidates for vascular scaffolding. Various methods have been applied for the preparation of nanofibers, among which electrospinning remains the most popular and versatile technique due to its simplicity offering many opportunities to address the different requirements of the target tissue.

Previously, we fabricated electrospun Tecophilic/gelatin (TP/gel) scaffolds and investigated the effects of scaffold stiffness and ligand

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density on phenotypic behavior of SMCs. Results suggested a blend ratio of 70:30 for TP:gel as the optimal composition (TP(70)/gel(30)) to obtain a relatively contractile phenotype of SMCs [11]. Further, we showed that the hydrophilic property of the composite scaffold induces non-thrombogenicity and its mechanical properties guarantee similarity in the mechanical behavior of the tubular scaffold to that of native vessels [12]. However, the success of a functional vascular graft is dependent on the phenotypic shifts of SMCs at the appropriate development stages using tissue engineering techniques [8]. SMCs possess remarkable plasticity that allows reversible changes in their phenotype, i.e.; between a contractile state and a synthetic state in response to alterations in local environmental cues, which plays a crucial role in vascular repair and remodeling [13]. The phenotypic plasticity of SMCs is potentially exploitable for tissue engineering of vascular grafts [8]. Synthetic phenotype of SMCs is needed during early stage to allow cells to proliferate rapidly, synthesize *de novo* ECM proteins and remodel the matrix. However, synthetic SMCs do not possess vasoactivity and their proliferation in the implanted graft leads to vessel wall thickening and narrowing of the vessel lumen, resulting in graft failure due to intimal hyperplasia and restenosis. Therefore, SMCs should then switch to a quiescent and contractile phenotype [8,14,15].

We aimed at investigating the hypothesis that oscillatory shear stress caused by dynamic culture condition can influence the behavior of SMCs seeded on electrospun TP(70)/gel(30) scaffolds. In this way, we aim to fulfil the phenotypic requirement of SMCs during the early phase of vascular tissue engineering. We further examined the effect of scaffold biodegradation and *de novo* matrix synthesis by SMCs on mechanical properties of the scaffolds under static and dynamic culture conditions during early days (10 days) of vascular regeneration process. We have meanwhile evaluated the effect of in vitro degradation on physical and mechanical properties of cell-free TP(70)/gel(30) TP(70)/gel scaffolds under static and dynamic conditions.

2. Materials and methods

2.1. Electrospun nanofibrous scaffold fabrication

Nanofibrous TP/gel scaffolds were fabricated by conventional electrospinning as described previously [11,12]. Briefly, TP (Lubrizol) and gelatin type A (Sigma Aldrich, 300 Bloom) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma Aldrich) to obtain a total concentration of 8% (*w*/*v*). The TP:gel weight ratio was kept constant at 70:30. The polymeric solution was ejected via a syringe pump at a flow rate of 1 ml h^{-1} through a spinneret charged to +10 kV generating nanofibers that were collected on a grounded collector at 12 cm from the spinneret tip. As demonstrated in earlier works and shown in Fig. 1(A), this results in the formation of randomly oriented nanofibers with an average fiber diameter of 409 ± 150 nm for electrospun TP(70)/gel(30) TP(70)/gel scaffolds [11,12].

2.2. Assays on cell-free scaffolds (acellular scaffolds)

2.2.1. Weight loss

Electrospun scaffolds were cut into 20 × 20 mm^2 square sheets, weighed (W_0) and divided into two groups. Subsequently, the specimens of each group were subjected to degradation under either static or dynamic conditions by incubation in smooth muscle cell medium (SMCM, Sciencell Research Laboratories) for 4, 7 and 10 days ($n = 3$ per group per time point) in a humidified atmosphere in 5% CO_2 at 37 °C. SMCM medium was changed every 3 days. Dynamic condition was applied by orbital shaker (Stuart Scientific) at 150 rpm. After each specific time point, the degraded samples were rinsed in PBS and dried using a vacuum desiccator. The weight of dried samples was determined (W_d) and the percentage weight loss was calculated as $100 \times (W_0 - W_d) / W_0$. The weight loss of the specimens subjected to in vitro degradation over 24 h under static condition was also determined.

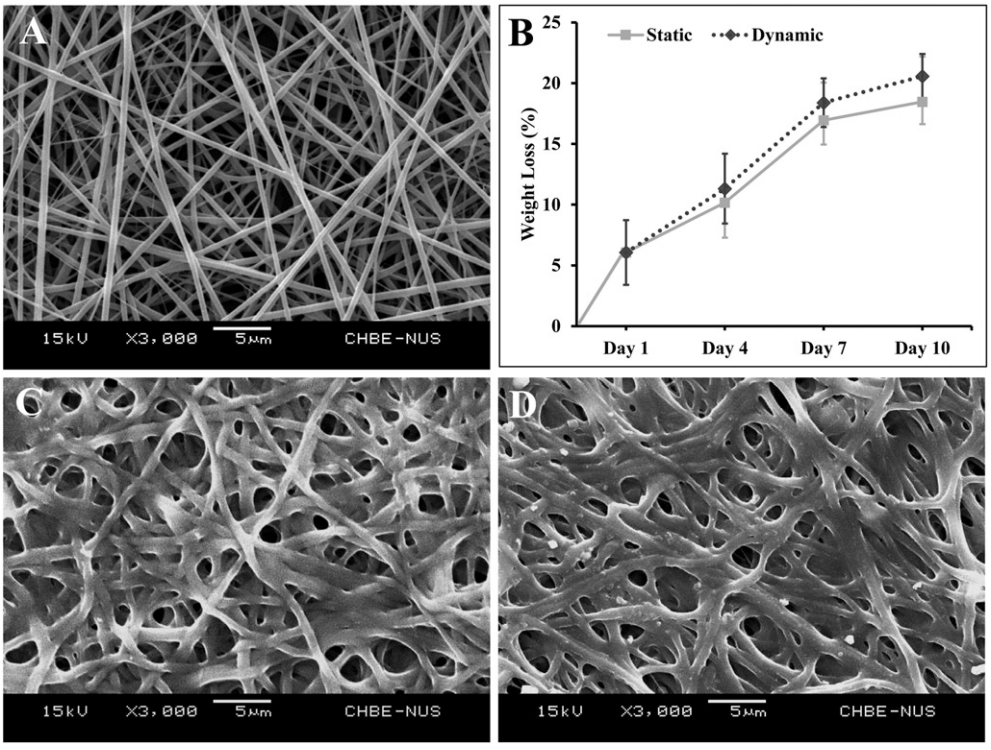

Fig. 1. (A) Morphology of electrospun TP(70)/gel(30) scaffold. (B) Degradation curves of scaffolds under static and dynamic conditions. Morphology of (C) statically and (D) dynamically degraded electrospun TP(70)/gel(30) scaffolds.