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Electrically conductive nanofibers with highly oriented structures and their potential application in skeletal muscle tissue engineering

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ABSTRACT

Recent trends in scaffold design have focused on materials that can provide appropriate guidance cues for particular cell types to modulate cell behavior. In this study highly aligned and electrically conductive nanofibers that can simultaneously provide topographical and electrical cues for cells were developed. Thereafter their potential to serve as functional scaffolds for skeletal muscle tissue engineering was investigated. Well-ordered nanofibers, composed of polyaniline (PANi) and poly(ε-caprolactone) (PCL), were electrospun by introducing an external magnetic field in the collector region. Incorporation of PANi into PCL fibers significantly increased the electrical conductivity from a non-detectable level for the pure PCL fibers to 63.6 ± 6.6 mS cm^{-1} for the fibers containing 3 wt.% PANi (PCL/PANi-3). To investigate the synergistic effects of topographical and electrical cues using the electrospun scaffolds on skeletal myoblast differentiation, mouse C2C12 myoblasts were cultured on random PCL (R-PCL), aligned PCL (A-PCL), random PCL/PANi-3 (R-PCL/PANi) and aligned PCL/PANi-3 (A-PCL/PANi) nanofibers. Our results showed that the aligned nanofibers (A-PCL and A-PCL/PANi) could guide myoblast orientation and promote myotube formation (i.e. approximately 40% and 80% increases in myotube numbers) compared with R-PCL scaffolds. In addition, electrically conductive A-PCL/PANi nanofibers further enhanced myotube maturation (i.e. approximately 30% and 23% or 15% and 18% increases in the fusion and maturation indices) compared with non-conductive A-PCL scaffolds or R-PCL/PANi. These results demonstrated that a combined effect of both guidance cues was more effective than an individual cue, suggesting a potential use of A-PCL/PANi nanofibers for skeletal muscle regeneration.

1. Introduction

Tissue engineering is an advanced interdisciplinary field that includes the design of artificial implant scaffolds for in vivo tissue regeneration [1]. Because the interactions between cells and biomaterial substrates play an important role in regulating cellular behavior and performance, much effort has been made to control cell responses by varying the topography, three-dimensional (3-D) geometry, or chemical composition of biomaterial scaffolds. Recent trends in scaffold design have focused on materials that are biomimetic and tissue-specific to provide appropriate guidance cues that mimic the native microenvironment [2,3].

Electrospun nanofibrous scaffolds have received much attention in tissue engineering because of their tunable porosities, large surface area to volume ratios, and similar physical structures to natural extracellular matrices (ECMs). In addition, the geometrical, mechanical, chemical, and electrical properties of the constituted fibers can be easily controlled to achieve the desired requirements and functionality. Recent publications have reported the development of functional nanofibrous scaffolds that provide various guidance cues, such as topographical [4], electrical [5], or biochemical cues [6,7], for specific cell types to promote cell function and tissue regeneration.

For certain applications in tissue engineering scaffolds with aligned patterns are useful to guide cell growth with the desired anisotropy [8]. Highly oriented electrospun nanofibers can be easily obtained using various collector designs [9,10]. Research groups have demonstrated that electrospun scaffolds composed of aligned nanofibers are beneficial topographical cues that enhance the extension and direct the out-growth of osteoblasts [5], cardiac myocytes [11], vascular cells [12], neural cells [13], and skeletal muscle cells [8] through contact guidance.

In addition to topographical cues, electrical signals are important stimuli that can control the adhesion and differentiation of certain cell types [14]. Electrospinning is a simple and effective technique to generate electrically active fibers by blending conductive materials such as polypyrrole [15], polyaniline (PANi) [16], or carbon nanotubes into polymers [5]. This electrically conductive nanofiber has been demonstrated to be a promising scaffold to electrically stimulate muscle [16], cardiac [17], and bone tissue [5].

Experimental research using functional nanofibers has provided valuable information regarding cell responses to individual guidance cues. However, the local environment that in vivo growing cells experience is inherently complex and contains a rich mixture

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of cues. Providing cells with the appropriate and multiple environmental stimuli is essential to the success of the tissue engineering approach. Patel et al. demonstrated that a bioactive and patterned nanofiber scaffold provides both topographical and biochemical cues to induce, enhance, and guide neurite out-growth and skin cell migration [18]. Yao et al. reported that surface micropatterned scaffolds with conjugated functional molecules can be used to guide neurite growth [19]. In this study, a nanofibrous scaffold that could simultaneously provide two types of guidance cues, electrical and topographical, to cells was proposed. This scaffold was composed of electrically conductive nanofibers with highly oriented structures, and was fabricated by electrospinning a blended solution of poly(ε-caprolactone) (PCL) and PANi.

PCL was chosen as the core material because of its good biodegradability and biocompatibility, and because its mechanical properties are suitable for tissue engineering [20,21]. Conductive PANi has been explored as a scaffold for cardiac and neuronal tissue engineering and its biocompatibility has been demonstrated by both in vitro and in vivo analyses [22–25]. To generate well-aligned nanofibers two parallel block magnets were used as the electrospinning collectors (Fig. 1). The practicality of using an external magnetic field to align and assemble the various materials has been previously demonstrated [9,26–28]. To optimize the electrospinning conditions the effects of the PANi content and the feed rate on the morphology and alignment of the PCL/PANi composite nanofibers were explored. The presence of PANi on the surface of the composite nanofibers was confirmed using Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). In addition, the electrical conductivity, in vitro degradation stability, and mechanical properties of the electrospun PCL/PANi nanofibers were investigated. Finally, to investigate potential use of the PCL/PANi nanofibers for skeletal muscle tissue engineering mouse C2C12 myoblasts were cultured on these scaffolds to study cell alignment, proliferation, and differentiation.

2. Materials and methods

2.1. Materials

PCL (molecular weight 70–90 kDa), PANi (emeraldine base, molecular weight 100 kDa), and camphor sulfonic acid (CPSA) were obtained from Sigma–Aldrich (St. Louis, MO). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was obtained from Alfa Aesar (Heysham, UK). All chemicals were used as received without additional treatment.

2.2. Electrospinning

The PANi solution was prepared by mixing 15 mg of PANi and 15 mg of CPSA in 5 ml of HFIP for 5 min at room temperature (RT), followed by sonication at 800 W for 7 h with a *pulse* every 3 s and *pulse* off every 1 s to obtain a homogeneous solution. PCL (500 mg) was dissolved in various volumes of HFIP (5, 3.3, and 1.7 ml) by stirring for 3 h at RT. The samples for electrospinning were prepared by mixing each solution with volume ratios of PCL to PANi of 5:0, 3.3:1.7, 1.7:3.3, and 0:5, followed by sonication as described for 4 h. For the solution with a volume ratio of 0:5 500 mg of PCL powder was directly mixed with 5 ml of PANi solution to maintain a total solute concentration of approximately 10% w/v. The calculated concentrations for the various samples are listed in Table 1.

For electrospinning a stainless steel plate and two parallel block magnets (60 × 20 × 30 mm, length × width × height) were separately used as the collector to obtain random or aligned nanofibers. Fig. 1 shows a schematic illustration of the set-up used in the magnetic field-assisted electrospinning (MFAES) process. A thick acrylic plate was placed in the middle of the two parallel magnets to prevent direct contact between them. The magnetic field at the center of the gap was maintained at 0.3 T, and the optimal distance between the two magnets was 30 mm. The field strength was determined using a Gaussmeter (Bell 5070, F.W. Bell, Orlando, FL, USA).

In a typical procedure the PCL/PANi blended solution was loaded into a glass syringe equipped with a 22G stainless steel needle. The syringe was then placed in a syringe pump (KDS 100, KD Scientific, Holliston, MA), and the needle connected to the positive output of a high voltage power supply (HER-30P1, Matsusada Precision Inc., Kusatsu, Japan) set at 20 kV. The distance between the tip of the needle and the collector was15 cm. The flow rate of the solution ranged between 0.1 and 4.0 ml h^{-1} to optimize the final morphology of the fibers. The nanofibers obtained were dried overnight under vacuum and used for characterization and the cell culture studies.

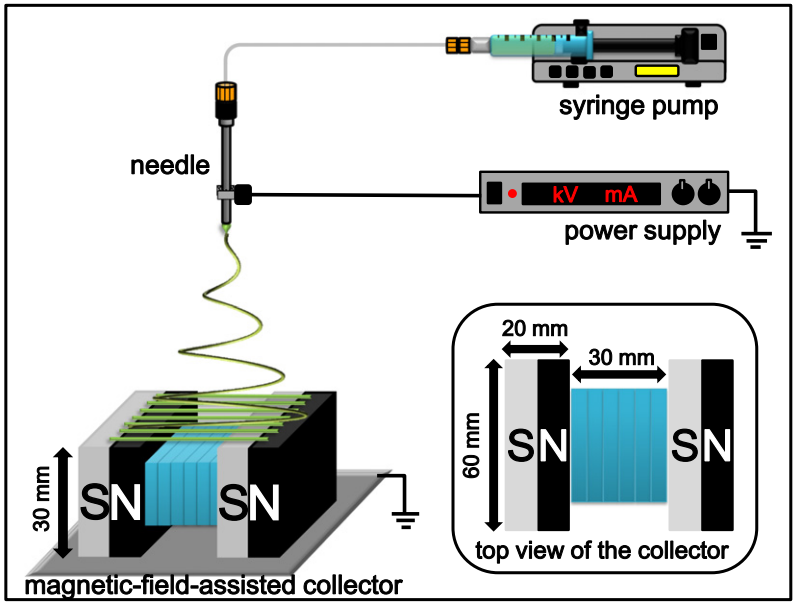

Fig. 1. Schematic illustrations of the set-up used in the magnetic field-assisted electrospinning (MFAES) method for preparing aligned nanofibers. (Insets) Specifications of the magnetic field-assisted collector.