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Aligned and random nanofibrous substrate for the in vitro culture of Schwann cells for neural tissue engineering

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Abstract

The current challenge in peripheral nerve tissue engineering is to produce an implantable scaffold capable of bridging long nerve gaps that will produce results similar to autograft without requiring the harvest of autologous donor tissue. Aligned and random polycaprolactone/gelatin (PCL/gelatin) nanofibrous scaffolds were fabricated for the in vitro culture of Schwann cells that assist in directing the growth of regenerating axons in nerve tissue engineering. The average fiber diameter attained by electrospinning of polymer blend (PCL/gelatin) ranged from 232 ± 194 to 160 ± 86 nm with high porosity (90%). Blending PCL with gelatin resulted in increased hydrophilicity of nanofibrous scaffolds and yielded better mechanical properties, approaching those of PCL nanofibers. The biocompatibility of fabricated nanofibers was assessed for culturing and proliferation of Schwann cells by MTS assay. The results of the MTS assay and scanning electron microscopy confirmed that aligned and random PCL/gelatin nanofibrous scaffolds are suitable substrates for Schwann cell growth as compared to PCL nanofibrous scaffolds for neural tissue engineering.
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Keywords: Electrospinning; Random and aligned nanofibers; PCL; Gelatin; Schwann cells

1. Introduction

Existing tissue engineering approaches focus on evolving an alternative route for nerve tissue regeneration using polymeric scaffolds and striving to find suitable biomaterials for tissue engineering. Due to the unavailability of ideal scaffold material and limited knowledge of cell and scaffold interaction, there have been few breakthroughs in nerve tissue engineering [1]. A scaffold that can mimic, and hence replace, the structure and function of extracellular matrix (ECM), i.e., with strong regenerative and cell supporting capacity, is central to nerve tissue engineering [2]. Nanofibrous scaffolds have replaced conventional strategies such as autografts, allografts and xenografts [3,4] for nerve tissue engineering due to the limited supply of donor nerve grafts in autografts [5,6], the chances of immunorejection in allografts and xenografts [7] and their use being limited to bridging only small nerve gaps and defects [8,9].

Nerve tissue regeneration requires an scaffold that should ideally be biodegradable, biocompatible and mechanically robust, having large surface area, high porosity and interconnected pores [10] and also act as a bridge to regenerate growing axons across larger nerve defects. Scaffold is also critical for regenerating cells as it allows the cells to proliferate, migrate, produce ECM to form functional tissues, and provides cells with their own microenvironment [11]. The high porosity of nanofibrous scaffolds provides more structural space for cell accommodation and enables the exchange of nutrient and metabolic waste between the scaffold and environment [12]. Electrospinning

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is one of the novel techniques for achieving nanofibrous scaffolds, mimicking the structural and functional morphology of ECM, which includes the use of very high voltage for the production of nanofibers [13].

Electrospinning is a simple and efficient technique used for the fabrication of high-porosity nanoscale scaffolds. Such scaffolds are effective in providing a large surface area for cell attachment, and allows cells to attain proper morphology [10]. The morphology, fiber diameter and porosity of electrospun nanofibrous scaffolds can be controlled by varying parameters, such as applied electric field strength; spinneret diameter; distance between the spinneret and the collecting substrate; temperature; feeding rate; humidity; air speed; and properties of the solution or melt, including the type of polymer, polymer molecular weight, surface tension, conductivity, and viscosity, temperature, concentration of the polymer, etc. [14,15]. Numerous natural and synthetic polymers have been investigated for the fabrication of nanofibrous scaffolds for nerve tissue regeneration. Yang et al. designed nanostructured porous poly(L-lactic acid) (PLLA) scaffolds intended for nerve tissue engineering [16], while polymers such as poly(glycolic acid) (PGA) [17], poly(L-lactic acid)-co-poly(ϵ-caprolactone) (PLA-CL) [18] have also been used for the regeneration of nerve tissue. Natural and synthetic polymers alone cannot meet all the requirements of a perfect scaffold: natural polymers lose their mechanical properties very early during degradation while synthetic polymers alone are less hydrophilic, lack binding sites for cell adhesion and release acidic degradation products [19]. To overcome the problems associated with individual polymers, hybrid materials (blends of two or more types of polymers) have been devised by researchers that assimilate the desirable characteristics of component materials.

An attempt to devise a near-perfect scaffold for growing Schwann cells for nerve tissue engineering was performed in the present study by using a blend of natural and synthetic polymer to impart favorable characteristics to the scaffold. Gelatin, a natural polymer and a product of partial hydrolysis of collagen [10], was blended with polycaprolactone (PCL), a synthetic and highly hydrophobic polymer, to fabricate aligned and random nanofibers for culturing Schwann cells, which are the supporting cells of peripheral nervous system (PNS) and play a key role in axonal regeneration. Gelatin contains many glycine, proline and hydroxyproline residues that help in cell adhesion and differentiation but it is also a soft material and has low tensile properties. PCL has good mechanical properties but its low hydrophilicity together with a lack of functional groups often results in low cell adhesion and proliferation on these scaffolds. Different types of physicochemical and post-processing surface modification techniques have been attempted to solve the problem of cell adhesion and proliferation on hydrophobic surfaces [20]. Moreover, nerve regeneration requires a scaffold with low degradation and swelling properties, in order to withstand nerve compression which otherwise impedes the outgrowth of regenerating nerves. Blending PCL with gelatin has furnished a better biocompatibility and hydrophilicity as well as mechanical properties suited for nerve tissue engineering. In an effort to closely mimic the ECM, aligned and random nanofibers were produced as potential substrates for Schwann cell growth, aiming at comparing the effect of fiber orientation and surface morphology on proliferation of Schwann cells. The aligned-nanofiber fabrication technique involves high rotation speed, providing thinner and closely packed nanofibers [10,14] that help in providing guidance cues to the regenerating axons, while the random nanofibers acquire high porosity, an even pore size and a surface topography containing ridges and grooves [16]. In this study the composite nanofibrous scaffold of PCL/gelatin was fabricated and evaluated for the properties such as hydrophilicity, functional groups, tensile strength, pore size distribution and growth of Schwann cells for neural tissue engineering.

2. Materials and methods

2.1. Materials

PCL (Mw 80,000) and gelatin (Type A) were brought from Sigma–Aldrich (St. Louis, MO, USA) and 2,2,2-trifluoroethanol (TFE) was obtained from Fluka (Germany). Rat Schwann cells were obtained from ATCC (USA) for cell culture studies. Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Sigma; fetal bovine serum (FBS), antibiotics and trypsin-EDTA were purchased from GIBCO Invitrogen (Carlsbad, CA, USA). Hexamethyldisilazane (HMDS) was bought from Sigma (Singapore) and used as sample drying agent for scanning electron microscopy (SEM) studies.

2.2. Methods

2.2.1. Fabrication of nanofibrous scaffolds

Solutions of PCL and PCL/gelatin with suitable concentrations were prepared for electrospinning. PCL (9% w/v) solution was made by dissolving PCL in TFE. Gelatin was dissolved first in TFE followed by the addition of PCL pellets, to make a 10% translucent solution and subsequently used for electrospinning to obtain PCL/gelatin aligned and random nanofibers. For electrospinning, the polymer solution was fed into a 3 ml standard syringe attached to a 27G blunted stainless steel needle with inner diameter of 0.4 mm using a syringe pump (KDS 100, KD Scientific, Holliston, MA) at a flow rate of 1.5 ml h^{-1} with an applied voltage of 17.5 kV (Gamma High Voltage Research, USA). Random fibers were collected on a flat collector plate wrapped with aluminum foil that was kept at a distance of 13 cm from the needle tip. Aligned nanofibers were formed using a rotating disk setup with the same parameters and this rotating disk operated at a speed of 4000 rpm for obtaining well-aligned nanofibers. These nanofibers were collected on 15 mm coverslips for cell cul-

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