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Peptide functionalized polyhydroxyalkanoate nanofibrous scaffolds enhance Schwann cells activity

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Abstract

Interactions between Schwann cells (SCs) and scaffolds are important for tissue development during nerve regeneration, because SCs physiologically assist in directing the growth of regenerating axons. In this study, we prepared electrospun scaffolds combining poly (3-hydroxybutyrate) (PHB) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) functionalized with either collagen I, H-Gly-Arg-Gly-Asp-Ser-OH (GRGDS), H-Tyr-Ile-Gly-Ser-Arg-NH2 (YIGSR), or H-Arg-Asn-Ile-Ala-Glu-Ile-Ile-Lys-Asp-Ile-OH (p20) neuromimetic peptides to mimic naturally occurring ECM motifs for nerve regeneration. Cells cultured on fibrous mats presenting these biomolecules showed a significant increase in metabolic activity and proliferation while exhibiting unidirectional orientation along the orientation of the fibers. Real-time PCR showed cells cultured on peptide-modified scaffolds had a significantly higher neurotrophin expression compared to those on untreated nanofibers. Our study suggests that biofunctionalized aligned PHB/PHBV nanofibrous scaffolds may elicit essential cues for SCs activity and could serve as a potential scaffold for nerve regeneration.
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Key words: Schwann cells; Scaffold; Electrospinning; Peptide

Introduction

Schwann cells (SCs) are currently being investigated as a component of nerve repair strategies because of their known ability to support nerve regeneration in both the central nervous system (CNS) and the peripheral nervous system (PNS), where enhanced nerve repair has been reported following cell transplantation.[1] *In vitro* and *in vivo* assessments have identified the use of bioengineered scaffolds seeded with SCs as a promising approach in synthetic nerve grafts to bridge nerve gaps.[2,3] The success of SCs in nerve regeneration is related to the production of cell adhesion molecules and neurotrophic factors, which mediate neurite attachment and growth.[4,5]

Scaffolds for nerve regeneration require appropriate biocompatibility and biodegradability, good pliability for suture and mechanical integrity, and *in vivo* physiological loading during axon regeneration across large nerve defects. Scaffolds should also provide contact guidance for cell migration and axon outgrowth along the gap defect to support nerve functional regeneration.[6]

Recently, electrospun nanofibrous scaffolds served as suitable environments for cell attachment and proliferation thanks to similar physical dimensions and cues compared to natural extracellular matrix (ECM).[7] Poly[(*R*)-3-hydroxybutyrate] (PHB) and poly[(*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyvalerate] (PHBV) are two

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extensively studied natural derived poly(hydroxyalkanoates) (PHAs) due to their good biocompatibility and mechanical properties.[8] In recent years, promising studies aimed at the engineering of tissues such as bone,[9] cartilage,[10] skin[11] and nerve[3] have used PHB or PHBV substrates as a scaffold.

Normally, cell affinity toward polymers is poor as a result of their low hydrophilicity and lack of surface cell recognition sites.[12] Therefore, surface treatment of polymeric scaffolds is necessary to improve their bioactivity to achieve functional tissue regeneration. While various studies improved the hydrophilicity and surface properties of PHAs scaffolds using techniques such as composite electrospinning,[13] plasma treatment,[14,15] photografting[16] and alkaline hydrolysis,[15,17] very few studies have been reported in the literature on the surface functionalization of PHAs electrospun fibrous scaffolds with peptides for neural tissue engineering applications. The majority employed biomolecules have been so far ECM proteins, on the basis that cells in native tissues are surrounded by and attach to this network of fibril proteins. Meng et al. fabricated nanofibrous scaffolds using a blended solution of PHBV/collagen, which showed to accelerate adhesion and growth of NIH-3T3 cells.[13] Alternatively, collagen can be immobilized on the surface of PHBV scaffolds, as reported by Tesema et al. and Baek et al.,[18,19] to improve their osteoblasts compatibility. Surface functionalization of PHBV-chitosan scaffolds grafted with hyaluronic acid (HA) has also been demonstrated by Hu et al., showing that antibacterial properties were maintained while protein adsorption was effectively reduced.[20] Within the context of nerve repair, Armstrong et al. and Novikova et al. showed that coating of PHB nerve conduits with ECM molecules such as laminin and fibronectin enhanced SCs activity to release neurite promoting factors,[3,21] highlighting the potential of adding ECM biomolecules to bioengineered nerve conduits in order to improve nerve regeneration. However, the complexity and source of ECM proteins can cause issues with consistency and control over cell response as well as final clinical translation.[22] Synthetically produced peptides represent a viable alternative, as shown by Wang et al.[23] with the introduction of RGD peptides on PHBV films; although not for a neural regeneration application, the viability of fibroblast-like NIH 3T3 cells was shown to improve.

Building on our previous work developing fibrous PHB/PHBV electrospun scaffolds with optimal physical properties and fiber alignment for improved SC activity, we compare here for the first time the biological activity of electrospun PHB/PHBV fibrous scaffolds which have been functionalized with relevant peptides: GRGDS, and the two laminin derived neuromimetic peptide sequences YIGSR and p20. These biomolecules were chosen based on their biological functionalities as cues present in the basal ECM and known to be involved in cell–cell and cell–ECM communications. We also show the immobilization of collagen type I for further comparison, following our earlier observations that SC adhesion and proliferation was enhanced when collagen was blended into the PHB/PHBV fibers.[24]

Methods

Materials

PHB with M_w of 437’000, PHBV with 5% wt poly (3-hydroxyvalerate) and M_w of 150’000, chloroform, *N*,*N*-dimethyl formamide (DMF), 2-(*N*-morpholino) ethanesulfonic acid (MES), sodium hydroxide (NaOH), *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) were acquired from Sigma Aldrich (USA). Purchased reagents for cell culture were as follows: fetal bovine serum (FBS) from Hyclone (USA), Dulbecco Modified Eagle Medium (DMEM), phosphate buffered saline (PBS), penicillin/streptomycin and trypsin-EDTA from Gibco BRL (USA). Acid soluble collagen type I powder of bovine origin was a generous gift from Kensey Nash Corporation (USA). Peptides for biofunctionalization consisted in H-Gly-Arg-Gly-Asp-Ser-OH (GRGDS; M_w: 490.47 g/m), H-Tyr-lle-Gly-Ser-Arg-NH2 (YIGSR; M_w: 594.67 g/m), and H-Arg-Asn-lle-Ala-Glu-lle-lle-Lys-Asp-lle-OH (RNIAEIIKDI (p20; M_w: 1184.40 g/m)) peptides were purchased from Bachem (Switzerland).

Electrospinning of aligned PHB/PHBV nanofibers

PHB/PHBV (1:1) solutions were prepared by dissolving the polymers in a chloroform (90)/DMF (10) solvent mixture at a concentration of 6% wt. Using a syringe pump (KDS 100, KD Scientific), the solution was fed at a rate of 1.5 ml/min through a 10 cc syringe with a 23 G needle placed 15 cm from a rotating mandrel collector with a speed of 5000 rpm. A high-voltage power supply was used to apply a voltage of 16 kV DC to produce nanofibers. Temperature and humidity were monitored during the process and ranged between 24 and 26 °C and 37% and 42%, respectively.

Covalent attachment of biomolecules on alkaline hydrolysed PHB/PHBV nanofibers

For biofunctionalization, PHB/PHBV nanofibrous mats were firstly treated with NaOH (1N) for 80 min at room temperature, washed and dried at 37 °C overnight to obtain hydrolysed scaffolds. Afterwards, the hydrolysed scaffolds were washed in MES buffer solution (0.1 M, pH 5.0) for 30 min at room temperature to be subsequently activated with 5 mg/ml EDAC and 2.5 mg/ml sulfo-NHS in MES buffer solution for 90 min at room temperature. Biofunctionalization with collagen (5 mg/ml PBS) and peptides (0.2 mg/ml PBS) solutions was performed for 24 hours at room temperature. Scaffolds were further rinsed with PBS and dried at 37 °C overnight (Figure 1).

Characterization of nanofibrous scaffolds

Scanning electron microscopy (SEM)

The morphology of electrospun fibers was observed using SEM (XL 30 ESEM-FEG, Philips). Fiber diameters were calculated from SEM micrographs by measuring 100 fibers using Manual Microstructure Distance Measurement software (NahaminPardazan Asia Co., Iran).

Monitoring of biofunctionalization method on scaffolds

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) of collagen immobilized and bulk PHB/PHBV nanofibrous scaffolds were performed over a range of 400-4000 cm^{-1} at a resolution of 2 cm^{-1} using a Nicolet spectrometer system.

The XPS spectra of GRGDS immobilized and bulk PHB/PHBV nanofibrous scaffolds were also obtained on VGEscalab 2201-XL Base System (Thermo VG Scientific, UK) with a take-

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