Materials Science and Engineering C 70 (2017) 1132–1140

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

NECL1 coated PLGA as favorable conduits for repair of injured peripheral nerve

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ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 26 February 2016
Accepted 14 March 2016
Available online 17 March 2016

Keywords:
Peripheral nerve injuries
NECL1
PLGA
Sciatic nerve
Nerve regeneration

ABSTRACT

Restoration of normal neurological function of transected peripheral nerve challenged regenerative medicine and surgery. Previous studies showed that Nectin-like molecule 1 (NECL1) is one of the important adhesion molecules on the axons and Schwann cells is located along the internodes in direct apposition to NECL1. In this study, we fabricated PLGA membrane pre-coated with NECL1, mimicking the natural axons to enhance the adhesion of Schwann cells. Investigation of the cellular response in vitro was performed by detecting cytotoxicity, proliferation, morphology, viability, specific markers and Scanning Electron Microscopy (SEM) of Schwann cells cultured in PLGA. Further, the NECL1-coated PLGA conduits were used for peripheral nerve repair after sciatic nerve defect was constructed. Results showed that PLGA-coated NECL1 enhanced cell proliferation compared with PLGA, as evidenced by MTT analysis, cell viability assay and histological evaluation. RT-PCR results showed that GDNF (glial cell line-derived neurotrophic factor), BDNF (brain-derived neurotrophic factor), CNTF (ciliary neurotrophic factor) and neurotrophic factors of axonal regeneration were highly expressed in PLGA/NECL1 group. S100, which is Schwann cell marker, was also elevated in PLGA-NCEL1 in both mRNA and protein expression as demonstrated by PCR and immunohistochemical examination. Moreover, in vivo study showed that implantation of PLGA/NCEL1 tubes in bridging the nerve defect can significantly improve Schwann cell aggregation and attachment and greatly enhance the functional recovery of nerve regeneration as compared with control and PLGA groups. Therefore, the novel blend of PLGA/NECL1 conduits proved to be promising candidate for tissue engineering scaffold.

1. Introduction

Peripheral nerve injuries can lead to lifetime loss of function [1], and its regeneration is still one of the most challenging tasks and concerns in neurosurgery, plastic and orthopedic surgery [2]. Fractures, hematomas, contusions, compressions always caused peripheral nerve injury characterized by the disruption of myelin sheaths and axons [3,4]. Although autografts remain the gold standard for nerve repair, various complications including neuroma, donor site morbidity, nerve site mismatch, and limited amounts of donor tissue confined the application [5,6] Tissue engineering technique by incorporating of cells into conduits for tissue repair holds promise in nerve regeneration.

As one of the main supportive cells in neural tissue engineering, Schwann cells are the myelin-forming cells of the peripheral nervous system, which play a crucial role in nerve regeneration through production of neurotrophic growth factors and secretion of extracellular matrices [7–10]. It was reported that Schwann cells have bioactivity and can produce nerve growth factors, secrete extracellular matrix and guide the growth of axons [11,12]. And they have been extensively employed in in vitro cultures and in vivo models as candidates for restoring defects in nerve bundles [13–15]. Superior in nerve regeneration, Schwann cells were aggregated into nerve conduits in order to improve nerve regeneration and functional recovery [16–18].

In an attempt to bridge nerve defects, nerve guides are necessary for nerve regeneration. They can also be used as cell substrates to provide cells with a friendly environment and to prevent them from leaking out of the lesion site. Materials have been used as drug delivery vehicle and tissue engineering scaffold need to be biocompatible and biodegradable [19]. Substances such as poly (glycolic acid) (PGA) and poly(lactic acid) (PLA) or polyhydroxybutyrate (PHA) and polycaprolactone (PCL) are under investigation as biodegradable-

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http://dx.doi.org/10.1016/j.msec.2016.03.043
0928-4931/

absorbable synthetic materials for scaffold fabrication [20–24]. It was reported that PCL/lignin–PMMA nanofibers membranes, a series of poly (methyl methacrylate) (PMMA) grafted lignin copolymers were blended with PCL, would be developed in biomedical and personal care fields [22]. Similarly, Dan Kai et al. reported the biodegradable and biocompatible lignin–PEGMA copolymers and they found that this potential applications lignin-based hydrogels would be developed in biomedical and personal care fields [25]. The recent development of poly (glycerol sebacate) (PGS) is significant in expanding its function as a suitable biodegradable polymer for various biomedical applications [26]. In a recent report, Hou and coworkers copolymerised modified gelatin with PLA–PEG–PLA under UV light illumination, which was later added to a-cyclodextrins (a-CDs) in order to obtain a new biodegradable gel [27,28]. Through acting as a complement to the embedded substances by enhancing their natural properties, such hybrid supramolecular hydrogels and nanoparticles hydrogels have shown great potential as therapeutic materials and delivery vehicles [28–30].

Among various nerve scaffolds, poly-(lactic acid-*co*-glycolic acid) (PLGA) is highly recommended as nerve conduit material because of its biodegradable and biocompatible properties. In addition, PLGA has been approved by the Food and Drug Administration (FDA) and widely accepted as drug delivery vehicle and suture material [31,32]. A numerous array of studies have been focused on PLGA employment in different contexts of cell based tissue engineering [33–35]. However, lack of cell recognition (binding) sites that may be unfavorable for cell anchorage has limited the potential applications of PLGA and several other synthetic polymers [36–38]. For this reason, surface of PLGA should be modified to better support cell growth for nerve regeneration.

An alternative is to introduce adhesion molecules on the cover of PLGA membrane. Nectins and Nectin-like molecules belong to the immunoglobulin superfamily of cell-cell adhesion molecules and play roles in the cell-cell communications [39]. Among them, Nectin-like molecule 1 (NECL1) is exclusively and highly expressed in the nervous system and plays a role in the formation of synapses, axon bundles, myelinated axons and cerebellar morphogenesis [40–42]. Studies showed that NECL4 is the main NECL expressed by myelinating Schwann cells and is located along the internodes in direct apposition to NECL1, which is localized on axons [43]. This suggested that NECL1 may promote the interaction of Schwann cells and extracellular matrix (ECM).

In this study, we fabricated PLGA film pre-coated with NECL1, mimicking the natural axons to enhance the adhesion of rat Schwann cells. Investigation of the cellular proliferation, morphology, attachment, viability, specific protein secretion, and Schwann cell-specific gene expression in vitro was performed by detecting. Further, the NECL1-coated PLGA conduits were used for peripheral nerve repair after sciatic nerve defect was created. This study may provide reference for application in nerve reconstruction in clinical treatment of peripheral nerve injury.

2. Materials and methods

2.1. Preparation of materials

A biocompatible and bioresorbable PLGA scaffold was purchased from Lakeshore Biomaterials, Birmingham, AL, USA. The average molecular weight of the PLGA copolymer was 5×10^4 Da (Mn GPC). The random PLGA films were cut into small squares of about 15 mm in each side. All PLGA samples were sterilized by immersion in 70% ethanol, air dried in a sterile culture hood and both sample sides exposed to UV for 30 min prior to cell seeding.

2.2. NECL1 treatment

Rat CADM3/NECL1 protein (purity ≥ 85%, as determined by SDS-PAGE) was purchased from Sino Biological Inc. (Beijing, China) and stored at −80 °C. Prior to the experiments, NECL1 was reconstituted in water to a concentration of 500 μg/ml. Do not vortex. For extended storage, it is recommended to further dilute in a buffer containing a carrier protein (example 0.1% BSA) and store in working aliquots at −20 °C to −80 °C until ready for use. The NECL1 stock solution was diluted with culture medium immediately before treatment. Cells were treated by NECL1 at various concentrations (0 ng/ml as control, 25 ng/ml, 50 ng/ml, and 100 ng/ml).

2.3. Preparation of PLGA conduit

PLGA films (about 15 mm in each side.) were sterilized by immersion in 70% ethanol, air dried in a sterile culture hood and both sample sides exposed to UV for 30 min. Plain and micropatterned PLGA films were precoated with NECL1 (100 ng/ml) and air-dried. A stainless steel bar with a triangular-shaped cross-section (each side 2.1 mm long) was inserted into a hollow PLGA tube of circular-shaped cross-section (about diameter 2 mm, length 15 mm) and heat sealing of the rolled conduit [1]. This internal diameter complies with optimal function in rat model [2]. Before implantation, each side of implants was sterilized under ultraviolet (UV) light for 30 min.

2.4. Cells culture

RSCs [RSC96, CCTCC, Shanghai, China], a spontaneously immortalized rat Schwann cell line derived from the long-term culture of rat primary Schwann cells, were purchased from the China Center for Type Culture Collection (CCTCC) and cultured in DMEM/F-12 supplemented with 10% (v/v) FBS and 1% (v/v) antibiotics in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C with the culture medium replaced every 3 days after plating. RSC96 cells were passaged with 0.25% trypsin when the cell confluence reaching about 80–90%. Confluence RSC96 cells cultures were treated at the indicated times with indicated concentrations of NECL1.

2.5. Cells seeding on scaffolds experiment

2.5.1. Cell cytotoxicity assay

RSCs viability was estimated using a colorimetric assay based on the conversion of tetrazolium dye (MTT) into a blue formazan product. The cells were then plated at 800 cells/well in 96 well cell culture cluster pretreated with various concentrations of NECL1 (0–300 ng/ml) for 3 days. 5 mg/ml MTT was added to cultures in each well and were incubated in the dark at 37 °C for 4 h. Then, culture medium was removed and treated with 150 μl DMSO to dissolve the formazan product. The cells were incubated in DMSO with shaking for 10 min. Samples' optical density (OD) were measured by a Multiskan GO Microplate Spectrophotometer at 570 nm. Five individual cultures were used for each test. The experiments were carried out in quintuplicate.

2.5.2. Cell proliferation analysis

Based on the result of cytotoxicity assay, we chose the three doses (25, 50, and 100 ng/ml NECL1) with more obvious positive effect, coupled with control group (0 ng/ml NECL1) for cell proliferation analysis. PLGA scaffolds were incubated with culture medium for 30 min. After trypsinization and determination of cell concentration, 150 μl of cell suspension containing 40 000 viable cells was transferred onto scaffolds. Cells were allowed to attach and propagate at 37 °C and 5% CO_2. At each time point (3, 5 and 7 days after cell seeding), 1 ml of 5 mg/ml MTT solution (Sigma-Aldrich Corp., St. Louis, MO, USA) was added to each scaffold for 3 h at 37 °C and 5% CO_2 in the dark. After formation of formazan during reduction process, DMSO was added for 10 min at 37 °C in the dark with moderate agitation to disrupt the cells and solubilize formazan. Samples' optical density (OD) were measured by a Multiskan GO Microplate Spectrophotometer at 570 nm. Five individual cultures were used for each test. The experiments were carried out in quintuplicate.