Materials Science & Engineering C 91 (2018) 715–726

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

The cellular response of nerve cells on poly-L-lysine coated PLGA-MWCNTs aligned nanofibers under electrical stimulation

Jing Wang[a,b], Lingling Tian[b], Nuan Chen[b], Seeram Ramakrishna[b,c], Xiumei Mo[a,*]

[a] State Key Laboratory of Modification of Chemical Fibers and Polymer Materials, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China

[b] Center for Nanofibers and Nanotechnology, E3-05-14, Department of Mechanical Engineering, Faculty of Engineering, National University of Singapore, 2 Engineering Drive 3, Singapore 117576, Singapore

[c] Guangdong-Hongkong-Macau Institute of CNS Regeneration (GHMICR), Jinan University, Guangzhou 510632, China

ARTICLE INFO

Keywords:
Nerve cells
MWCNTs
Surface modification
Aligned nanofibers
Electrical stimulation

ABSTRACT

Tissue engineering scaffold provide an effective alternative for peripheral nerve repair. Nanofibrous nerve conduits fabricated with various synthetic and natural materials have great potential to support nerve regeneration as a bridge between adjacent ends. The physical, chemical and electrical properties of the scaffolds affect the outcome of nerve regeneration and recovery of function. In this paper, a surface modified, electrically conductive, aligned nanofibrous scaffold composed of poly(lactic-*co*-glycolic acid) (PLGA) and multi-walled carbon nanotubes (MWCNTs), referred to as L-PC_A was fabricated for nerve regeneration. The morphology, surface chemistry and hydrophilicity of nanofibers were characterized by Scanning Electron Microscopy (SEM), Energy-dispersive X-ray (EDX) and water contact angle, respectively. The mechanical property of the nanofibrous scaffold was also evaluated using a universal materials tester. The effects of these scaffolds on PC12 cell adhesion, proliferation and neuronal differentiation were all evaluated. A hydrophilic surface was created by poly-L-lysine coating, which was able to provide a better environment for cell attachment. Furthermore aligned fibers were proved to be able to guide PC12 cells and DRG neurons growing along the fiber direction and be beneficial for neurite outgrowth. The cellular responses of PC12 cells and DRG neurons on L-PC_A scaffold under electrical stimulation were evaluated by neurofilament proteins expression. As a result, the PC12 cells and DRG neurons stimulated with electrical shock showed longer neurite length, indicating that electrical stimulation with a voltage of 40 mV based on the scaffold with MWCNTs could enhance the neurite extension. Moreover, the cellular response of Schwann cells including cell attachment, proliferation and MBP expression were also enhanced with the synergistic effect of aligned nanofibers and electrical stimulation. In summary, the L-PC_A nanofibrous scaffold supported the cellular response of nerve cells in terms of cell proliferation, differentiation, neurite outgrowth, and myelination in the presence of electrical stimulation, which could be a potential candidate for nerve regeneration application.

1. Introduction

Peripheral nerve injuries (PNI) are the common reasons of human disabilities, which often lead to the loss of mobility and sensory obstacle [1]. Various methods have been applied for therapies to restore the loss of function. End to end coaptation can be used for short nerve lesion, whereas nerve grafts are needed to bridge the transected nerves for the long distance nerve defection [2]. Currently, nerve autografts are the clinical standard for the treatment of PNI with gap > 5–10 mm [3], however, this method need remove a piece of nerve from a secondary site on the body, which results in additional surgical pain, as well as sensory function loss of donor site [4]. Additionally, the drawbacks such as limited donor resource, insufficient donor nerve length, and mismatch of diameter between donor nerve and recipient site restrict its application [5]. These associated drawbacks also motivate the search of alternative treatment options with little additional sacrifice and more therapeutic effect for nerve injuries.

Tissue engineering scaffolds provide an effective alternative for peripheral nerve repair. Nerve conduits fabricated with various synthetic and natural materials have great potential to support nerve regeneration as a bridge between adjacent ends. The physical, chemical and electrical properties of the materials affect the outcome of nerve regeneration and recovery of function. Electrically conductive materials have received considerable attention to influence cellular behaviors of

* Corresponding author.
E-mail address: xmm@dhu.edu.cn (X. Mo).

https://doi.org/10.1016/j.msec.2018.06.025
Received 27 October 2017; Received in revised form 27 April 2018; Accepted 11 June 2018
Available online 12 June 2018

nerve cells for potential uses as tissue engineering scaffolds. Carbon nanotubes (CNTs) have been investigated for neural regeneration due to the surface nanotopography which mimics the extracellular matrix (ECM) and their intrinsic conductivity. Within the last ten years, numerous studies reported the impact of both single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) on neuronal behavior, particularly on their ability to promote neurite extension [6]. Mattson et al. demonstrated the ability of MWCNTs-layered substrates to support the long-term survival of cultured dissociated hippocampal neurons [7]. Recent studies pointed that the electrical conductivity of CNTs played a critical role in mediating their impact in both neuronal growth and electrical behavior. In fact, some studies have reported that the conductivity of CNTs exerts a critical role in neuronal growth and in boosting neuron electrical performance [8, 9].

It is known that bioelectricity exits and participates in maintaining biological functions such as signaling in the nervous system in human body. Nerve cells are sensitive and intricately tied to electrical behaviors [10]. Electrical stimulation could change protein adsorption and nerve cell interactions with conducting biomaterials, thus improving neuronal extension and outgrowth [11, 12]. It has reported that electrical stimulation to PC12 cells enhanced neurites extension by 90% compared with cells without electrical stimulation [11]. Other studies also found that electrical stimulation could promote the nerve growth factor (NGF) induced neurite outgrowth and signaling [13]. Although the detailed mechanisms of electrical stimulation to improve neurite outgrowth are not understood completely, it provides an effective way to potentially promote nerve tissue regeneration. Nanofibrous scaffolds with biocompatibility and electrical conductivity are suitable for electrical stimulation on nerve cells in nerve tissue engineering.

Electrospun nanofibers have been well investigated for peripheral nerve regeneration and gained commendable success. Various kinds of synthetic and natural materials, such as poly (lactic acid) (PLA), polycaprolactone (PCL), and poly(lactic-*co*-glycolic acid) (PLGA) have been fabricated into nanofibrous scaffolds for nerve tissue repair [14–16]. For designing a desirable electrospun nanofibrous scaffolds for nerve tissue engineering, topography and surface chemistry are crucial parameters besides biocompatibility and electrical conductivity [17]. The aligned electrospun fibers have been proved to affect the cellular behaviors of nerve cells, which could guide the direction of neurites outgrowth from the cells [18]. For example, the elongation and neurite outgrowth of neural stem cells on aligned electrospun PLLA nanofibers were improved compared to that on random fibers [19]. Surface property, including surface chemistry and hydrophilicity, is another important factor influencing the initial cell adhesion and anchorage, and then affecting the cell proliferation and viability on scaffolds [20].

Based on the above considerations, PLGA blended with MWCNTs to fabricate an electrically conductive nanofibrous scaffold with appropriate topographic guidance cues and surface property. The performance of scaffold in vitro was evaluated using PC12 cells, Schwann cells and Dorsal root ganglion (DRG) neurons under electrical stimulation. PLGA nanofibers with various MWCNTs ratios have been fabricated in our previous study, and the effect of scaffold with different MWCNTs rations on nerve cell behaviors has also been evaluated. Our results found that the scaffolds significantly promoted nerve cellular behaviors including both proliferation and differentiation when the content of MWCNTs was 8% w/w to PLGA. Herein, PLGA and MWCNTs were mixed in a weight ratio of 100:8 and further fabricated into aligned nanofibers via electrospinning and high-speed rotating drum collection. Then the nanofibers were coated with Poly-L-lysine solution to increase the number of positively-charged sites for cell binding. The proliferation of both PC12 and Schwann cells were evaluated, while the differentiation of PC12 cells and neurite outgrowth of DRG neurons were performed on scaffolds with electrical stimulation when exogenous NGF was added to the culture medium.

2. Materials and methods

2.1. Materials

The 75/25 poly(lactic-*co*-glycolic acid) (PLGA) (inherent viscosity 0.75dL/g) was purchased from Jinan Daigang Biomaterial CO., Ltd., China. Graphitized Multi-Wall Carbon Nanotubes COOH (out diameter: 10–20 nm; length: 1–30 μm; purity: > 99.9 wt%; COOH content: 1.0–2.0 wt%) was purchased from Cheap Tubes Inc., Cambridgeport, VT, USA. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFP), glutaraldehyde and Dulbecco's modified eagle's medium (DMEM/F12) were purchased from Sigma, Singapore. Rat PC12 cells in the adherent type (ATCC® CRL-1721.1) and rat Schwann cells (S42 ATCC® CRL2942™) were obtained from ATCC, USA, while fetal bovine serum (FBS), horse serum (HS) and trypsin/EDTA were purchased from GIBCO Invitrogen, USA. Schwann cell medium was purchased from Gene-Ethics Asia Pte Ltd., Singapore. Neurobasal Medium and B27 supplement factors were also obtained from GIBCO Invitrogen, USA. Nerve growth factor (NGF) was purchased from Millipore, Singapore. Alamar Blue (AbD Serotec) was purchased from Chemoscience, Singapore.

2.2. Fabrication of aligned PLGA/MWCNTs nanofibers

PLGA was dissolved into HFP with a concentration of 23% w/v, followed by adding MWCNTs into PLGA/HFP solution with a MWCNTs to PLGA weight ratio of 8–100. Furthermore, 100 μL of span 80 was added to improve the dispersion of MWCNTs. Then, the suspensions were stirred continuously for 24 h. Finally, the suspensions were ultrasonicated for 1 h before electrospinning. The electrospinning process was the same as that described in the previous work [21], and aligned PLGA/MWCNTs composite nanofibers were obtained on an Al foil-wrapped high-speed (4000 rpm) rotating drum. The obtained aligned composite nanofibrous scaffold was referred as PC_A. The random PLGA/MWCNTs nanofibers as control were directly electrospun on an Al foil, and the random nanofibrous scaffold was referred as PC_R.

2.3. Surface modification of PLGA/MWCNTs nanofibers with poly-L-lysine

The aligned and random PLGA/MWCNTs nanofibrous scaffolds were all sterilized under UV light for 5 h. Then, the scaffolds were washed by PBS for 3 times and incubated in 500 μL poly-L-lysine solution (15 μg mL^{-1}), followed by being placed in an incubator for coating of 12 h. Subsequently, the residual poly-L-lysine solution was removed, and the scaffolds were put back to the incubator to allow them to be dry. The coated random and aligned nanofibrous scaffolds were referred as L-PC_R and L-PC_A, separately.

2.4. The characterization of PLGA/MWCNTs nanofibers

2.4.1. Observation of surface morphology

The nanofibers were sputter-coated with gold (JEOL JFC-1200 Fine coater, Tokyo, Japan) and the surface morphologies were visualized by FESEM (Model S-4300, Hitachi, Tokyo, Japan). The average diameter of the fibers was calculated with image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA).

2.4.2. Evaluation of surface hydrophilicity

The water contact angles of nanofibrous scaffolds were measured by VCA Optima surface analysis system (AST products, Billerica, MA) to identify the effect of poly-L-lysine on the hydrophilicity of scaffolds. The method was the same to that described in the previous work [22]. Briefly, the nanofiber mat was placed on the testing plate and kept smooth. Then, 0.05 mL of distilled water was dropped slowly onto the surface of samples. The images of water drop on the nanofiber mat were recorded by a camera software in the testing system after the droplet was stable. After that, the water contact angle was measured with the