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Enhancement of neurite adhesion, alignment and elongation on conductive polypyrrole-poly(lactide acid) fibers with cell-derived extracellular matrix

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ABSTRACT

Extracellular matrix (ECM) can promote peripheral nerve repair. In this study, a conductive fiber-film (CFF) with core-sheath structure and conductivity of ~10 S cm^{-1} was prepared by electrospinning of aligned poly(L-lactide acid) (PLLA) fibers and electrochemical deposition of polypyrole (PPy) nanoparticles. Then the multiple components of ECM, including laminin, fibronectin and collagen, were coated on the surface of CFF by culturing and lysing L929 cells to fabricate the bioactive scaffold of ECM-linked CFF (ECM-CFF). The electrical stimulation (ES) of 100 mV/cm for 14 days and 2 h per day did not significantly decrease the conductivity of ECM-CFF. The results of PC12 cells test indicated that, cells adhesion rate, neurite-bearing cell rate and neurite alignment rate on ECM-CFF were ~95%, ~77%, ~70%, respectively, significantly larger than the corresponding values on bare CFF (17%, 29% and 14%, respectively). The neurites length on ECM-CFF (~79 mm) was also larger than that on bare CFF (~25 mm). ES of 100 mV/cm onto PC12 cells through ECM-CFF could significantly promote neurite extension in first 3 days of the neurite growth. These results indicated that, the combination of ECM-CFF with ES could improve the nerve regeneration by encouraging neural-cell adhesion, neurite growth and extension.

1. Introduction

Electrical stimulation (ES) can obviously influence nerve growth due to the inherent nature of nerve tissue in transmitting electrochemical signals throughout the nervous system [1,2]. So conductive biomaterials have been extensively investigated for peripheral nerve repair [3]. Polypyrrole (PPy), one conductive and biocompatible polymer, has been applied in nerve regeneration as the material of ES [4,5]. It is desirable to fabricate a mimetic biomaterial with morphological cue to guide neurites regeneration [6,7] and to enhance nerve cell adhesion and recognition, reducing scar tissue after peripheral nerve lesions [8,9].

It was proved that conductive and aligned polymeric nanofibers could effectively guide neurite growth along the fiber axis [10], and the neurite length was further increased under ES of the conductive materials [5]. However, the electrical resistance of PPy-coated fiber-film prepared via in situ chemical oxidation method was too high to achieve good functional ES [11,12]. However, the electrochemical deposition is a convenient method to prepare PPy film with good electrical conductivity for nerve regeneration [13]. Abidian et al. have fabricated random PPy-coated fibers by electrochemical deposition for the nerve regeneration [14], drug release [15] and biosensor [16].

In order to enhance neural interface, the prepared materials need to be modified with different biomolecules. When nerve growth factor (NGF), laminin and collagen I were immobilized on conductive PPy film, respectively, the neurite lengths of differentiated PC12 cells cultured on these modified PPy films were larger than those on unmodified film [17–19]. Koh et al. blended laminin into PLLA fibers via electrospinning, and found that this fiber scaffold could promote axonal extension [20]. Recently, some reports focused on modified scaffold with only one protein/bioactive molecule of extracellular matrix (ECM). However, ECM of peripheral nerve tissue is comprised of many proteins and bioactive molecules, which can be secreted by fibroblasts or schwann cells [21,22]. Some ECM scaffolds from tissues and organs (such as urinary bladder or heart valves) [23,24], were utilized as tissue repairs materials, because the structures of the corresponding tissues/organs were remained. However, there are problems of autologous tissue/organ scarcity, host response and

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potential pathogen transmission when allogenic and xenogenic tissues/organs are used. By comparison, the combination of synthetic materials and cultured host cells to fabricate scaffold with ECM, can avoid risk of disease transmission. Liao et al. [25] reported that electrospun poly(ε-caprolactone) microfiber scaffolds coated with cartilaginous ECM could support the chondrogenic differentiation of mesenchymal stem cells. Kang et al. [26] reported that human umbilical vein endothelial cells-derived ECM on a three dimensional beta-tricalcium phosphate scaffold improved osteogenic differentiation of more human mesenchymal stem cells (MSCs). Gu et al. [27,28] reported that a nerve guidance conduit coated with schwann cell-derived (or MSCs-derived) ECM could achieve better regeneration of peripheral nerve than bare nerve guidance conduit. Although ECM derived from schwann cells includes many neurotrophic factors, such as NGF, bran-derived neurotrophic factor and glial celline-derived neurotrophic factor, the sharp boundary between nerve tissue and schwann cells is a barrier of axon regeneration [29]. Liu et al. [30,31] reported that the residual biomolecules of acellular muscle (containing laminin, fibronectin and collagen) could promote the repair of spinal cord injury in rats. These studies suggest that non nerve cell-derived ECM could form a good cellular micro-environment to promote cell differentiation and neurite growth. L929 cells, which could also excreted ECM of laminin, fibronectin and collagen [32], are more easily adhered and cultured on composite materials than nerve cells. However, there is no report about effect of L929 cell-derived ECM on aligned conductive fibers on neurites outgrowth and extension.

In this study, a conductive PPy-PLLA fiber-film with aligned core-sheath structure were fabricated by electrospinning of aligned PLLA and electrochemical deposition of PPy. Subsequently, L929 cells were cultured on the aligned conductive fibers, then the materials with cells were decellularized to obtain ECM-coated conductive fibers-film (ECM-CFF). Effect of ECM and aligned fibers under ES on adhesion and differentiation PC12 cells, neurite alignment and elongation were analyzed.

2. Materials and methods

2.1. Electrospinning of aligned fiber-film

First, dissolving PLLA (0.24 g) in dichloromethane/dimethyl formamide (DCM/DMF, v/v = 9/1, 4 mL) to obtain a homogenous solution with PLLA concentration of 6.0% (w/v). Then the aligned PLLA fibers were directly electrospun on the indium tin oxide-coated PET films ($20 \times 20\,mm^2$) placed on the rotating drum. The electrospinning conditions included an electrical field of $1\,kV\,cm^{-1}$, a flow rate of $1.0\,mL\,h^{-1}$ of PLLA solution and the speed of 600 rpm of the drum, to obtain well aligned fibers. Finally the prepared fiber-films were air-dried for further use.

2.2. Electrochemical deposition of conductive PPy sheath

The electrochemical deposition was performed on the aligned PLLA fiber-film ($20 \times 20\,mm^2$) with a conventional two-electrode configuration at room temperature (RT) by a constant voltage resource (Qianfeng Electronic Co., China) under galvanostatic mode. PLLA fiber-film as the working electrode and the aluminum sheet ($20 \times 20\,mm^2$) as the counter electrode, were placed in an aqueous solution of 0.2 M Py and 0.05 M DBS with a current density of $0.5\,mA\,cm^{-2}$ for 15 min, and PPy nanoparticles (NPs) were deposited on the surface of PLLA fibers. After washed with deionized water and alcohol, respectively, the fabricated conductive fibers-films (CFF) were dried in a vacuum oven at RT for further use.

2.3. Coating ECM on conductive fiber-film

A special device was assembled to exert ES on seeded cells (Fig. S1). According to the product instruction, two parts in polydimethyl-siloxane (PDMS) encapsulants are mixed thoroughly at the ratio of 10:1 (glue/curing agent, wt/wt). Subsequently, the mixed encapsulant was degased in the vacuum for 1 h, then coated on the glass slide to form a thin adhesive layer, and heated at 60 °C for 1 h to form the half-dry pre-cured glue. Each CFF was placed on pre-cured glue, and fixed on the glass slide through the low permeability and complete curing of encapsulant in another 2 h. A glass well (12 mm of diameter, 12 mm of length) was attached on CFF with pre-curing PDMS as a sealant (Fig. S1a). In ES test, two aluminum tapes were placed perpendicularly to the fiber axis and tightly clipped (Fig. S1b). All samples were sterilized by ethylene oxide for further use.

L929 cells were cultured in RPMI 1640 medium complemented with 10% newborn calf serum and 1% penicillin-streptomycin solution at 37 °C in a humidified 5% CO_2. When the cultures reached to 90% confluence, the cells were detached from the flasks using 0.25% trypsin. Then cells were seeded on each well with CFF at a density of 1×10^4 cells·well^{-1}, and cultured for 7 days, and the media were changed every other day. Subsequently, the composite scaffolds with cells were washed in PBS, and L929 cells on the scaffolds were lysed in a mixture solution of 0.5% Triton X-100 and 20 mM NH_4OH for 5 min [33], exposing uniformly ECM coated on the surface of fibers. Finally, ECM-coated conductive fiber-film (ECM-CFF) was gently washed five times with PBS, and kept in 4 °C for further use.

2.4. Characterizations of various fiber-films

The morphology of bare conductive fiber-film (bCFF) and ECM-CFF was observed under scanning electronic microscopy (SEM, S4800, Hitachi). Then the size of fiber-film was obtained by analyzing SEM micrographs with ImageJ. Four-point probes resistivity measurement system (RTS-9, China) was used to analyze the conductivity of CFF at the perpendicular and parallel fiber axis directions. Alignment degree among fibers was also assessed with previously reported method [34]. Fourier transmission infrared spectra (FTIR) was obtained to analyze the composition of ECM-CFF, bCFF, PLLA fiber-film and PPy film with an attenuated total reflection system. A CHI660D electrochemical workstation was used to record impedance curves of working electrode of ECM-CFF after the daily ES of 100 mV/cm was exerted to ECM-CFF. A solution of phosphate buffered saline (PBS, 0.1 M, pH = 7) was used as the electrolyte in a three-electrode cell configuration. The counter electrode was platinum sheet ($2 \times 3\,mm^2$) and an Ag/AgCl electrode was used as reference electrode. An AC sinusoidal signal with 5 mV amplitude was used to analyze impedance in a frequency range of 10^{-1} to 10^5 Hz. Cyclic voltammogram was also recorded with CHI660D electrochemical workstation. The potential on the electrodes from −0.8 to 0.8 V *vs* Ag/AgCl with scanning rate of $100\,mV\,s^{-1}$ was exerted.

To confirm the components and distribution of L929-derived ECM on the scaffold, immunofluorescent staining was performed according to the instructures. Laminin, collagen IV and fibronectin were chosen as biomarkers of ECM components, according to the previous report [35]. In order to avoid staining non-special components, ECM-CFF samples were blocked in 5% BSA-PBS for 1 h at RT, and stained overnight at 4 °C with primary polyclonal antibodies: rabbit anti-rat against laminin (1:200, Boster), rabbit anti-rat against collagen (1:200, Boster), and rabbit anti-rat against fibronectin (1:200, Boster), respectively. Then appropriate fluorescein–labelled secondary antibodies (1:50, Boster) were