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Polypyrrole-Coated Electrospun PLGA Nanofibers for Neural Tissue Applications

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Abstract

Electrospinning is a promising approach to create nanofiber structures that are capable of supporting adhesion and guiding extension of neurons for nerve regeneration. Concurrently, electrical stimulation of neurons in the absence of topographical features also has been shown to guide axonal extension. Therefore, the goal of this study was to form electrically conductive nanofiber structures and to examine the combined effect of nanofiber structures and electrical stimulation. Conductive meshes were produced by growing polypyrrole (PPy) on random and aligned electrospun poly(lactic-co-glycolic acid) (PLGA) nanofibers, as confirmed by scanning electron micrographs and X-ray photon spectroscopy. PPy-PLGA electrospun meshes supported the growth and differentiation of rat pheochromocytoma 12 (PC12) cells and hippocampal neurons comparable to non-coated PLGA control meshes, suggesting that PPy-PLGA may be suitable as conductive nanofibers for neuronal tissue scaffolds. Electrical stimulation studies showed that PC12 cells, stimulated with a potential of 10 mV/cm on PPy-PLGA scaffolds, exhibited 40–50% longer neurites and 40–90% more neurite formation compared to unstimulated cells on the same scaffolds. In addition, stimulation of the cells on aligned PPy-PLGA fibers resulted in longer neurites and more neurite-bearing cells than stimulation on random PPy-PLGA fibers, suggesting a combined effect of electrical stimulation and topographical guidance and the potential use of these scaffolds for neural tissue applications.

Keywords

polypyrrole; nanofibers; nerve tissue engineering; electrical stimulation; PC12 cells; hippocampal neurons

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1. Introduction

Electroconducting polymers (i.e., polypyrrole (PPy), polythiophene (PT), polyaniline (PANI), poly(3,4-ethylenedioxythiophene) (PEDOT)) exhibit excellent electrical and optical properties and have been explored in the past few decades for a number of applications including microelectronics, polymer batteries, and actuators [1,2]. In particular, because of its ease of synthesis, cytocompatibility, and good conductivity, PPy has been the most extensively studied for biological and medical applications, such as biosensors and tissue engineering scaffolds [3]. This electroconducting polymer has been recognized as a promising scaffold material to electrically stimulate neurons and nerve tissues for therapeutic purposes such as nerve tissue engineering scaffolds and neural prostheses [3,4]. Schmidt et al. first electrically stimulated PC12 cells through PPy films and observed the promotion of neurite outgrowth from the cells, demonstrating the potential use of electroconducting polymers for nerve tissue engineering scaffolds [5]. Subsequent studies have focused on improving the electroconducting polymer scaffolds by incorporating various cues, such as neurotrophins [6], cell adhesive molecules [7,8], and topographical features [9], emphasizing the importance of multiple cues for improved modulation of neuronal responses [10]. For example, Gomez et al. electrochemically synthesized PPy microchannels to fabricate electroconductive, topographical substrates for neural interfacing and found that PPy microchannels facilitated axon establishment of rat embryonic hippocampal neurons [9]. However, these studies involve relatively planar substrates which are ideal model surfaces for characterizing cell responses to electric fields, but lack the three-dimensional architecture necessary for organization of a functional nerve tissue.

Electroconducting nanofibers may be a means to translate model studies on well-defined topographical features into scaffold materials for nerve regeneration. Nanofibers present unique topographical surfaces of which features (i.e., fiber diameter and orientation) affect cellular behaviors of many cells [11–13]. For example, Yang et al. found that immortalized neural stem cells cultured on aligned poly(L-lactic acid) nanofibers extended longer neurites than cells on random nanofibers and aligned microfibers of the same composition [12]. Several attempts to synthesize conducting nanofibers for tissue engineering applications have been described in the literature. Lelkes and colleagues electrospun polyaniline-gelatin blends to produce electroconducting nanofibers, which displayed good conductivities ranging from 0.01 to 0.02 S/cm. These fibers supported the growth and proliferation of cardiac rat myoblasts [14]. Also, carbon nanofiber composites of polycarbonate urethane were produced for neural and orthopedic interfaces [15]. Depending on compositions of the blends, mechanical, and electrical properties as well as cell adhesion were varied. As an alternative, electroconducting polymers, mostly PPy, have been deposited on fiber templates, which can be achieved by in situ chemical oxidation of PPy in a polymerizing solution or by oxidation of monomers deposited on the substrates in vapor phase followed by oxidant treatment. This PPy deposition method enables the simple production of conducting fibers using template fibers [16,17]. For example, Zhang and colleagues deposited PPy on woven fabrics of poly(ethylene terephthalate) (> 20 μm in diameter, 10^2 – 10^5 ohm/square) for in vitro cytocompatibility studies for the application of vascular prostheses [18,19]. However, there have been fewer studies on nano-scaled fibers for tissue engineering scaffolds. More importantly, application of electrical potential of nerve cells using electroconducting micro-/nano- fibers has not been explored yet.

In this article, we produced polypyrrole-coated electrospun PLGA nanofibers (PPy-PLGA), as electroconducting nanofibers, for neural tissue applications. Because these components, PPy and PLGA, are non-cytotoxic in vivo as well as in vitro [20–22], the PPy-PLGA product was expected to be appropriate for ultimate use as implantation materials. To retain the fibers under submicron size and to take advantage of submicron scaled features, we deposited nano-thick PPy onto PLGA nanofibers. Also, oriented fibers were electrospun as template meshes for the

PPy-coating to study contact guidance (i.e., neurite/axon alignment). For in vitro neuronal culture, we cultured two different types of neurons – PC12 cells and rat embryonic hippocampal neurons. We also performed electrical stimulation of PC12 cells on these cytocompatible electroconductive nanofibers according to previous protocols [6,23] to demonstrate their potential uses as nerve tissue engineering scaffolds for the regeneration of injured peripheral and central nerves.

2. Materials and Methods

2.1 Materials

All chemicals, cell culture supplements, and disposable tissue culture supplies were purchased from Sigma (St Louis, MO), Hyclone (Logan, UT), and BD (Brookfield, NJ), respectively, unless otherwise noted.

2.2 Electrospinning

The 75/25 poly(lactic-co-glycolic acid) (PLGA) (inherent viscosity 0.55–0.75 dL/g, Lactel Biodegradable Polymers, Birmingham, AL) was used for electrospinning as described previously [13]. In brief, PLGA was dissolved in hexafluoro-2-propanol (HFIP). Aluminum foil was wrapped around the 7.6 cm drum. The polymer solution (7.0 or 6.5 wt% PLGA) was electrospun with a syringe equipped with a 22 gauge steel needle using a 15 kV potential, a throw distance of 15 cm, and a syringe flow rate of 3 mL/h. Random fibers (RF) were electrospun from 6.5 wt% polymer solution on a stationary collector, and aligned fibers (AF) were obtained from 7.0 wt% polymer solution on a rotating drum at a linear velocity of 6.4 m/s to achieve similar diameters with different fiber orientations because fibers generally exhibit a smaller diameter on a rotating collector [13]. After electrospinning, PLGA meshes were air dried for 2 days to remove residual HFIP. Meshes were cut into 15 × 15 mm squares, and then carefully removed from the aluminum foil.

2.3 Polypyrrole Coating

Differently oriented PPy-PLGA meshes, PPy-RF and PPy-AF, were synthesized by coating PPy on random and aligned PLGA, respectively. To do this, pyrrole was purified by passing it through a column of activated basic alumina before use. A PLGA mesh (15 × 15 mm) was put into 2 mL aqueous solution of 14 mM pyrrole and 14 mM sodium para-toluene sulfonate (pTS) (Aldrich) in a 15 mL polypropylene tube, followed by ultrasonication for 30 s to allow the mesh to be saturated with pyrrole solution. The mesh was incubated at 4°C for 1 h. Then, 2 mL ferric chloride solution (38 mM) was added to the tube and incubated with shaking at 4°C for 24 h for the polymerization and deposition of PPy on the PLGA mesh. The polymerization of PPy from pyrrole involves the incorporation of chlorine and pTS, of which elements can be detected using XPS. Black PPy-coated mesh was sonicated for 1 min, washed with copious amounts of deionized water, and then transferred onto a clean glass slide. PPy-PLGA was dried in a vacuum oven at room temperature for 2 days.

2.4. Fiber Characterization

2.4.1. Scanning Electron Microscopy (SEM)—SEM was used to characterize fiber diameter and orientation of electrospun PLGA meshes and PPy-PLGA meshes. SEM images of electrospun PLGA fibers and PPy-PLGA fibers were acquired with a Zeiss SUPRA 40 VP Scanning Electron Microscope (Carl Zeiss SMT, Thornwood, NY). Non-coated PLGA samples were sputter-coated with 5 nm of palladium using a Cressington Scientific Instruments Model 208HR (Cranberry, PA) prior to taking SEM images. PPy-PLGA fibers were imaged using SEM without metal coating. The SEM images were analyzed using ImagePro Plus software (ICube, Crofton, MD) or ImageJ (NIH) for fiber diameter and macroscale fiber

orientation as previously described [13]. PPy shell thickness was calculated from the differences of fiber diameters between non-coated fibers and PPy-PLGA fibers. The degree of fiber alignment was characterized by the wrapped normal distribution and reported as angular standard deviation (ASD), in which a smaller value of ASD represents a greater alignment of the individual fibers.

2.4.2. Surface Resistance—Surface resistance of PPy-PLGA meshes was measured as previously described [24]. Two silver wires separated by 1 cm were placed onto the sample. Resistance (R) was measured between the two silver electrodes using a digital multimeter (DM-8A, Sperry Instrument, Milwaukee, WI). Surface resistance (R_s) was calculated as follows:

$$R_s = R \times W/D,$$

where W is the sample width and D is the distance between the two silver electrodes.

2.4.3. X-ray Photon Spectroscopy (XPS)—XPS was used to characterize the surface compositions of PPy-coated PLGA fibers. High-resolution spectra of elements were obtained using a Kratos AXIS Ultra XPS system (Chestnut Ridge, NY). A monochromatic Al $K\alpha_1$ source was employed. Typical operating conditions were 1×10^{-9} Torr chamber pressure, and 15 kV and 150 W for the Al X-ray source. High-resolution elemental scans were collected with a pass energy of 20 eV at takeoff angles of 90 degrees between the sample and analyzer. Calibration of the binding energy was performed by setting C-C/C-H components in C_{1s} peak at 284.6 eV. Peak deconvolution was performed using XPSPEAK software (The Chinese University of Hong Kong).

2.4.4. Stability Test—Biodegradation and stability of PPy-PLGA in a physiological solution were determined by SEM and measurements of surface resistance. Samples were incubated in phosphate-buffered saline (PBS) at 37°C for 2 weeks. Resistance was measured from 5 samples of PPy-RF as described above. For SEM images, the samples were washed with deionized water twice to remove salts and dried in a vacuum chamber for 24 h prior to imaging.

2.5 Cell Culture

2.5.1 Sample Preparation—PPy-PLGA meshes were tested for their ability to support cell attachment and differentiation. Each mesh was placed on a thin poly(dimethylsiloxane) (PDMS, Sylgard® 184, Dow Corning, Midland, MI) film on a glass slide. Two silver wires (round 30 gauge, Hauser & Miller, St. Louis, MO) were then placed on both sides of the mesh, followed by covering them with a thin PDMS well (1 cm $\times$ 1 cm $\times$ 1 mm inner well dimension) to serve as a sealant and to prevent the wires from direct contact with medium. A Plexiglas well (1 cm $\times$ 1 cm $\times$ 1 cm inner well dimension) was placed on top of the assembled system (Figure 1). For the aligned fibers, two electrodes were placed perpendicular to the major direction of the fibers. The assembly was tightly clipped and sterilized by exposure to UV for 1 h. The samples were incubated overnight in a sterile solution of rat tail type I collagen (0.1 mg/mL in deionized water) for PC12 cells and poly-D-lysine (0.1 mg/mL in deionized water) for hippocampal cells, respectively. The substrates were washed twice with sterile deionized water and incubated in sterile PBS solution for 2 days to remove unreacted compounds.

2.5.2 PC12 Cell Culture and Immunostaining—PC12 cells were maintained at 37°C in a humid, 5% CO₂ incubator in F-12K culture medium (Sigma) containing 15% heat-inactivated horse serum (Hyclone), 2.5% fetal bovine serum (Hyclone), and 1% Penicillin-Streptomycin solution (Sigma). Cells were passaged weekly using a 0.25% trypsin-EDTA solution (Sigma).

Cells were primed by culturing them in medium containing 50 ng/mL nerve growth factor (NGF) three days prior to an experiment. The primed PC12 cells were inoculated at a density of 2×10^4 cells per well and cultured for 2 days. Four substrates for each condition were tested ($n=4$).

Electrical stimulation was studied to demonstrate that conducting nanofibers (PPy-PLGA) may be beneficial as neuronal tissue engineering scaffolds. Electrical stimulation of PC12 cells was performed on random and aligned PPy-PLGA meshes according to the experimental conditions as previously reported [6,23]. PC12 cells were inoculated at 2×10^4 cells per well and allowed to attach to the PPy-PLGA meshes. After 24 h in culture, a constant electrical potential of 100 mV/cm or 10 mV/cm was applied across two electrodes for 2 h in the incubator using an AFRDE5 bipotentiostat (a Pine Instrument, Raleigh, NC). Cells were analyzed 24 h after electrical stimulation. Four substrates for each condition were employed ($n=4$).

After culture, the PC12 cells were fixed using 4% paraformaldehyde and 4% sucrose in PBS buffer for 15 min. The cells were permeabilized in 0.1% Triton X-100 (Fluka) and 2% bovine serum albumin (Sigma) in PBS for 15 min, followed by blocking with 2% bovine serum albumin in PBS for 30 min at room temperature. PC12 cells were stained with Alexa Fluor 488-labeled phalloidin (Invitrogen) for 30 min for actin filaments and with 4',6-diamidino-2-phenylindole dilactate (DAPI, Invitrogen) nuclear stain, washed with PBS buffer twice, and stored at 4°C until analysis.

2.5.3 Hippocampal Neuron Culture and Immunostaining—Embryonic hippocampal neurons were isolated from commercial rat hippocampus (E-18, BrainBits Springfield, IL) according to the supplier's protocol. In brief, hippocampal tissue was treated in papain (Warthington, Likewood, NJ) solution (4 mg/mL in Hibernate E medium (BrainBits)) and triturated using a fire-polished Pasteur pipette. Cells were collected by centrifugation (200 g, 1 min) and suspended in Neurobasal medium, supplemented with 2% B-27 supplement (Invitrogen), 0.5 mM L-glutamine (Fisher), 0.025 mM glutamic acid (Sigma), and 1% Penicillin-Streptomycin solution (Sigma). The cells were inoculated into each well (2×10^4 cells per well) and cultured in medium at 37°C in a humid, 5% CO₂ incubator for 24 h. Four substrates per each condition were employed ($n=4$).

Embryonic hippocampal neurons cultured on the meshes were fixed in a solution of 4% paraformaldehyde and 4% sucrose in PBS for 20 min at room temperature. The cells were permeabilized with 0.1% Triton X-100 and 3% goat serum (Sigma) in PBS for 20 min, and washed twice with PBS, followed by treatment with blocking solution (3% goat serum in PBS buffer) for 1 h at 37°C. Mouse tau-1 antibody (Chemicon, Temecula, CA) (1:200 in blocking solution) was added to the sample, incubated at 4°C overnight, and washed with PBS twice (10 min each). The cells were stained with Alexa Fluor 488-labeled goat anti-rat IgG (Invitrogen) (1:200 dilution in blocking solution) at 4°C for 5 h, washed with PBS twice (10 min, each), and stored at 4°C until analysis.

2.6 Immunofluorescence and Image Analysis

Fluorescence images of PC12 cells and hippocampal neurons were acquired using a fluorescence microscope (IX-70, Olympus, Center Valley, PA) equipped with a color CCD camera (Optronics MagnaFire, Goleta, CA). The fluorescence images were processed and analyzed using Adobe Photoshop and Image J (NIH) software.

The numbers of cells on the sample meshes were determined by counting nuclei stained with DAPI dye from the randomly acquired fluorescence images. Axon/neurite length was measured as a linear distance between the cell junction and the tip of an axon/neurite. For PC12 cells, data was collected for neurite lengths greater than 5 μ m [25]. Neurite outgrowth was reported

in terms of median length because neurite lengths were not normally distributed [5,6,25]. Also, the percentages of PC12 cells with neurites and the numbers of neurites per cell (for cells that expressed at least one neurite) were calculated. More than 600 PC12 cells were analyzed for each condition. For hippocampal neurons, differentiation is marked by the formation and elongation of single axons. Therefore, axon establishment (neuron polarization) – defined when neurite processes were at least twice as long as the length of the cell body – and axonal length were measured for embryonic hippocampal neurons on fibers [9,26]. The averages and standard errors of the means were calculated for the percentages of polarized hippocampal neurons and average axon lengths. More than 100 neurons were analyzed per substrate.

2.7 Assessment of Cellular Morphology

Cellular morphologies on the fibers were visualized using SEM. The fixed cells were dehydrated using increasing ethanol/water concentrations (30% for 45 min; 50% for 30 min, 70%, 85%, 90%, 95%, and absolute ethanol for 10 min each). Samples were dehydrated with hexamethyl disilazane (HMDS) (Sigma) and dried in air overnight. Platinum/palladium was coated on the sample with 10 nm in thickness using a sputter coater. SEM images were obtained with Zeiss SUPRA 40 VP Scanning Electron Microscope.

2.8 Statistics

Averages and standard deviations were calculated and reported from at least four samples per each condition. The statistical significance between two groups was determined using a Student's *t*-test ($p < 0.05$) for the numbers of the cells, percentages of neurite-bearing PC12 cells, numbers of neurites per PC12 cell, percentages of polarized hippocampal neurons, and axon lengths of hippocampal neurons. For the evaluation of PC12 neurite extension, median lengths were calculated and reported for each condition because the measured neurite lengths were not normally distributed. Statistical differences between medians were calculated with a Mann-Whitney U test [27] ($p < 0.05$).

3. Results and Discussion

3.1 Electrospinning PLGA Nanofibers

Template PLGA nanofibers for PPy-coating were used in this study because of PLGA's well-investigated cytocompatibility, biodegradability, and suitability for various biomedical applications [11,20]. The PLGA nanofiber meshes were obtained by electrospinning PLGA (75/25) solution as previously described [13]. Highly aligned nanofibers (AF) and random nanofibers (RF) were collected from PLGA polymer solution on a rotating and stationary collector, respectively. The SEM images of the electrospun fibers indicate that both meshes (AF and RF) have uniform fibrous features on the surface and that AF meshes exhibit highly aligned fibers (Figure 2). The fiber diameters, which were determined by analyzing the SEM images, were $0.25 \pm 0.11 \mu\text{m}$ for AF and $0.36 \pm 0.13 \mu\text{m}$ for RF. For degree of fiber orientation, the AF had a value of angular standard deviation (ASD) of 32.9° , whereas the RF had a larger ASD (54.7°). High ASD reflects randomly oriented fibers. These nanofibers, with nanometer scale dimensions, provide highly porous, nanofibrous topographies capable of modulating cellular responses in various cells [11,28].

3.2 Synthesis of PPy-Coated PLGA Nanofibers

To produce electroconducting nanofibers, PPy deposition was performed on the electrospun PLGA nanofibers (RF and AF) in a polymerizing solution containing pyrrole, pTS, and FeCl_3 as a monomer, a dopant, and an oxidizing reagent, respectively. As pyrrole was polymerized, polymeric products (PPy) aggregated in solution or deposited on the fiber surfaces, depending on reaction conditions, forming conducting shells. Various polymerization

conditions were explored to arrive at an optimal set of parameters to produce conductive and clean meshes (Table 1). The reaction conditions (i.e., reactant concentrations, reaction times) strongly influenced coating properties and electrical properties of the PPy-PLGA fibers, such as uniformity, conductance, and morphologies of the PPy shells, which is consistent with other literature [16,29]. As shown in Figure 3, incomplete coverage of PPy, which resulted in non-conducting meshes, was observed at low concentrations of the reactants and/or a short reaction time. On the other hand, high concentrations of the reactants resulted in PPy aggregates in solution and non-uniform deposition of the PPy covering on the fibers. The PPy-PLGA products, synthesized under the optimized condition (7 mM pyrrole, 7 mM pTS, 19 mM ferric chloride, and 24 h), consist of PLGA fiber cores and PPy shells with complete coverage and no PPy aggregates (Figure 3 and Figure 4). PPy-coated AF meshes (PPy-AF) retained the aligned fiber structures, which were similar to their non-coated template meshes (AF) (Figure 2). The SEM images of the PPy-PLGA were acquired without treatment of the samples with metal coating, indicating qualitatively good electrical conductance of the fibers. Some PPy aggregates were observed on the fiber surfaces, but they were loosely bound and could be removed by successive washes. The PPy-PLGA fibers had fiber diameters of 520 ± 150 nm for PPy-RF and 430 ± 180 nm for PPy-AF, with 85 ± 41 nm shell thickness. Nanometer-scale thick PPy deposition was accomplished on the template nanofibers, of which features were retained (i.e., fiber orientations).

3.3 Characterization of PPy-coated PLGA Nanofibers

To characterize electroconducting properties of the PPy-PLGA meshes, surface resistance (R_s) was measured. Random PPy-PLGA had an $R_s = 1.7 \pm 0.6 \times 10^4$ Ohm/square. This value was insensitive to the direction of measurement, which was reasonable because the PPy-RF fibers were randomly oriented and had multiple contacts among coated fibers. On the other hand, R_s values of aligned PPy-PLGA varied with direction of the fibers: $R_s = 7.4 \pm 3.2 \times 10^3$ Ohm/square along the fiber direction and $R_s = 9.0 \pm 6.0 \times 10^4$ Ohm/square perpendicular to the major fiber direction. These results suggest that current is conducted primarily along the fiber axis.

To characterize the surfaces of the fibers, XPS analysis was performed. Table 2 summarizes the elemental compositions of the sample surfaces. For the PPy-PLGA, nitrogen, chlorine, and sulfur atoms were detected, whereas they were absent in the uncoated PLGA samples. Doping levels of the PPy components were calculated from the atomic ratio of Cl and S to N from the high resolution XPS spectra. The doping level of PPy-PLGA was 0.27 and close to that for oxidized PPy (about 0.3), as previously investigated [3]. pTS and Cl ions accounted for 80% and 20%, respectively, in total molar amounts of dopant. High-resolution spectra of the PPy-PLGA were decomposed according to previous reports [30]. The C_{1s} spectrum of PPy-PLGA (Figure 5) revealed substantial changes in the spectrum after PPy-coating, which can be attributed to new signals at 285.8 eV (C-N), 287.2 eV (C=N), and 288.9 eV (C=N⁺). Peaks, related to the PLGA cores, were diminished, indicating PPy-coating shields the surface of PLGA. In addition, Figure 5b showed changes in the high resolution spectra of N_{1s} after PPy-coating with new signals at 397.8 eV (=N-), 399.8 eV (-NH-), 401.2 eV (C-N⁺), and 402.2 eV (C=N⁺). No significant peaks were observed in the N_{1s} spectrum of the PLGA sample, as expected. These XPS spectra obtained from the PPy-PLGA are consistent with those from pristine PPy in other literature [24,34], demonstrating surface deposition of PPy on the PLGA fibers.

3.4 Stability of PPy-coated PLGA Nanofibers

In the present study, we selected PLGA as a model material that is biodegradable and non-cytotoxic for the production of nanofiber templates. Degradation of implanted scaffolds is beneficial to enable tissue regeneration and integration and to avoid subsequent surgical removal of scaffolds [31]. However, rapid degradation of PLGA fibers in vivo would lead to

a quick loss of electrical and mechanical properties of the conducting nanofibers, which may limit long-term performance of the scaffolds for certain applications. Thus, electrical and mechanical stability and biodegradation of PPy-PLGA meshes were assessed in a physiological solution (PBS at 37°C) for 2 weeks. After 2 weeks, surface resistance increased from 11.4 Ohm/square to 23.6 Ohm/square. This increase in the surface resistance likely results from de-doping and/or over-oxidation of PPy, which have been reported previously [24,32,33], and not from fragmentation of the PPy shell. This is confirmed by SEM analysis, which showed no significant degradation of the PLGA fiber cores, no substantial delamination or fragmentation of PPy shells, and nearly identical morphologies to the as-prepared PPy-PLGA fibers (Figure 6). We speculate that PPy shells may partly contribute to structural stability by preventing the inner PLGA fibers from coming in contact with water (less opportunity of hydrolysis). Degradation (residence time) of the scaffolds in vivo could be further modified by coating PPy on different biodegradable polymer fibers which have different degradation rates (e.g., poly- ϵ -caprolactone, polydioxanone, copolymers having various compositions of polylactide and polyglycolide) [31].

In addition, PPy doped with small anions has been reported to be brittle, which would be an obstacle for long-term performance with electrical stimulation through the scaffolds [34]. As we mentioned above, our 2 week stability test showed little fragmentation or delamination of the PPy shells, which may be partly attributed to mechanical stabilization of the PPy coating by the underlying PLGA cores. The more ductile PLGA likely imparts flexibility to the overall composite structure, similar to previous observations in which PLGA films laminated to films of PPy improved the overall handling and mechanical properties of PPy [5]. Yet, it may be still desirable to improve the mechanical properties and adhesiveness of PPy to template fibers for various biomedical applications. Use of a larger dopant (e.g., polystyrene sulfonate) will minimize de-doping [32] and chemical conjugation of pyrrole onto the template nanofibers prior to PPy polymerization may provide a strong interface between the PPy coating and the core fibers [19].

3.5 Cell Culture Experiments

In an effort to study properties of the PPy-PLGA scaffolds for neuronal applications, we cultured PC12 cells and embryonic hippocampal neurons on the scaffold materials. PC12 cells are the most widely studied cell type for neuritogenesis in response to various extracellular cues such as neurotrophins and electrical stimulation [5,6]. This cell line has been used as an experimental model of the sympathetic nervous system [35]. On the other hand, the embryonic hippocampal neuron is of CNS origin and plays an important role in learning-and-memory and processing of spatial information in the brain [36]. Embryonic hippocampal neurons have been widely used for initial axon establishment in neuronal development [37]. It has been also noted that the cellular responses of these neurons are influenced by external electrical fields [38] and topographical surfaces of substrates (i.e., pores, grooves) [9,39]. Therefore, we cultured PC12 cells and hippocampal cells on the PPy-PLGA meshes for in vitro cell culture to demonstrate the utility of these meshes bearing nanofibrous topographical features and electrical activities for nerve tissue engineering applications. Non-coated PLGA fibers, displaying similar fiber features (fiber diameters and orientations) to the PPy-PLGA fibers, were also used as controls for comparison.

The PC12 cells were cultured on both PPy-PLGA (PPy-RF and PPy-AF) and uncoated PLGA (RF and AF) in an NGF-containing medium for 2 days. Cell numbers, counted from the numbers of DAPI-stained nuclei from random fluorescence images, were 148 ± 15 (RF), 111 ± 39 (PPy-RF), 112 ± 27 (AF), and 128 ± 18 per image (PPy-AF). These differences were not statistically different, indicating that the PPy-coating does not affect the adhesion or viability of the PC12 cells. Fluorescence images of PC12 cells stained for actin filament (Figure 7) show

that the cells formed neurites on all substrates. Analysis of neurite outgrowth was performed from at least 300 neurites. The results indicated that median lengths of the neurites on the meshes were 12.3 μm (RF, $n=507$), 12.8 μm (PPy-RF, $n=424$), 16.0 μm (AF, $n=391$), and 15.3 μm (PPy-AF, $n=523$), where n denotes the numbers of analyzed neurites. Longer neurites were formed on the aligned fibers (AF and PPy-AF) than on the random fibers (RF and PPy-RF), $p<0.01$. The median lengths were similar between the PPy-PLGA fibers and their template PLGA fibers having similar fiber dimensions: $p=0.32$ between RF and PPy-RF; $p=0.31$ between AF and PPy-AF. These results suggest that fiber features played important roles in neurite outgrowth regardless of the PPy-deposition. Other types of neurons have shown similar cellular responses to fiber orientation, including dorsal root ganglia explants [40] and neural stem cells [12], which formed longer neurites/axons on aligned nanofibers and extended their neurites along the fiber strands. Most neurites were aligned along the major direction of the meshes (AF and PPy-AF) (Figure 7). SEM images show that PC12 cells on PPy-PLGA (both PPy-RF and PPy-AF) formed intimate contact with multiple fibers (Figure 8). Cells on the aligned fibers were also found to form more elliptical morphologies elongated in the major fiber direction.

Likewise, embryonic hippocampal neurons were also cultured on the fiber samples and immuno-stained for analysis (Figure 9). No significant difference was found between PLGA fibers and PPy-coated fibers in terms of the number of the cells and the fraction of cells with established axons (i.e., polarized neurons). After 24 h in culture, $27.8 \pm 3.2\%$, $28.9 \pm 9.7\%$, $30.1 \pm 4.3\%$, and $28.8 \pm 8.8\%$ of the hippocampal neurons established axons on RF, PPy-RF, AF, and PPy-AF, respectively. Average axon lengths were similar with a range of 60–65 μm for all the samples. These results suggest that nano-thick PPy-coating on PLGA nanofibers did not affect initial differentiation (i.e., axon establishment) and resulted in similar results among the substrates. Fiber orientation did not influence axon establishment or axon elongation of the hippocampal neurons. This finding is consistent with our previous studies with various PLGA fibers (400 nm – 2.4 μm in diameter and different degrees of fiber orientation) for axon formation and elongation of embryonic hippocampal neurons [41], where we found that axon establishment and average length were not significantly different among the fibers, although more axons were found on the cells cultured on the fibers compared to smooth PLGA substrates. Contact guidance of hippocampal axons was also observed on the aligned fibers (AF and PPy-AF) similarly to the PC12 cells. Most axons were found to grow along the major direction of the aligned fibers (Figure 9). Consequently, the growth and differentiation of hippocampal neurons (i.e., neuron polarization and axon elongation) were supported by the PPy-PLGA fibers as well as the non-coated PLGA fiber controls. As results, the PPy-PLGA fibers supported the attachment and differentiation of PC12 cells and embryonic hippocampal cells in comparison with the uncoated PLGA fibers, which may be attributed to cytocompatibility of the individual components (PPy and PLGA) [20–22].

Electrical stimulation of neurons on electroconducting scaffolds was performed to demonstrate the use of PPy-PLGA meshes as potential nerve tissue engineering scaffolds delivering electrical cues through nanofibers. We electrically stimulated PC12 cells on PPy-RF and PPy-AF fibers at the potentials of 10 mV/cm and 100 mV/cm according to previously described protocols [6,23] and analyzed neurite lengths, percentages of neurite-bearing cells, and numbers of neurites per cell [15,23,25]. Statistically significantly more neurite-bearing PC12 cells and longer neurites were observed with electrical stimulation compared to unstimulated controls ($p<0.01$) (Figure 10). Percentages of neurite-bearing cells were $18.0 \pm 2.3\%$ (0 mV/cm, PPy-RF), $34.5 \pm 1.9\%$ (10 mV/cm, PPy-RF), $28.6 \pm 3.5\%$ (100 mV/cm, PPy-RF), $28.8 \pm 3.2\%$ (0 mV/cm, PPy-AF), $40.4 \pm 3.9\%$ (10 mV/cm, PPy-AF), and $37.4 \pm 2.5\%$ (100 mV/cm, PPy-AF). On random PPy-PLGA fibers, the median lengths of neurites were 12.7, 18.9, and 15.6 μm for unstimulated cells, stimulated cells at 10 mV/cm, and at 100 mV/cm, respectively. This trend was similar for neurite lengths of 14.9 (unstimulated), 21.1 (10 mV/cm), and 17.0 μm (100

mV/cm) on aligned PPy-PLGA fibers. Neurite lengths for PC12 cells electrically stimulated via the conducting nanofibers were similar to those from previous studies of electrical stimulation of PC12 cells on plain PPy films. Schmidt et al. found longer median neurite lengths for electrically-stimulated PC12 cells (18.1 μm) on PPy films compared to unstimulated cells on the same substrates (9.5 μm) after 2 days in culture [5]. Gomez et al. also observed that the median neurite length for electrically stimulated PC12 cells was 12 μm , whereas the median length for unstimulated controls was 8.0 μm [6]. Electrical stimulation using aligned fibers also resulted in further promotion of neurite outgrowth compared to random fibers, in terms of neurite lengths and fractions of neurite-bearing cells, which suggests that fiber alignment and electrical stimulation act together to enhance neurite extension of PC12 cells. Interestingly, lower potential (10 mV/cm) resulted in more neurite-bearing cells than higher potential (100 mV/cm) on the random fibers ($p=0.035$), whereas this effect was not significant on the aligned fibers ($p=0.253$). However, cells stimulated with 10 mV/cm extended significantly longer neurites than cells stimulated with 100 mV/cm on both random and aligned fibers ($p<0.01$). Analysis of neurite numbers per cell indicated that a single cell had 1.4–1.8 neurites on average and that the differences were not significant for various conditions including fiber orientation and electrical stimulation, suggesting that nanofiber features and electrical stimulation do not dramatically influence the numbers of neurites. These results indicate that level of electrical potential has an impact on degree of stimulation and that a lower potential may be more favorable for promoting neurite outgrowth of PC12 cells. Zhang et al. found a similar trend with PC12 cells at various electrical currents, in which more neurite-bearing PC12 cells were observed below 10 μA ; promotion of neurite formation diminished as currents increased above 10 μA [42]. The exact effects of electrical stimulation are not fully understood; however, some mechanisms are postulated as follows: redistribution of membrane proteins responding to electrical field/current [43], decrease in membrane potentials more likely to cause membrane depolarization of neurons [44], and preferential deposition of biomolecules such as fibronectin on electrodes [23]. These non-cytotoxic, electroconducting nanofibrous scaffolds are suitable for electrically stimulating neurons and potentially enhancing nerve tissue regeneration.

4. Conclusion

Electrically conductive biomaterial scaffolds hold great promise in biomedical applications including neural tissue interfacing. We fabricated electroconducting nanoscaffolds using a simple method involving nano-thick deposition of PPy on electrospun PLGA fibers. The PPy-PLGA displayed electrical activity and the nanofibrous features. The nano-scaled sizes and fiber orientation of the template fibers were retained with PPy coating so that the conducting nanofibers provide general advantages of conventional electrospun nanofibers, such as high surface area to volume ratio, interconnecting pores, and nanofibrous topographies. The PPy-deposited fibers were characterized using various techniques including XPS and surface conductance measurement. In vitro cell culture using PC12 cells and embryonic hippocampal neurons demonstrated that compatible cellular interactions on the fabricated PPy-PLGA meshes are appropriate for neuronal applications and present topographies for modulating cellular interactions comparable to the PLGA control nanofibers. Finally, electrical stimulation of PC12 cells on the conducting nanofiber scaffolds improved neurite outgrowth compared to non-stimulated cells; the lower electrical potential of 10 mV/cm encouraged more neurite outgrowth than the higher potential of 100 mV/cm. In addition, further increases in neurite length and percentage of neurite-bearing cells were observed with electrical stimulation on aligned conducting nanofibers. This work will aid to design neuronal tissue interfaces integrated with topographical and electrical cues for use in nerve tissue scaffolds and for neural interfacing.

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References

1. Gurunathan K, Murugan AV, Marimuthu R, Mulik UP, Amalnerkar DP. Electrochemically synthesized conducting polymeric materials for applications towards technology in electronics, optoelectronics and energy storage devices. *Mater Chem Phys* 1999;61:173–91.
2. Jager EWH, Smela E, Inganas O. Microfabricating Conjugated Polymer Actuators. *Science* 2000;290:1540–5. [PubMed: 11090345]
3. Guimard NK, Gomez N, Schmidt CE. Conducting polymers in biomedical engineering. *Prog Polym Sci* 2007;32:876–921.
4. Ateh DD, Navsaria HA, Vadgama P. Polypyrrole-based conducting polymers and interactions with biological tissues. *J R Soc Interface* 2006;3:741–52. [PubMed: 17015302]
5. Schmidt CE, Shastri VR, Vacanti JP, Langer R. Stimulation of neurite outgrowth using an electrically conducting polymer. *Proc Nat Acad Sci USA* 1997;94:8948–53. [PubMed: 9256415]
6. Gomez N, Schmidt CE. Nerve growth factor-immobilized polypyrrole: bioactive electrically conducting polymer for enhanced neurite extension. *J Biomed Mater Res* 2007;81:135–49.
7. Sanghvi AB, Miller KP, Belcher AM, Schmidt CE. Biomaterials functionalization using a novel peptide that selectively binds to a conducting polymer. *Nat Mater* 2005;4:496–502. [PubMed: 15895095]
8. Song HK, Toste B, Ahmann K, Hoffman-Kim D, Palmore GT. Micropatterns of positive guidance cues anchored to polypyrrole doped with polyglutamic acid: a new platform for characterizing neurite extension in complex environments. *Biomaterials* 2006;27:473–84. [PubMed: 16112728]
9. Gomez N, Lee JY, Nickels JD, Schmidt CE. Micropatterned Polypyrrole: Combination of Electrical and Topographical Characteristics for Stimulation of Cells. *Adv Func Mater* 2007;17:1645–53.
10. Li GN, Hoffman-Kim D. Tissue-Engineered Platforms of Axon Guidance. *Tissue Eng* 2008;14(1): 33–50.
11. Pham QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications. *Tissue Eng* 2006;12:1197–1211. [PubMed: 16771634]
12. Yang F, Murugan R, Wang S, Ramakrishna S. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. *Biomaterials* 2005;26:2603–10. [PubMed: 15585263]
13. Bashur CA, Dahlgren LA, Goldstein AS. Effect of fiber diameter and orientation on fibroblast morphology and proliferation on electrospun poly(D, L-lactic-co-glycolic acid) meshes. *Biomaterials* 2006;27:5681–8. [PubMed: 16914196]
14. Li M, Guo Y, Wei Y, MacDiarmid AG, Lelkes PI. Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. *Biomaterials* 2006;27:2705–15. [PubMed: 16352335]
15. Webster TJ, Waid MC, McKenzie JL, Price RL, Ejiofor JU. Nanobiotechnology: carbon nanofibres as improved neural and orthopaedic implants. *Nanotechnol* 2004;15:48–54.
16. Kuhn, HH.; Child, AD. Electrically conducting textiles. In: Skotheim, TA.; Elsenauer, RL.; Reynolds, JR., editors. *Handbook of Conducting Polymers*. New York: Marcel Dekker; 1998. p. 993-1104.
17. Nair S, Nataragan S, Kim SH. Fabrication of electrically conducting polypyrrole-poly(ethylene oxide) composite nanofibers. *Macromol Rapid Commun* 2005;26:1599–603.
18. Jakubiec B, Marois Y, Zhang Z, Roy R, Sigot-Luizard MF, Dugré FJ, et al. In vitro cellular response to polypyrrole-coated woven polyester fabrics: Potential benefits of electrical conductivity. *J Biomed Mater Res* 1998;41:519–26. [PubMed: 9697023]
19. Zhang Z, Roy R, Dugré FJ, Tessier D, Dao LH. In vitro biocompatibility study of electrically conductive polypyrrole-coated polyester fabrics. *J Biomed Mater Res* 2001;57:63–71. [PubMed: 11416850]

20. Kohn, J.; Abramson, S.; Langer, R. Bioresorbable and bioerodible materials. In: Ratner, BD.; Hoffman, AS.; Schoen, FJ.; Lemons, JE., editors. *Biomaterials science*. London, UK: Elsevier Academic Press; 2004. p. 115-26.
21. Wang X, Gu X, Yuan G, Chen S, Zhang P, Zhang T, et al. Evaluation of biocompatibility of polypyrrole in vitro and in vivo. *J Biomed Mater Res* 2004;68(3):411–22.
22. Jiang XP, Marois Y, Traoré A, Tessier D, Dao LH, Guidoin R, et al. Tissue reaction to polypyrrole-coated polyester fabrics: An in vivo study in rats. *Tissue Eng* 2002;8(4):635–47. [PubMed: 12202003]
23. Kotwal A, Schmidt CE. Electrical stimulation alters protein adsorption and nerve cell interactions with electrically conducting biomaterials. *Biomaterials* 2001;22(10):1055–64. [PubMed: 11352099]
24. Jiang X, Tessier D, Dao H, Zhang Z. Biostability of electrically conductive polyester fabrics: An in vitro study. *J Biomed Mater Res* 2002;62:502–13.
25. Leach JB, Brown XQ, Jacot JG, DiMilla PA, Wong JY. Neurite outgrowth and branching of PC12 cells on very soft substrates sharply decreases below a threshold of substrate rigidity. *J Neural Eng* 2007;4:26–34. [PubMed: 17409477]
26. Dotti CG, Sullivan CA, Banker GA. The establishment of polarity by hippocampal neurons in culture. *J Neurosci* 1988;8(4):1454–68. [PubMed: 3282038]
27. Rostener, B. *Fundamentals of Biostatistics*. Vol. 6. Belmont, CA, USA: Thomson Brooks/Cole; 2006.
28. Murugan R, Ramakrishna S. Nano-featured scaffolds for tissue engineering: a review of spinning methodologies. *Tissue Eng* 2006;12:435–47. [PubMed: 16579677]
29. Malinauskas A. Chemical deposition of conducting polymers. *Polymer* 2001;42:3957–72.
30. De Giglio E, Guascito MR, Sabbatini L, Zamboni G. Electropolymerization of pyrrole on titanium substrates for the future development of new biocompatible surfaces. *Biomaterials* 2001;22:2609–16. [PubMed: 11519780]
31. Yang S, Leong KF, Du Z, Chua CK. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Eng* 2001;7(6):679–678. [PubMed: 11749726]
32. Fonner JM, Forciniti L, Nguyen H, Byrne JD, Kou YF, et al. Biocompatibility implications of polypyrrole synthesis techniques. *Biomed Mater* 2008;3(3):034124. [PubMed: 18765899]
33. Cui X, Wiler J, Dzaman M, Altschuler RA, Martin DC. In vivo studies of polypyrrole/peptide coated neural probes. *Biomaterials* 2003;24(5):777–87. [PubMed: 12485796]
34. Green RA, Lovell NH, Wallace GG, Poole-Warren LA. Conducting polymers for neural interfaces: challenges in developing an effective long-term implant. *Biomaterials* 2008;29(24–25):3393–9. [PubMed: 18501423]
35. De Giglio E, Guascito MR, Sabbatini L, Zamboni G. Electropolymerization of pyrrole on titanium substrates for the future development of new biocompatible surfaces. *Biomaterials* 2001;22:2609–16. [PubMed: 11519780]
36. Tischler AS. Chromaffin cells as models of endocrine cells and neurons. *Ann NY Acad Sci* 2002;971:366–70. [PubMed: 12438154]
37. Burgess N, Miguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. *Neuron* 2002;35(4):625–41. [PubMed: 12194864]
38. Fukata Y, Kimura T, Kaibuchi K. Axon specification in hippocampal neurons. *Neurosci Res* 2002;43(4):305–15. [PubMed: 12135774]
39. McCaig CD, Rajnicek AM, Song B, Zhao M. Has electrical growth cone guidance found its potential? *Trends Neurosci* 2002;25(7):354–9. [PubMed: 12079763]
40. Sapelkin AV, Bayliss SC, Unal B, Charalambou A. Interaction of B50 rat hippocampal cells with stain-etched porous silicon. *Biomaterials* 2006;27:842–6. [PubMed: 16098578]
41. Corey JM, Lin DY, Mycek KB, Chen Q, Samuel S, Feldman EL, et al. Aligned electrospun nanofibers specify the direction of dorsal root ganglia neurite growth. *J Biomed Mater Res A* 2007;83(3):636–45. [PubMed: 17508416]
42. Lee JY, Bashur CA, Gomez N, Goldstein AS, Schmidt CE. Enhanced polarization of embryonic hippocampal neurons on micron scale electrospun fibers. *J Biomed Mater Res A*. In press
43. Zhang Z, Rouabhi M, Wang Z, Roberge C, Shi G, Roche P, et al. Electrically conductive biodegradable polymer composite for nerve regeneration: electricity-stimulated neurite outgrowth and axon regeneration. *Artif Organs* 2007;31(1):13–22. [PubMed: 17209956]

44. Patel N, Poo MM. Orientation of neurite growth by extracellular electric fields. *J Neurosci* 1982;2(4):483–96. [PubMed: 6279799]
45. Patel N, Poo MM. Perturbation of the direction of neurite growth by pulsed and focal electric fields. *J Neurosci* 1984;4(12):2939–47. [PubMed: 6502213]

Figure 1. Schematic of PPy-PLGA mesh assembly (side view and top view) for in vitro cell culture and electrical stimulation studies. Silver wires were not included in the assemblies for cytocompatibility studies.

Figure 2.

Scanning electron micrographs of electrospun PLGA nanofibers, (a) and (b), and their PPy-coated fibers, (c) and (d). (a) Randomly-oriented nanofibers (RF), (b) aligned nanofibers (AF), (c) PPy-coated randomly oriented fibers (PPy-RF), and (d) PPy-coated aligned fibers (PPy-AF).

Figure 3.

Representative SEM images of PPy-coated PLGA fibers synthesized under various conditions; (a, c, e) at low magnification and (b, d, f) at high magnification. (a, b) Incomplete PPy-coating with a short reaction time (7.0 mM pyrrole, 7.0 mM pTS, 19 mM FeCl₃, and 8 h). (c, d) Complete coating without aggregates produced under an optimized condition (7.0 mM pyrrole, 7.0 mM pTS, 19 mM FeCl₃, and 24 h). (e, f) PPy aggregates are produced with a high concentration of reactant (7.0 mM pyrrole, 14.0 mM pTS, 19 mM FeCl₃, and 12 h).

Figure 4. PPy-coated PLGA meshes. (a) Photographs of uncoated PLGA meshes (white, left) and PPy-PLGA meshes (black, right). (b) SEM micrograph of single strands of PPy-PLGA fibers. (c) SEM image of section of the PPy-PLGA meshes.

Figure 5.

High resolution (a) C_{1s} and (b) N_{1s} XPS spectra of PLGA and PPy-PLGA. The C_{1s} spectrum of PPy-PLGA shows typical signals of PPy at 284.6 eV ($\underline{C}-H_x/\underline{C}-C$), 285.8 eV ($\underline{C}-N$), 287.2 eV ($\underline{C}=N$), 288.9 eV ($C=N^+$), and 290.1 eV ($\pi-\pi^*$). Intense peaks at 287.1 eV and 288.7 eV in the PLGA spectrum diminished after PPy-coating. (b) New peaks were detected in the N_{1s} spectrum after PPy-coating at 397.8 eV, 399.8 eV, 401.2 eV, and 402.2 eV, which were assigned to $=\underline{N}-$, $-\underline{NH}-$, $C-\underline{N}^+$, and $C=\underline{N}^+$, respectively.

Figure 6. SEM images of PPy-coated PLGA fibers incubated in PBS solution at 37°C for 2 weeks at (a) a low magnification and (b) a high magnification. No fragmentation of the PPy shell and no degradation of PLGA fibers were observed after 2 weeks of incubation.

Figure 7. Immunostaining of PC12 cells cultured on (a) RF, (b) AF, (c) PPy-RF, and (d) PPy-AF. PC12 cells were cultured for 2 days with exogenous NGF in the medium and stained with phalloidin-Alexa 488 for F-actin. Arrows indicate major directions of the aligned fibers of (b) and (d) in the images. Scale bars are 50 μm.

Figure 8.
SEM images of PC12 cells cultured on (a) PPy-RF and (b) PPy-AF for 2 days.

Figure 9.

Fluorescence images of hippocampal neurons cultured on (a) RF, (b) AF, (c) PPy-RF, and (d) PPy-AF. Immunostaining for tau-1 (axonal marker) was employed to stain hippocampal cells and axons after 1 day in culture. Arrows indicate major directions of the aligned fibers of (b) and (d) in the images. Scale bars are 50 μm .

Figure 10.

Electrical stimulation of PC12 cells through PPy-PLGA (PPy-RF and PPy-AF) fibers at 0 mV/cm and 10 mV/cm. Representative fluorescence images of electrically stimulated cells: (a) PPy-RF at 0 mV/cm (unstimulated); (b) PPy-RF at 10 mV/cm; (c) PPy-AF at 0 mV/cm; (d) PPy-AF at 10 mV/cm. Scale bars are 50 μm. (e) Median neurite lengths and (f) percentages of neurite-bearing PC12 cells when unstimulated and when electrically-stimulated (10 mV/cm) on random (PPy-RF) and aligned (PPy-AF) PPy-PLGA fibers. At least 300 neurites were analyzed from four substrates for each condition.

Table 1
Reaction parameters and properties of PPy-coated PLGA (PPy-RF)^a.

Morphology	Pyrrole (mM)	FeCl ₃ (mM)	pTS (mM)	Reaction time (h)	R _s (ohm/sq.)
Aggregates	14	38	0	24	3.0±1.7 ×10 ³
	7	38	0	24	9.8±9.3 ×10 ⁴
	7	19	17.5	24	1.6±1.1 ×10 ⁴
	7	19	0	48	6.9±5.8×10 ⁵
Incomplete coating	3.5	9.5	0	24	N/A ^b
	7	19	0	8	N/A ^b
Coating	7	19	0	24	4.7±2.7 ×10 ⁵
	7	19	1.2	24	7.6±1.9 ×10 ⁴
	7	19	7	24	2.4±2.0 ×10 ⁴

^aReaction was performed at 4°C.

^bNot measurable

Table 2
Elemental compositions and doping levels of PLGA and PPy-coated PLGA (PPy-RF) using XPS analysis.

Sample	Elements (atomic %)				Doping level ^d
	C	N	Cl	S	
PLGA	99.7	0.2	0	0	-
PPy-PLGA ^b	84.9	12.0	1.0	2.2	0.27

^a doping level = (Cl+S)/N

^b PPy-RF used