Supplementary Materials for
3D Bio-nanofibrous Mats as Platforms for Cell Culturing

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Materials and Reagents

Iron(III) ρ-toluenesulfonate hexahydrate, tetrahydrofuran formaldehyde, Na_2HPO_4, NaH_2PO_4, and NaCl were all obtained from Sigma-Aldrich. Poly(styrene-β-isobutylene-β-styrene) (SIBS) was supplied by Boston Scientific. Pyrrole monomer from Merck was distilled and stored at -18°C before use. Gold coated mylar was from CP Films Inc. Rat PC12 cells were obtained from American Type Tissue Collection. Eagle's minimal essential medium (DMEM), glutamine and horse serum were purchased from GIBCO. Fetal bovine serum (FBS) and collagen was provided by Thermoelectron and Purecol respectively. Nerve growth factor (NGF) and lexa Fluor 488 phalloidin were obtained by Invitrogen.

Synthesis of SIBS/PPy composite nanofibers

10% (w/w) Fe(III) ρ-toluenesulfonate hexahydrate was dissolved in tetrahydrofuran with magnetic stirring for 30 minutes. 10% (w/w) SIBS was subsequently added into the solution and magnetic stirred over 3 hours. SIBS/Fe(III) composites were then electrospun onto a gold coated mylar collector which was placed in a sealed **chamber** full of pyrrole monomer vapor. **The vapor chamber is made of plastic and transparent. An air flow was introduced into the vapor chamber to control humidity, which had an influence to the electrospinning process.** The electrospinning was carried out from a 5 ml glass syringe with a Precision GlidTH 19 G needle and the feed rate was controlled at 100 μl/min by a NE-1000 syringe pump (New Era Pump Systems Inc.). 20kV voltage was generated by a Gamma high voltage power supply (ES50P-10W/DAM) between the tip of the needle and the grounded target (i.e. gold coated mylar) which were maintained at a separation of 13 cm. Polypyrrole was consequently polymerized on SIBS/Fe(III) nanofibers via vapor phase polymerisation (VPP) during electrospinning process.

Raman Spectroscopy

The presence of PPy in the composite fibers were confirmed using a Raman JYHR800 at 632.8 nm, compared with a PPy film prepared using VPP technique under the same conditions. Four typical bands (Peak A, B, C and D) were found in the spectrum of SIBS/PPy electrospun fibers (Figure 1a), while **no band was observed in the spectrum of SIBS only fibers (Figure 1c).** Some shifts were observed from PPy (Figure 1b) to PPy/SIBS, indicating the interaction between SIBS and PPy in the composite fibers.

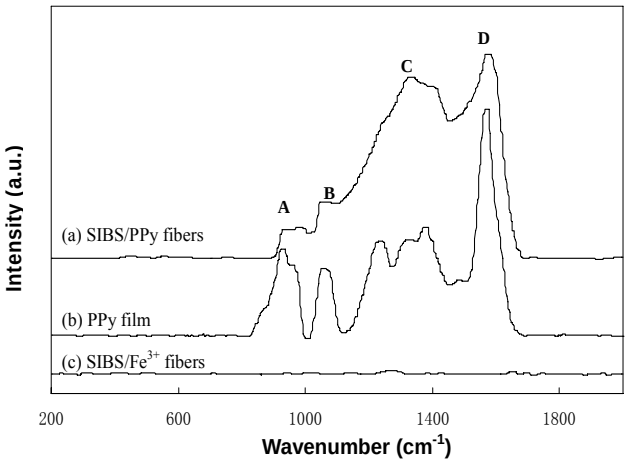

Figure 1. Raman spectra of (a) SIBS/ PPy electrospun fibers, (b) the PPy film prepared by VPP and (c) SIBS/Fe^{3+} fiibers, excitation wavelength 632.8 nm.

Cyclic Voltammetry

SIBS/PPy electrospun fibers were peeled off from the gold coated mylar. Pt was then sputter coated onto the fiber surface at 30 mA for 20 minutes using a Dynavac Magnetron Sputter Coater (Model SC100MS). The SIBS/PPy fibrous electrode coated with Pt was measured using cyclic voltammetry, compared with a Pt sputter coated glass at the same geometric size. Electrochemical characterisation was carried out in a divided three-electrode cell using a 720C Electrochemical Analyzer (CH Instruments) with an Ag/AgCl reference electrode and a Pt mesh as the auxiliary electrode. Solutions were deoxygenated by nitrogen gas for 15 minutes prior to the electrochemical experiments. Cyclic voltammograms were obtained in phosphate buffer solution (PBS, containing 0.2M Na_2HPO_4, 0.2M NaH_2PO_4 and 0.15M NaCl, PH 7.4). The potential was cycled between -0.6 V and 0.4 V (vs. Ag/AgCl) at a scan rate of 20 mVs^{-1}.

PC12 cells culture and differentiation

SIBS/PPy electrospun fibers were soaked in 70% ethanol for 30 minutes and allowed to dry in the laminar flow hood, followed by exposing under UV light for 20 minutes. The cells were seeded at a density of 10,000 cells per cm^2 in DMEM medium with 2 mM glutamine, 5% FBS and 10% horse serum. The differentiation was carried out in DMEM supplemented with glutamine, 1% horse serum and 50 ng/ml NGF (7s). The substrate

was soaked in 0.3 mg/ml collagen/0.01 M HCl solution overnight to coat a collagen cover on the surface before cell culture.

Fluorescence staining of PC12 cell

PC12 cells were fixed in 3.7% paraformaldehyde/PBS for 10 minutes at room temperature and subsequently washed by PBS for three times. Cells were then stained using 0.2 μM Alexa Fluor 488 phalloidin (Molecular probes) for 20 minutes at room temperature. Cells were excited at 488 nm and fluorescence was collected using an emission window set at 500–540 nm. Images were obtained using a Leica DMIRBE inverted microscope with a Leica TCS SP confocal system (Leica Microsystems).

SEM sample preparation

PC12 cells grown on PPy/SIBS fibers were fixed in 3.7% paraformaldehyde/PBS solution for 15 minutes and then exposed to increasing concentration of ethanol (30%, 50%, 70%, 90% and 100%) for 30 minutes respectively, and finally, mounted onto a stub to determine morphology using a Leica Cambridge 440 stereoscan scanning electron microscope (SEM). This SEM was also used to measure the morphology of SIBS/PPy electrospun fibers.