Supplementary Materials for 3D Bio-nanofibrous Mats as Platforms for Cell Culturing
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Materials and Reagents
Iron(III) \(\rho\)-toluenesulfonate hexahydrate, tetrahydrofuran formaldehyde, \(\text{Na}_2\text{HPO}_4\), \(\text{NaH}_2\text{PO}_4\), and \(\text{NaCl}\) were all obtained from Sigma-Aldrich. Poly(styrene-\(\beta\)-isobutylene-\(\beta\)-styrene) (SIBS) was supplied by Boston Scientific. Pyrrole monomer from Merck was distilled and stored at -18 \(^\circ\)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 Thermoelctron 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) \(\rho\)-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 Glid\textsuperscript{Tm} 19 G needle and the feed rate was controlled at 100 \(\mu\)l/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), indicating the interaction between SIBS and PPy in the composite fibers.

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 \(\text{Na}_2\text{HPO}_4\), 0.2M \(\text{NaH}_2\text{PO}_4\) and 0.15M \(\text{NaCl}\), 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.