3D Bio-nanofibrous PPy/SIBS mats as platforms for cell culturing†

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Received (in Cambridge, UK) 13th March 2008, Accepted 9th May 2008
First published as an Advance Article on the web 17th June 2008
DOI: 10.1039/b804283g

3D bio-nanofibrous polypyrrole/poly(styrene-β-isobutylene-β-styrene) mats, prepared *via* a vapor-phase polymerisation modified electrospinning process, provide excellent platforms for PC12 cells attachment and growth, indicating potential applications in areas requiring good mass transport such as nerve growth guidance channels.

Electrospun biocompatible and/or biodegradable polymer nanofibers are considered as desirable tissue engineering scaffolds considering their attractive three-dimensional networks, interconnected microporous structures and high surface area-to-volume ratio.[1–3] Numerous studies have been carried out in using electrospun fibers such as biodegradable poly(lactic-*co*-glycolic acid),[4] aliphatic polyesters (*e.g.* poly(ε-caprolactone)),[5] and biocompatible/bioresorbable dextran membranes,[6] in the tissue engineering areas. Poly(styrene-β-isobutylene-β-styrene) (SIBS) is a biostable[7] and biocompatible block copolymer which has been used as a drug-eluting coating for cardiac stents[8] and substrates for cell cultures.[9] Nanofibrous mats based on SIBS were prepared using electrospinning with the addition of single-wall carbon nanotubes (SWNTs) to facilitate the electrospinning process while retaining the biocompatibility of the materials.[10] The electrochemical properties of the resulting fibrous electrodes, however, were limited by the poor conductivity of electrodes, resulting in poor electronic stimulation on cell adhesion and growth. Due to its chemical and thermal stability, highly electroactive and biocompatible polypyrrole (PPy) is an attractive material for cell culture work.[11] In this work, we prepare 3D nanofibrous PPy/SIBS mats by using a vapour-phase polymerisation (VPP) modified electrospinning method in which a thin conducting and electroactive polypyrrole film is deposited (*via* VPP) onto SIBS nanofibres during the electrospinning process.

Fig. 1 illustrates the method and setup for the preparation of PPy/SIBS nanofibrous mats. SIBS was mixed with the oxidant, iron(III) *p*-toluenesulfonate (Fe(III)pTs), in tetrahydrofuran at various concentrations of 10–20% (w/w) SIBS and 5–20% (w/w) Fe(III)pTs. The SIBS/Fe(III)pTs blend was subsequently electrospun onto gold-coated Mylar in a sealed box filled with pyrrole monomer vapor, in a vapor-phase polymerisation (VPP) process of polypyrrole. Nanofibrous networks of PPy/SIBS composites can be obtained with the control of the concentration of SIBS and Fe(III)pTs.

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† Electronic supplementary information (ESI) available: Experimental section. See DOI: 10.1039/b804283g

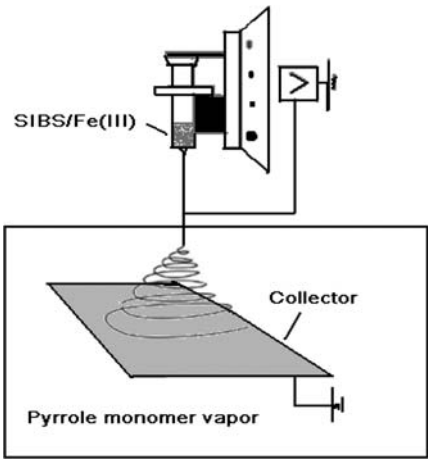

Fig. 1 Electrospinning setup demonstrating the synthesis of composite nanofibers of PPy/SIBS.

Fig. 2 shows the SEM image of a PPy/SIBS mat with an uniform and well-defined interconnected fibrous network obtained from 10% (w/w) SIBS and 10% (w/w) Fe(III) THF solution. It shows the 3D microporous fibrous structure of the resulting PPy/SIBS mat, providing an attracting tough surface for cell attachment and proliferation. On the basis of SEM images, the diameter of the electrospun fibers was found in the range of 80–120 nm. The average diameter of PPy/SIBS was estimated to be around 90 nm, which is higher than that (70 nm) of SIBS/Fe^{3+} due to the polymerisation of PPy. The inset image is the optical photo of PPy-coated SIBS

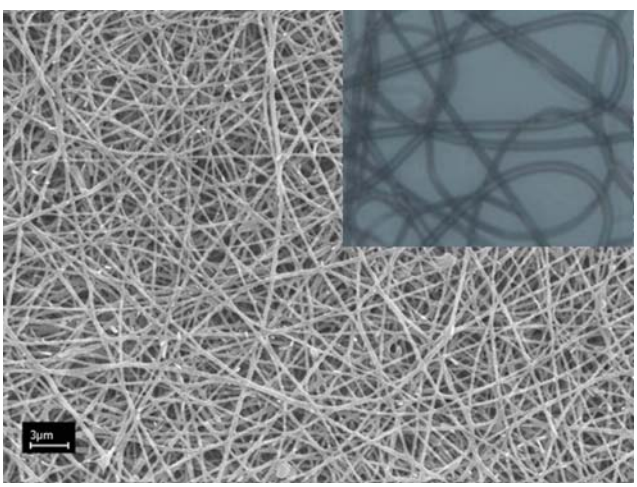

Fig. 2 SEM image of PPy/SIBS composite electrospun fibers. The inset is the optical image of PPy-coated SIBS nanofibres.

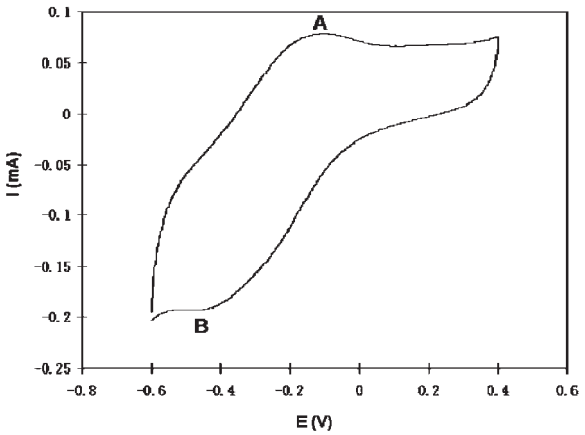

Fig. 3 Cyclic voltammogram of a PPy/SIBS nanofibrous mat in phosphate buffer solution (PH = 7.4). Scan rate = 20 mV s^{-1}.

nanofibres, which clearly shows SIBS nanofibres were uniformly coated by PPy films (the outside dark layer). This suggested that PPy had been successfully deposited onto SIBS fibres during the electrospinning process.

The presence of PPy in the resulting fibrous structure was also confirmed by Raman spectroscopy. Typical bands due to the presence of PPy were found in the spectrum of the resulting electrospun PPy/SIBS nanofibrous mat. Combined with SEM results that micropores were observed in the resulting structure, it could be concluded that PPy had successfully deposited along the surface of individual SIBS fibers while the microporous structure of PPy/SIBS fibrous mat was maintained.

Fig. 3 shows the cyclic voltammogram (CV) of the PPy/SIBS fibrous electrode in phosphate buffer solution (PBS) at a scan rate of 20 mV s^{-1}. A stable redox couple (labelled A/B) was observed, which could be attributed to the redox of the PPy backbone in the composite fibrous mat while no redox response was observed from a pure SIBS fibre mat under identical conditions. This suggests that the PPy film coated outside the SIBS nanofibres is highly electroactive and electrochemically stable in PBS buffer solution. It could be concluded that a highly electroactive PPy/SIBS nanofibrous mat was successfully prepared *via* a VPP modified electrospinning process.

PC12 is a cell line derived from pheochromocytoma of the rat adrenal medulla. PC12 cells respond to nerve growth factor by sprouting neuritis and biochemically differentiating into sympathetic ganglion-like cells, making them as a model system for neuronal differentiation. In this study, PC12 cells were seeded onto a 3D nanofibrous mat of PPy/SIBS and subsequently stained with Alexa Fluor 488 phalloidin. Phalloidin is normally used to investigate the distribution of F-actin in cells by labeling phalloidin with fluorescent analogs and using them to stain actin filaments for light microscopy. Fig. 4(a) shows the SEM image of a cell cultured PPy/SIBS fibrous mat, which indicated that PC12 cells had been successfully well adhered to the surface of the PPy/SIBS fibrous mat. The fluorescence microscope images (Fig. 4(b)) displayed not only the clear neuronal differentiation (left) of PC12 cells on the PPy/SIBS nanofibers, but also the particular growth of neuritis from PC12 cells (green lines) in the inner layer of the 3D fibrous network (right) after 144 h of culture.

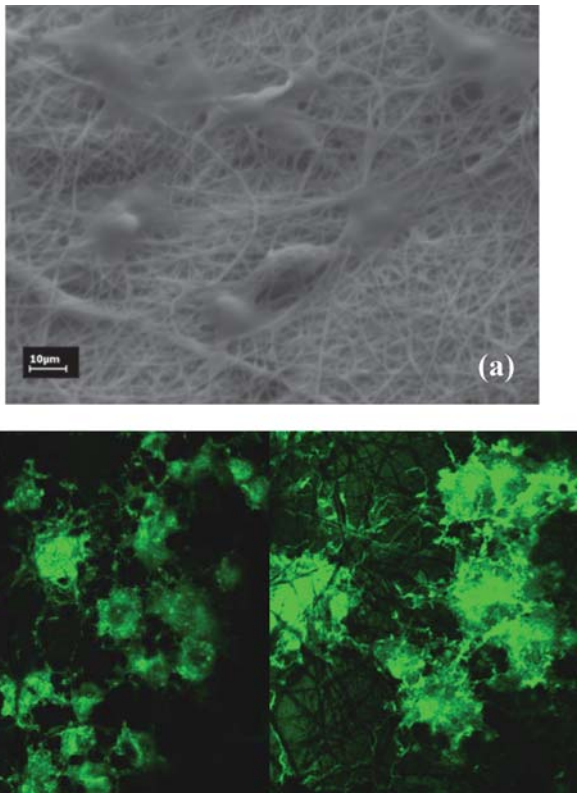

Fig. 4 (a) SEM image of PC12 cells adhered to a PPy/SIBS nanofibrous mat; (b) fluorescence microscope images of phalloidin stained PC12 cells grown on PPy/SIBS nanofibers.

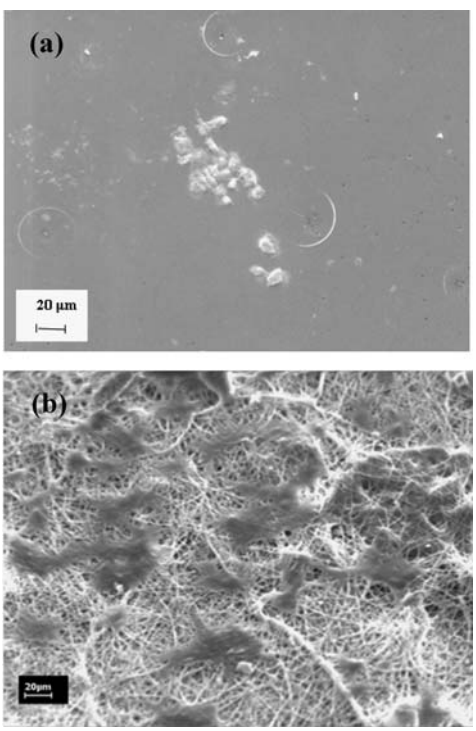

Fig. 5 SEM micrographs illustrating (a) PC12 cells on pristine gold coated Mylar and (b) PC12 cells on a PPy/SIBS nanofibrous mat. Cells were cultured at a seeding density of 10 000 cells cm^{-2} for 144 h.

PC12 cells grown on PPy/SIBS fibers, compared with cells grown on pristine gold coated Mylar were investigated using SEM to determine the morphologies and density of PC12 cells. Fig. 5(a) shows that PC12 cells on pristine gold Mylar are rounded in shape, indicating apoptosis between cells and the substrate. The cells adhered on PPy/SIBS fibers (Fig. 5(b)), however, exhibit a spreading polygonal shape, suggesting good phenotypic spreading of PC12 cells on electrospun PPy/SIBS nanofibers. PC12 cells are also found to be well interconnected with the nanofibers and neurites of the cells are oriented along the nearest fiber direction. Furthermore, a higher density of cells and more firm adhesion to the substrate were obtained on PPy/SIBS nanofibers than those on pristine gold coated Mylar, suggesting the excellent stimulation of 3D nanofibrous matrix after 144 h of culturing. This could be concluded due to the cytocompability with the PPy/SIBS nanofibers.

Our preliminary work demonstrates the successful synthesis of blended conductive, electroactive and biocompatible 3D nanofibrous PPy/SIBS mats. The results indicate that three-dimensional, well interconnected fibrous networks provide excellent substrates for PC12 cell attachment and neuritis growth, indicating potential applications in areas requiring good mass transport such as nerve growth guidance channels. The useful combination of properties including conductivity and electroactivity of PPy, biostability and biocompatibility of SIBS and good mechanical properties, give rise to possible investigation on cell cultures that require electrical stimulation and a wide range of applications of biomaterials used in electrochemical areas such as biofuel cells.

Further studies on electrical stimulation for cell growth are ongoing and will be described in a full paper.

We are grateful for the financial support from the Australian Research Council. SIBS was supplied by Boston Scientific, USA.

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