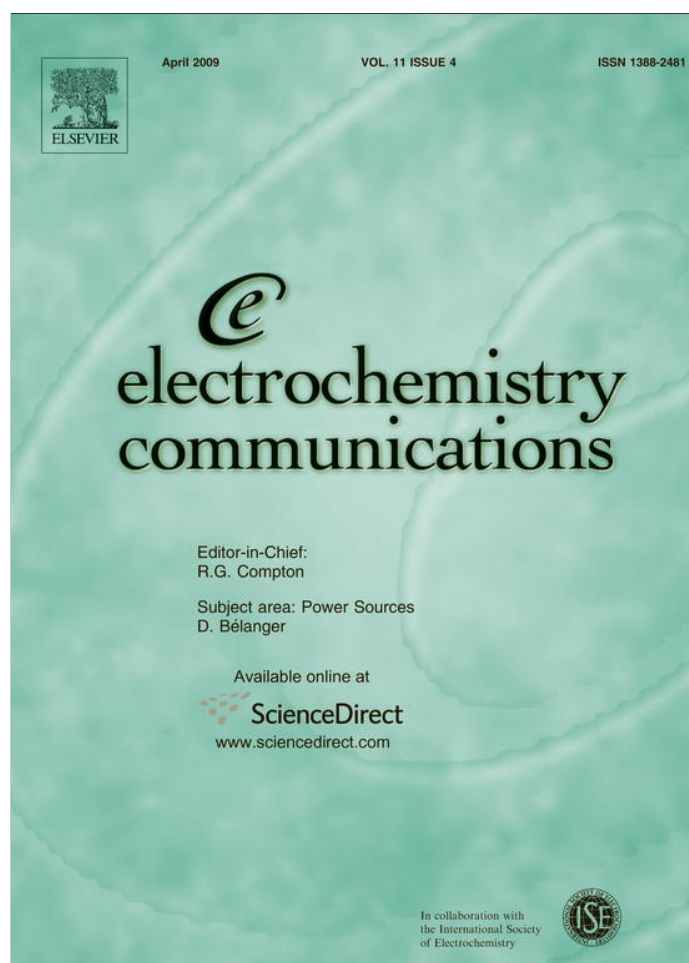


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Carbon nanofiber doped polypyrrole nanoscaffold for electrochemical monitoring of cell adhesion and proliferation

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ABSTRACT

A novel nanoscaffold of carbon nanofiber (CNF) doped polypyrrole (PPy) is prepared on indium-tin oxide through controllable one-step electropolymerization. This highly conducting matrix provides a biocompatible and robust substrate for adhesion and subsequently proliferation of ECA-109 cells on a modified electrode, which increase the electron transfer resistance and thus lead to the method for cell immobilization and sensitive monitoring of cell growth. This strategy offers advantages of simple and low-cost fabrication, economic and convenient detection, and possesses potential application in cytological study.

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1. Introduction

Local interaction of cells with substrates plays a key role in cellular adhesion and proliferation [1]. The surface properties such as roughness, hydrophobicity, topography and component can affect these processes [2]. Polypyrrole (PPy), a conducting polymer with easy preparation, altering surface characteristics, relative stability, high conductivity and cytocompatibility, has been used to coat a grown carbon nanofiber (CNF) array for fluorescent imaging study of the morphology and growth rate of PC12 neural cells on 3D nanostructure [3]. This work used CNFs with anionic groups as sole dopant to conveniently produce a CNF-PPy nanoscaffold on electrode surface by one-step electropolymerization, and developed an electrochemical technique for monitoring the cellular adhesion and proliferation.

CNFs have been used as neural biomaterials for adhesion, proliferation, and long-term function studies of astrocytes using fluorescence microscopy due to their high conductivity [4]. Our previous work prepared a biocompatible CNFs-chitosan architecture for cytosensing [5]. Here the incorporation of CNFs into conducting PPy matrix confers an enhanced performance with significant electrical properties to individual components [6,7]. The composite film formed on indium-tin oxide (ITO) glass slide shows to act as

both a cell-anchoring substrate and a reporting platform for impedimetric sensing of cell adhesion and proliferation, thus providing a simple, low-cost and disposable electrochemical avenue for cytologic study.

2. Experimental

2.1. Materials and reagents

Pyrrole was purchased from Sigma-Aldrich Inc. (USA) and distilled prior to use. All solutions were prepared with deionized water of 18 M Ω purified from a Milli-Q purification system, and all other reagents are of analytical grade. CNFs were obtained from WPI (Sarasota, FL) and were boiled in 30% nitric acid for 24 h to obtain carboxylic group-functionalized CNFs. After centrifugation and wash with deionized water until the pH reached 6.0, the CNFs were dispersed in 0.1 mol l⁻¹ pyrrole solution.

2.2. Cell culture

ECA-109 cells were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (Sigma), penicillin (100 μ g ml⁻¹) and streptomycin (100 μ g ml⁻¹) at 37 °C in a humidified atmosphere containing 5% CO₂. After 48 h, the cells were trypsinized, separated and re-suspended in the medium. Cell number was determined using a Petroff-Hausser cell counter (USA).

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2.3. Preparation of CNF-PPy/ITO and cell proliferation

An ITO glass slide with $10 \times 10 \text{ mm}^2$ area as electrode surface was used for electropolymerization and cell culture. To obtain hydroxylated surface, the slide was immersed in 1:1:5 (v/v) $\text{H}_2\text{O}_2/\text{NH}_4\text{OH}/\text{H}_2\text{O}$ solution for 1 h. The CNF-PPy composite film was formed at +0.7 V for 200 s in 0.1 mol l^{-1} pH 2.0 pyrrole solution containing 2.0 mg ml^{-1} CNFs. PPy films without CNF incorporation were also produced as control using 0.5 mol l^{-1} KCl as dopant.

$0.2 \text{ ml } 2.0 \times 10^6 \text{ cells ml}^{-1}$ ECA-109 cell suspensions were introduced onto CNF-PPy/ITO, and cultured under the conditions described above. Afterwards the cell-proliferated CNF-PPy/ITO was taken out for electrochemical impedance spectroscopic (EIS) measurements.

2.4. Apparatus

Cyclic voltammetric measurements were performed on a CHI 660B electrochemical analyzer (CHI, Inc.). EIS measurements were carried out on a PGSTAT30/FRA2 system (Autolab, Netherlands) in 0.01 mol l^{-1} pH 7.4 PBS containing 10 mmol l^{-1} $\text{Fe}(\text{CN})_6^{3-/4-}$ and 0.1 mol l^{-1} KCl using the modified ITO as working, platinum wire as auxiliary, and saturated calomel electrode as reference electrodes. The impedance spectra were recorded within the frequency range of $1\text{--}10^6 \text{ Hz}$ with 5 mV amplitude of the applied sine wave potential. Fourier-transform infrared spectroscopy (FTIR) was carried out on a NEXUS 870 FTIR spectrometer (Nicolet, USA). The static water contact angles were measured with a Rame-Hart-100 Contact Angle Goniometer (NJ, USA) using droplets of deionized water at $25 \text{ }^\circ\text{C}$. The morphology was observed under TE2000-U inverted fluorescence microscopy (Nikon, Japan). All the experiments were performed at room temperature.

3. Results and discussion

3.1. Electropolymerization of CNF-PPy nanoscaffold

Oxidation treatment of CNFs can produce plentiful oxygen-containing groups on the surface of CNFs, and thus improve greatly the hydrophilicity and biocompatibility of the material [5], which are in favor of cell adhesion and proliferation and can be used as the charge-balancing counter ions for electropolymerization of PPy without need of any other dopant. At +0.7 V, the radical cations of pyrrole monomers were formed and reacted with other monomers to form oligomeric products and then a polymer on ITO surface. Meanwhile the CNFs were doped in the polymer scaffold. The electropolymerization conditions significantly affected the behavior of the nanocomposite film, which was examined in pH 7.4 PBS after polymerization (Fig. 1). The CNF-PPy/ITO formed at pH 2.0 showed two redox peaks at -0.297 and -0.666 V , which was a common feature of PPy [8]. The maximum reduction peak current occurred at the electropolymerization potential of +0.7 V (Fig. 1A). More positive potentials might produce dication and pyrrolinones, leading to a decrease of conjugation length and the formation of more loosen PPy structure [9] and decreasing the response. After electropolymerization for 200 s, the redox peaks reached relatively steady values (Fig. 1B), which was chosen as optimal electropolymerization time.

The film formed in pH 2–3 solution exhibited more stable and enhanced electron transfer behavior compared with one obtained at pH 7 (Fig. 1C), verifying that low pH favored polymerization.

The CNFs as counter ions could greatly improve the redox properties of the resulting composite film (Fig. 1D). In comparison with pure PPy film formed with KCl as a source of counter ions,

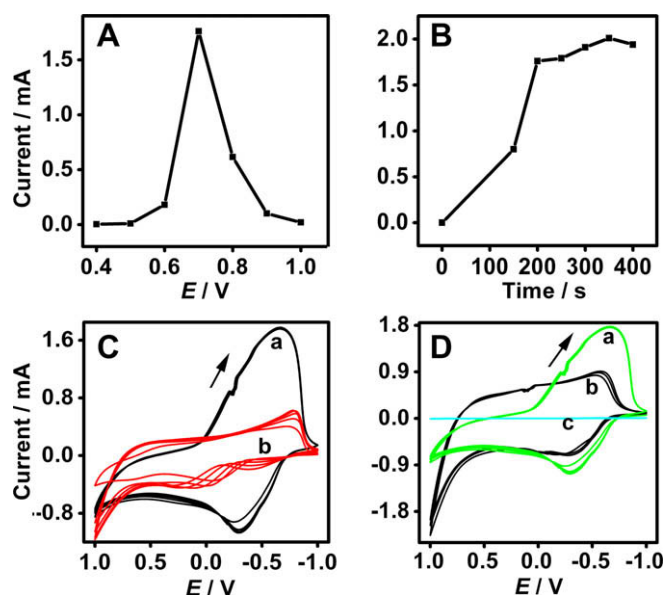


Fig. 1. Effects of electrodeposition potential (A) and time (B) on reduction peak current of CNF-PPy/ITO in pH 7.4 PBS and cyclic voltammograms of (C) CNF-PPy/ITO prepared at pH 2.0 (a) and 7.0 (b) and (D) CNF-PPy/ITO prepared at pH 2.0 (a), PPy/ITO prepared at pH 2.0 (b) and bare ITO (c) in pH 7.4 PBS. Scan rate: 50 mV s^{-1} .

the composite film was notably more adhesive to the ITO surface. Along with the scanning process, the PPy film on ITO without CNF doping gradually fell and its reduction peak current decreased, showing unstable electrochemical response. The composite nanoscaffold showed obvious enhancement in voltammetric response because of the promotion of the oxidation–reduction process and lessening of the electrokinetic polarization by doping of CNFs as electron conductor with extensive oxygen-functionalized sites on walls [8,10].

3.2. Characterization of the CNF-PPy composite nanoscaffold

The CNFs showed the peaks of hydroxyl group at 3401 cm^{-1} , carboxyl group at 1726 cm^{-1} , and carboxylate group at 1584 and 1383 cm^{-1} (curve a, Fig. 2). The pure PPy showed the bands of typical pyrrole ring vibration at 1544 and 1460 cm^{-1} and the bands of $=\text{C-H}$ in plane vibration at 1293 , 1091 and 1042 cm^{-1} [11] (curve b, Fig. 2). The peaks at 3440 and 1634 cm^{-1} might be assigned to N–H and $\text{C}=\text{C}$ stretch vibration, respectively. The spectrum of CNF-PPy

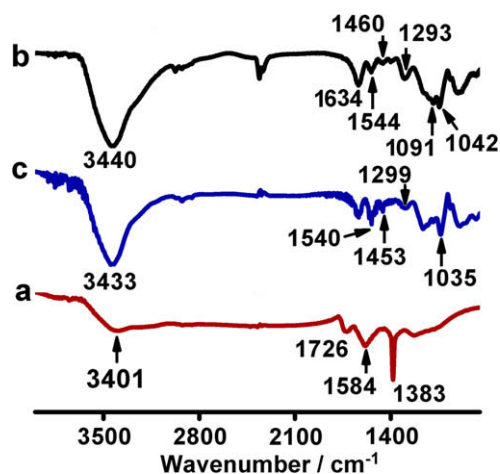


Fig. 2. Infrared spectra of soluble CNF (a), PPy (b) and CNF doped PPy (c) powder.

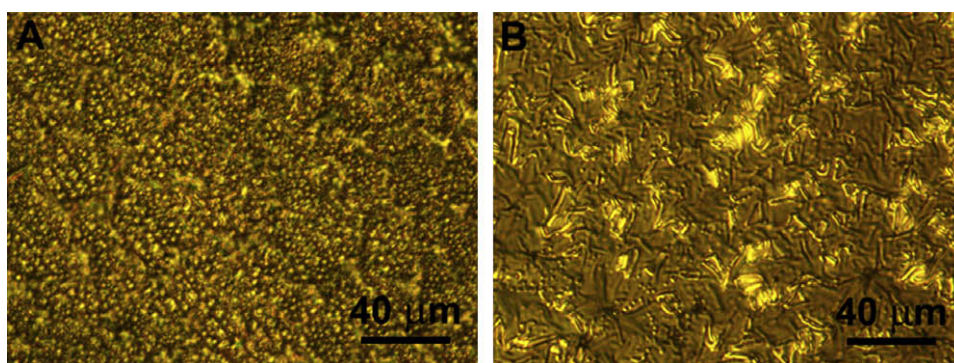


Fig. 3. Discrepancy images of PPy (A) and CNF-PPy composite nanoscaffold (B) covered ITO slides.

displayed more similarity with that of PPy (curve c, Fig. 2), with the characteristic vibration at 1540, 1453, 1299 and 1035 cm^{-1} , verifying the existence of PPy in the composite film, but the peak for N–H shifted in red to 3433 cm^{-1} , indicating the interaction with the reactive hydroxyl functional groups of CNFs.

The morphology of CNF-PPy and PPy scaffold covered ITO showed significant difference. CNF-PPy showed well-distributed interwoven texture, unlike the particle morphology of pure PPy (Fig. 3). This observation was actually in accordance with the proposed doping of CNFs, and confirmed the incorporation of CNFs into the polymer and the formation of high-quality composite nanoscaffold.

The biocompatibility of a support for loading biomolecules or cells and preserving their bioactivity is positively related to its hydrophilicity [12], which can be characterized with the contact angle measurement of the substrate. The CNF-PPy and PPy covered ITO glass slides gave the contact angles of 52.3° and 57.7°, respectively. The smaller contact angle of CNF-PPy/ITO demonstrated better hydrophilicity of the composite film, which was in favor of promoting cell adhesion and proliferation. Thus the designed composite nanoscaffold provided a biocompatible and conducting platform for cell growth research, especially by electrochemical technique.

3.3. Monitoring of cell adhesion and proliferation on CNF-PPy/ITO

With the composite nanoscaffold in hand, cell adhesion and proliferation was monitored by impedance technique based on

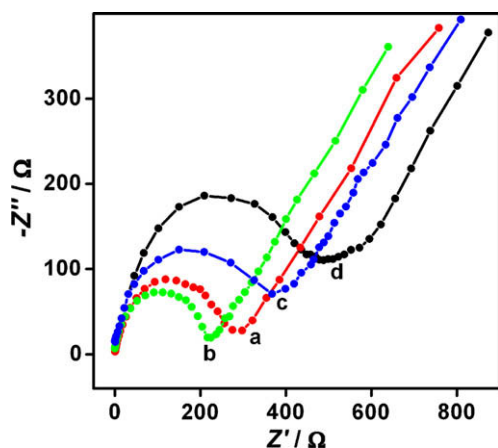


Fig. 4. Electrochemical impedance spectra recorded at PPy/ITO (a), CNF-PPy/ITO before (b) and after incubation in $0.2 \text{ ml } 2 \times 10^6 \text{ ECA-109 cells ml}^{-1}$ for 2 h (c) and 3d (d).

the measurement of electron transfer resistance (R_{et}) with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. The redox process of the probe showed a resistance of 277 Ω at PPy covered ITO (Fig. 4a). The assembly of the CNF-PPy composite layer on the ITO surface significantly accelerated the electron transfer owing to the excellent electronic conductivity of CNFs, thus decreased the R_{et} to 237 Ω (Fig. 4b), which decreased the background and enhanced the detection sensitivity for the following cell adhesion. After incubation of the modified ITO in a culture solution containing ECA-109 cells for 2 h, the cells adhered on ITO would hinder the access of the redox probe to the surface, due to the insulating properties of the cell membrane [13], causing an obvious increase in R_{et} to 433 Ω (Fig. 4c). As control, after the CNF-PPy covered ITO slides were incubated in culture medium without cells for the same time, no obvious change could be observed. Thus the magnitude of increase of the R_{et} depended on the surface coverage of the cells. After 3-day proliferation, the R_{et} further increased to 582 Ω (Fig. 4d). The results demonstrated that CNF-PPy covered ITO surface offered a biocompatible substrate for cell immobilization and growth, and an effective reporting platform for electrochemical interrogation of cell adhesion and proliferation. The CNFs doped in PPy matrix increased the electronic conductivity, thus increased the detection sensitivity.

4. Conclusions

This work focuses on the combination of the properties of carbon nanofibers and polypyrrole to construct a composite nanoscaffold on ITO glass by controllable one-step electropolymerization for impedimetric monitoring of cell adhesion and proliferation. It is the first example of using anionic CNFs as sole conductive dopant in the electropolymerization of a conducting polymer. The designed biocompatible architecture is demonstrated to be very effective for cell immobilization, and sensitive for electrochemical study of cell proliferation due to the remarkable conductivity. This strategy offers advantages of simple and low-cost fabrication, convenient and sensitive electrochemical detection, and possesses potential application in cytology.

Acknowledgments

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