THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

Preparation of a Hydrophobic Polythiophene Film to Improve Protein Adsorption and Proliferation of PC 12 Cells

Da-Feng Li, Hua-Jie Wang, Jian-Xi Fu, Wei Wang, Xue-Shun Jia, and Jin-Ye Wang

J. Phys. Chem. B, **2008**, 112 (51), 16290-16299 • DOI: 10.1021/jp8041704 • Publication Date (Web): 21 November 2008

Preparation of a Hydrophobic Polythiophene Film to Improve Protein Adsorption and Proliferation of PC 12 Cells

Da-Feng Li,[†,‡,||] Hua-Jie Wang,[§,||] Jian-Xi Fu,[†] Wei Wang,[†] Xue-Shun Jia,[‡] and Jin-Ye Wang*[,†,§]

Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai, 200032, PR China, Department of Chemistry, Shanghai University, 99 Shangda road, Shanghai, 200444, PR China, College of Life Science and Biotechnology, Shanghai Jiaotong University, 1954 Huashan Road, Shanghai, 200030, PR China

Received: May 12, 2008; Revised Manuscript Received: August 26, 2008

High quality films of polythiophene with different alkyl side chains were successfully synthesized by a novel method in the presence of sodium dodecylbenzenesulonate (SDBS) under N_2 atmosphere on the PTFE (polytetrafluorethylene) substrate. The as-prepared films were characterized by scanning electron microscopy (SEM), conductivity measurement, and water contact angle measurement. The morphologies of the films were homogeneous with micro-/nanostructures, and their conductivities were high enough for biomedical applications. Hydrophobicity of the films could be adjusted easily by inducing alkyl side chains with different length, which could control protein adsorption in succession. Hydrophobic polythiophene film with a long alkyl side chain had a higher ability of protein adsorption and PC 12 cell proliferation. The biocompatibility study of the synthesized films in vitro proved that the synthesized films were not cytotoxic to two cell lines used and could support cell attachment and proliferation well. Polythiophenes films prepared by in-situ deposition will be good candidates for biomedical applications.

1. Introduction

It is well-known that conducting polymers are composed of π-conjugated polymer chains and counterions processed by a doping process. Conducting polymers have electrical and optical properties similar to those of metallic and inorganic semiconductors, and also exhibit the attractive properties associated with conventional polymers, such as an ease of synthesis and processing. This unique combination of properties has given these polymers a wide range of applications, such as anticorrosion coatings, batteries, sensors, separation membranes, antistatic coating, electromagnetic interference shielding, and so on.[1−6] More recently, there is also a growing interest in conductive polymers for diverse biomedical applications, including biosensors, tissue engineering applications, neural probe applications, and others.[7] Common classes of organic conductive polymers include polyactylene (PA), polypyrrole (PPy), polyaniline (PANi), polythiophene (PTh), and poly(*p*-phenylenevinylene) (PPV). Among of them, there have been many reports about PPy and PANi as electro-active scaffolds or substrates for culturing electrically excitable cells, such as neural or cardiac cells.[8−13]

Compared to studies on PPy and PANi, it has been only quite recently that polythiophene and its derivatives have been explored as electroactive scaffolds for cell culture. Khan and co-workers prepared homopolymers of oligosiloxane by modifying polythiophene and copolymers with 3-methylthiophene and found that the conductivity of the PTh homopolymers (V−VIII) and copolymers doped with I_2 was very low or nonconductive.[14] According to an in vitro biocompatibility study with Hela cells (human ovarian cancer cells), the attachment of cells on poly (3-methylthiophene) homopolymers was not observed and cells were not counted due to the powder form of the copolymers. Also, the paper did not give a clear result against the control. In another series of studies, Martin and co-workers modified a neural microelectrode with poly (3,4-ethylenedioxythiophene) (PEDOT), poly(hydroxymethylated-3,4-ethylenedioxythiophene), or PEDOT/sulfonatoalkoxy EDOT coatings, all of which lowered the impedance of the coated electrode over a wide range, while increasing the charge capacity compared to the bare electrode.[15−17] The same group also reported that the neural probe coated by the order surfactant-templated PEDOT exhibited lower impedance and higher charge capacity than uncoated gold, nodular PEDOT, and nodular PPy-coated electrodes. In addition, human neuroblastoma cells can adhere and perform neurite extension on the coated electrode, though the surfactant poly $(oxyethylene)_{10}$-oleyl ether they used is toxic to cells.[18] Development of electrically conducting biomaterials intended for direct, functional contact with electrically active tissue, such as the nervous system, heart and skeletal muscle,[19,20] signified a new electrode paradigm for creating soft, low impedance implantable electrodes. Recently, del Valle et al. reported that the steel electrodes coated by PEDOT films showed good biocompatibility with Hep-2 cells, and revealed that the electroactivity of PEDOT in different biological fluids is significantly enhanced by the attached cellular monolayer.[21] Neuron-to-electrode attachment is vitally important in the performance of deep stimulating electrodes used in the treatment of neural diseases such as Parkinson's and is a concern in the performance of prosthetic devices such as retinal and cochlear implants. This involves electrode materials selection and design, surface chemical modification strategies and optimization of cell culture conditions.[22] Self-assembled method for surface modifications of neural electrodes has proved to be more controllable than with the electrodeposited method.[23]

* Corresponding author: Tel.: 0086-21-54925330. Fax: 0086-21-54925330. E-mail:jywang@mail.sioc.ac.cn.
[†] Chinese Academy of Sciences.
[‡] Shanghai University.
[§] Shanghai Jiaotong University.
[||] These authors contributed equally to this work.

10.1021/jp8041704 CCC: $40.75
Published on Web 11/21/2008

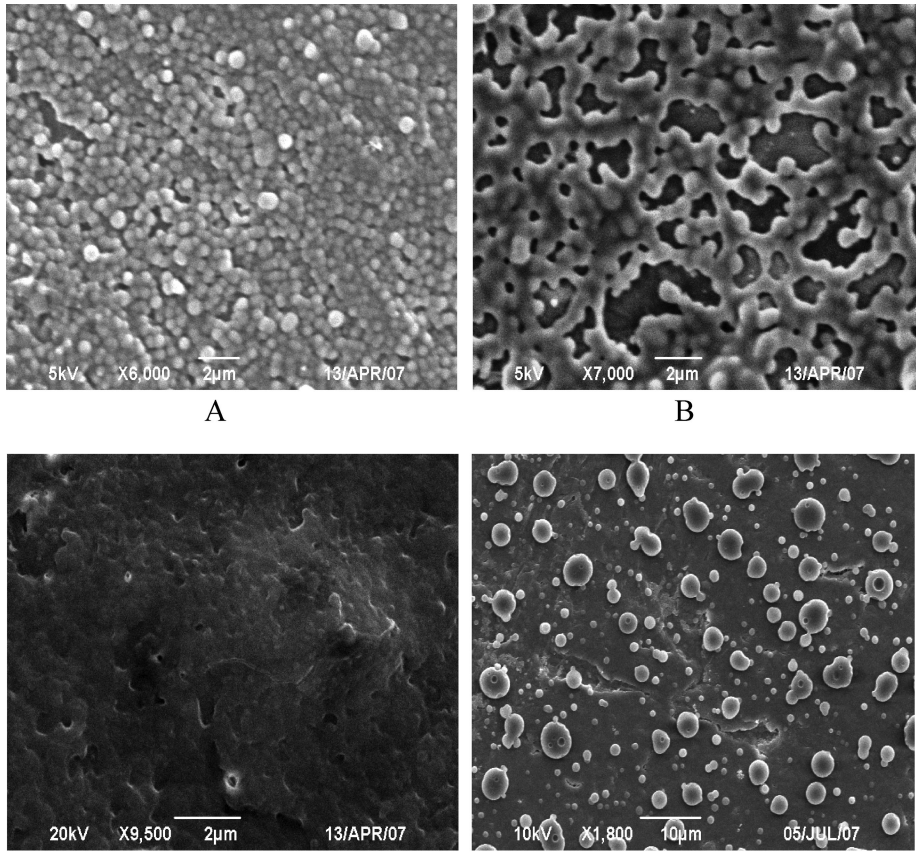

Figure 1. Scanning electron micrographs of the as-prepared polythiophene films. (A) PTh film; (B) PMTh film; (C) PDDTh film from direct polymerization; (D) PDDTh film from the casting.

SCHEME 1: Chemical Structure of Polythiophenes

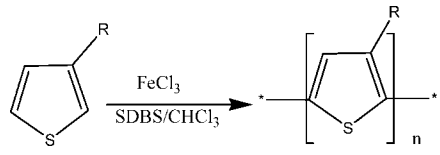

TABLE 1: Conductivity of Polythiophene Films Deposited on the PTFE Substrate

	PTh	PMTh	PDDTh
surface resistivity[a] (Ω/square)	1.2×10^4	2.0×10^4	4.0×10^4
thickness of film[b] (μm)	0.515	0.512	0.650
conductivity ($S \cdot cm^{-1}$)	1.62	0.98	0.42

[a] The values expressed as means ($n = 6$). [b] The values expressed as means ($n > 3$).

Since the first report on the electrochemical synthesis of polythiophene in 1981, PTh and its derivatives have received a great deal of attention due to their high electroconductivity, environmental stability in both doped and dedoped states, and their possible applications.[24,25] Though electropolymerization can be used to produce conducting polymer films, conductive substrates are required. Also, some other methods, such as oxidative polymerization, spin casting from PEDOT/polystyrene sulfonate solution, chemical vapor deposition, matrix-assisted pulsed laser evaporation, and plasma polymerization, can yield conducting polymer films.[26−31] In most cases, low reproducibility, low conductivity, or the requirement for complicated special instruments restricted the applications of these methods. Recently, much attention has been given to the preparation of conductive and transparent conductive films using a dipping polymerization method.[32,33] This simple and straightforward deposition method, which did not require special instrumentation, offered a simple and potentially low cost method to produce smooth and continuous conductive polythiophene films on a variety of different substrates (such as metal, glass, and silicon) with reproducible thickness. In the present work, a preliminary study was carried out on the chemical polymerization of thiophene, 3-methylthiophene, and 3-dodecylthiophene in the presence of sodium dodecylbenzenesulonate (SDBS). The conditions of deposition were optimized and the resulting films on polytetrafluorethylene (PTFE) were characterized by scanning electron microscopy (SEM), conductivity measurements, and water contact angle measurements. To assess the biocompatibility of the new polymers films, the attachment and proliferation of PC 12 pheochromocytoma cells and NIH 3T3 fibroblasts were studied. The micro BCA protein assay and electrophoresis were used to analyze the protein adsorption on the film surfaces.

2. Materials and Methods

2.1. Materials. Thiophene, 3-methylthiophene, and 3-dodecylthiophene were purchased from Aldrich (St Louis, MO). Ferric chloride ($FeCl_3$) and SDBS were purchased from SCRC (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and used as received. PTFE was washed with acid, base, and acetone independently before use. Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (St Louis, MO). Human plasma fibronectin (FN) was purchased from Chemicon (Temecula, CA). A micro BCA protein assay reagent kit was purchased

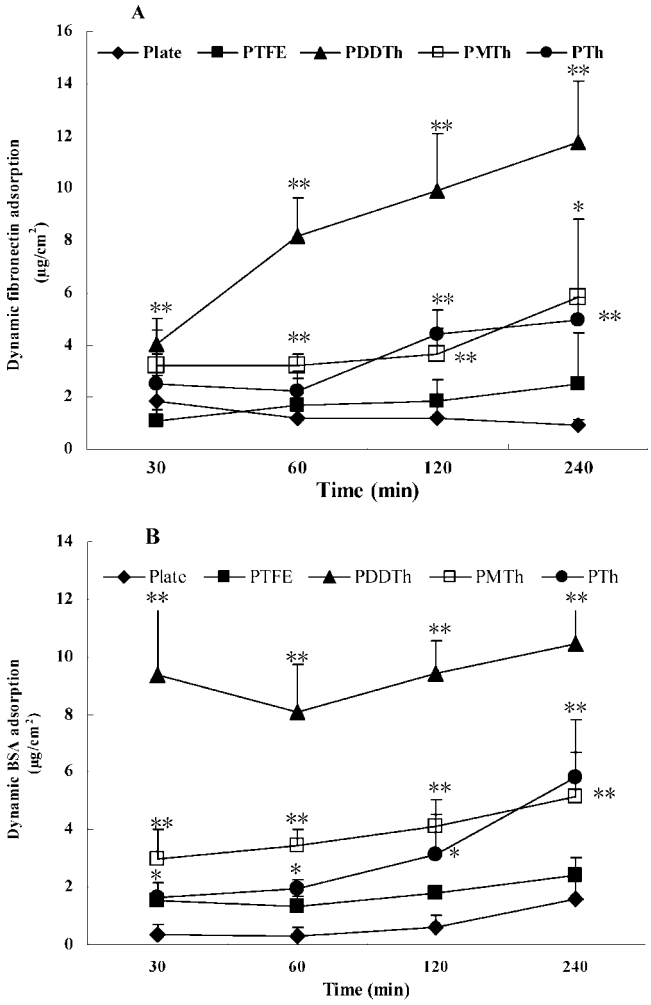

Figure 2. Dynamic adsorption of proteins on plate, PTFE, PDDTh, PTh, and PMTh film surfaces: (A) fibronectin; (B) BSA. The * and ** symbols indicate significant differences compared with plate control and PTFE, respectively ($p < 0.05$, $n = 3$).

TABLE 2: Water Contact Angles of Polythiophene Films ($n = 3$)

sample	angle (deg)
PTh	75.0 ± 2.14
PMTh	115.5 ± 1.46
PDDTh	133.3 ± 4.03

from Pierce (Rockford, IL). A silver stain kit was purchased from Fluka (Buchs, Switzerland). Acridine orange fluorescent dye was purchased from Shanghai Chemical Reagent Corporation. All cell culture media and supplements were from Gibco (Langley, OK).

2.2. Synthesis and Processing of Polythiophene (PTh), Poly (3-methylthiophene) (PMTh) and Poly (3-dodecylthiophene) (PDDTh) Films. The mixture of $FeCl_3$ (1.5497 g), SDBS (0.3334 g), and chloroform (45 mL) was stirred at room temperature for 8−10 h, and then transferred to a Schlenk flask (100 mL) under N_2 atmosphere. A solution of thiophene (0.25 mL) in chloroform (5 mL) was added dropwise to the stirred $FeCl_3$ solution under N_2 atmosphere within one minute. Then the substrate PTFE was immediately immersed into the polymerization bath without stirring. Thin films of polythiophenes on PTFE were drawn out of the reaction bath 18 h later and rinsed with distilled water and methanol and dried in a vacuum drier at room temperature. The molar ratio [monomer]/[oxidant] was 1:3. For PMTh, 3-methylthiophene (0.246 mL), $FeCl_3$ (1.2543 g), SDBS (0.4445 g), and chloroform (50 mL) were added. The procedure of synthesis and processing were similar to that of PTh. For PDDTh, the method of polymerization used in this case was according to the reported method.[34] In a 25 mL, two-necked, round-bottom flask, anhydrous $FeCl_3$ (0.4868 g) was suspended in chloroform (15 mL) under N_2 atmosphere. A solution of 3-dodecylthiophene (0.28 mL) in $CHCl_3$ (5 mL) was added dropwise over 30 min under N_2 atmosphere. The resulting black mixture was stirred for 12 h. After stirring, the reaction mixture was poured into methanol (200 mL), the precipitate was collected by filtration and washed with water, methanol until the filtrate was colorless, dried in a vacuum drier at room temperature. To a mixture of PDDTh (5 mg) and SDBS (7 mg), THF (1 mL) was added and the solution was stirred 12 h at room temperature. High quality films of PDDTh on PTFE were obtained through casting from the above solution and dried in a vacuum drier. Chemical doping was performed by dipping the film in a nitromethane (50 mL) solution including $FeCl_3$ (1.5498 g) for 4 h at room temperature. After doping, all films were rinsed several times with nitromethane and dried in vacuum drier.

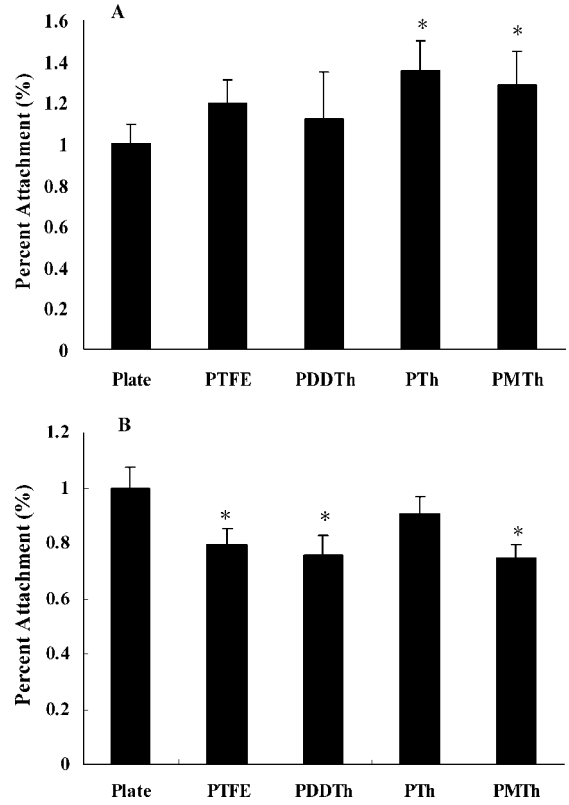

Figure 3. Attachment of PC 12 pheochromocytoma cells (A) and NIH 3T3 fibroblasts (B) on plate, PTFE, PDDTh, PTh and PMTh. Cells were seeded at 10^4 cells/cm^2 and allowed to attach for 2 h. The data were expressed as means ± SD ($n = 4$). The * symbol indicates the significant differences compared with plate control ($p < 0.05$).

2.3. Thickness of Thin Films. Film thickness was measured by SEM (JSM-6390LV, JEOL, Japan) via edge-on view of a film deposited on a commercial glass slide.

2.4. Conductivity Measurements. Surface conductivities of the as-prepared films were measured using a 4-probe technique with a low-impedance instrument (MCP-T610, Mitsubishi Chemical Corporation, Japan).

2.5. Surface Morphology Characteristics. Surface morphologies of films were observed using SEM (JSM-6700F, JEOL, Japan) after being coated with gold in vacuum.

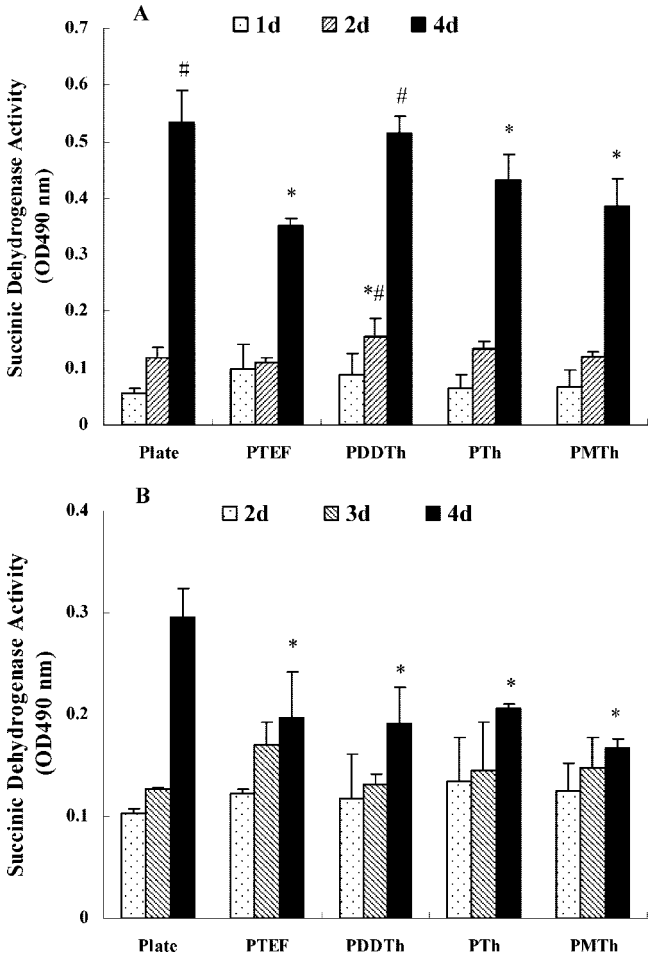

Figure 4. Proliferation of PC 12 pheochromocytoma cells (A) and NIH 3T3 fibroblasts (B) on plate, PTFE, PDDTh, PTh, and PMTh, determined by MTT method based on succinic dehydrogenase activity. Cells were seeded at 10^4 cells/cm^2 and allowed to proliferate for 4 days. The data were expressed as means ± SD ($n = 3$). The * and # symbols indicate the significant differences compared with plate control and PTFE control, respectively ($p < 0.05$).

2.6. Aqueous Contact Angle Measurements. Wettabilities of the synthesized polythiophene films were evaluated as static water contact angles using a contact angle goniometer (JC2000A, Shanghai, China). Briefly, a droplet of water (0.5 μL, ultrapure grade) was put on the surfaces of different materials and the images were photographed. Temperature and moisture were constant during the course of the experiments (23 °C and 68%, respectively). The angle between the baseline of the drop and the tangent at the drop boundary was measured. Measurements were performed at least in triplicate on three different batches of materials.

2.7. Dynamic Adsorption of BSA and FN. BSA and FN were used to determine the adsorption abilities of the synthesized film surfaces. Protein solution (200 μL of 100 μg/mL) was pipetted onto film surfaces in a 96-well plate. The adsorption study was conducted in a 37 °C incubator for 30, 60, 120, and 240 min, respectively. The films were then rinsed carefully with PBS buffer to remove the nonadherent protein. An aqueous solution (200 μL of 1%) of sodium dodecyl sulfate (SDS) was added to desorb the proteins on the film surfaces. The plate was then left shaking for 30 min at room temperature. The desorption fraction was quantified with the micro BCA protein assay reagent kit.

2.8. Cell Cultures. PC 12 pheochromocytoma cells were used to study cell adhesion, spreading and proliferation on the polythiophene film surfaces. The PC 12 cells were routinely cultured in tissue culture flasks with high glucose-Dulbecco's modified eagle's medium (HG-DMEM), containing 100 units/mL penicillin, 100 μg/mL streptomycin sulfate, and 20% fetal bovine serum and incubated at 37 °C in a humidified atmosphere with 95% air and 5% CO_2. The culture medium was refreshed every two days. The synthesized polythiophene films on PTFE substrate were used for this study, both PTFE substrate and culture plate (flat bottom with lid, Corning) were as the control. When the cells became almost confluent after 5 days, they were detached by treatment with 0.25% trypsin for 3 min at 37 °C. Before cell seeding, the different substrates were placed into 96-well culture plate and sterilized by UV for 20 min, then were equilibrated with prewarmed (37 °C) HG-DMEM medium for 2 h. After removing the medium from the wells by pipetting, the cells were counted to 10^4 cells/cm^2 and 200 μL of the cells suspension were poured onto each substrate.

NIH 3T3 fibroblasts were also used as the model to further evaluate the cell compatibility of polythiophene films. The culture medium was replaced with RPMI 1640 medium, and the other culture conditions were the same with those of PC 12 pheochromocytoma cells.

2.9. Cell Adhesion and Proliferation. Cell adhesion and proliferation were measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT) based on succinic dehydrogenase activity at OD 490 nm ($n = 3$); 630 nm was chosen as the reference wavelength. This was done at intervals of 2 h, 1, 2, and 4 days for PC 12 pheochromocytoma cells, or 2 h, 2, 3, and 4 days for NIH 3T3 fibroblasts. The wells were carefully washed with PBS, 20 μL of MTT was added in 180 μL of culture medium, and the cell culture was continued for 4 h. Then, the solution was removed and the wells were washed twice with PBS. The mixture of methanol and isopropanol (200 μL) was pipetted into the wells and OD values were read on a microplate reader (Stat Fax-2100, Awareness Technology, USA).

2.10. Cell Morphology Analysis. At the prescribed time points, all substrates were rinsed in PBS and the cells attached to the surfaces were stained with acridine orange fluorescent dye in PBS (pH 7.2) for 5 min and examined by a fluorescence microscope (IX71, Olympus, Japan). Moreover, cells were taken out randomly and examined by a scanning electron microscope after being cultured for 2 or 3 days. Briefly, after washing with PBS (pH 7.2), the cells attached on films were fixed by immersing the materials into 2.5% solution of glutaraldehyde in PBS and allowing them to stand in the fixative at 4 °C for more than two hours. Finally, a standard dehydration in ethanol graded series was performed. The samples were mounted on stubs and coated in vacuum with gold, then were examined by SEM.

2.11. Statistical Analysis. The number of independent replicas was listed individually for each experiment. Where applicable, all data were means ± standard deviation. The data were analyzed by one-way factorial ANOVA and multiple comparisons. Significant effects of treatment were defined using Fisher's method as a Post-Hoc test, with $p < 0.05$ considered as being statistically significant.

3. Results and Discussion

3.1. Morphologies of the Polythiophene Films. The surface morphologies of the synthesized films are shown in Figure 1. The as-prepared PTh film was found to be homogeneous, compact, and composed of spherical particles averaging 200 to 300 nm (Figure 1A). As shown in Figure 1B, the poly

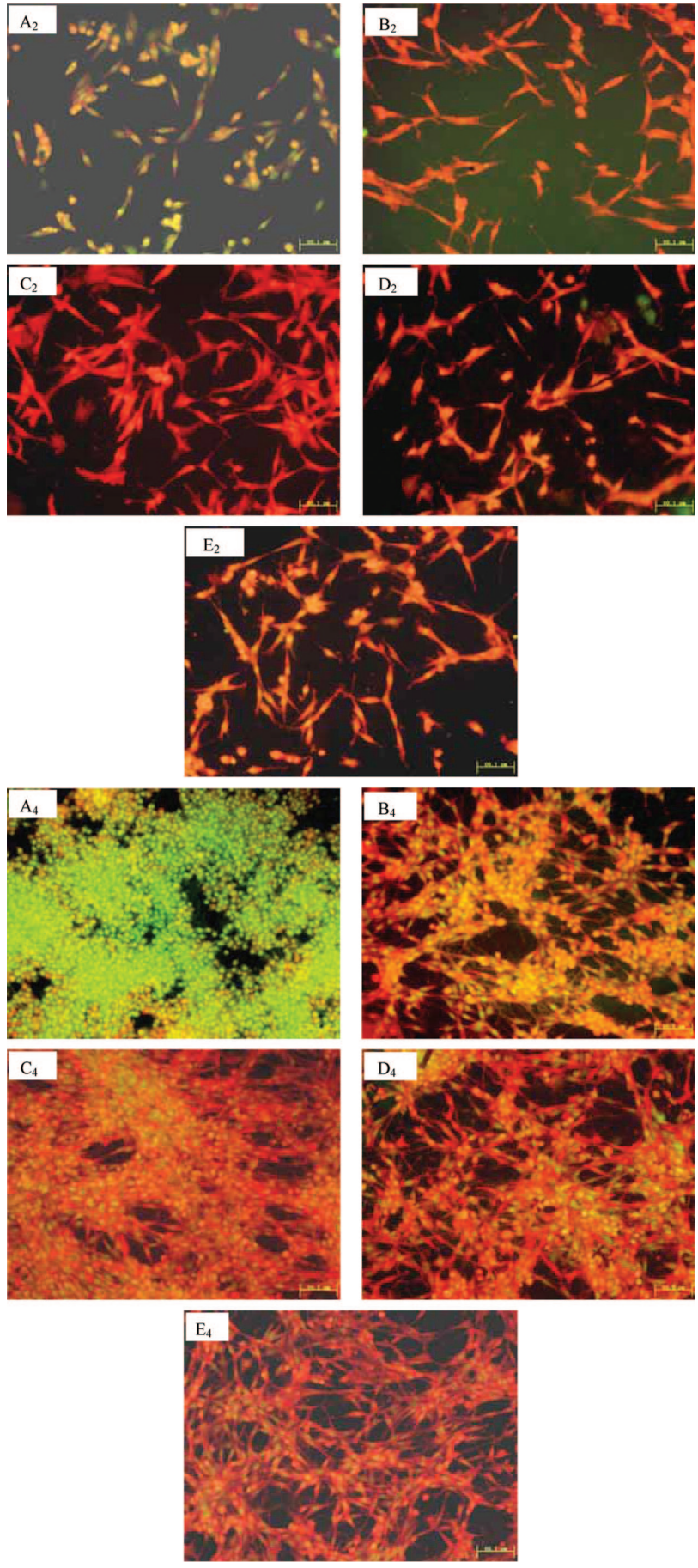

Figure 5. Morphologies and distributions of PC 12 pheochromocytoma cells cultured on plate (A), PTFE (B), PDDTh (C), PTh (D), and PMTh (E) for 2 days (A_2-E_2) and 4 days (A_4-E_4). All scale bars represent 60.1 μm.

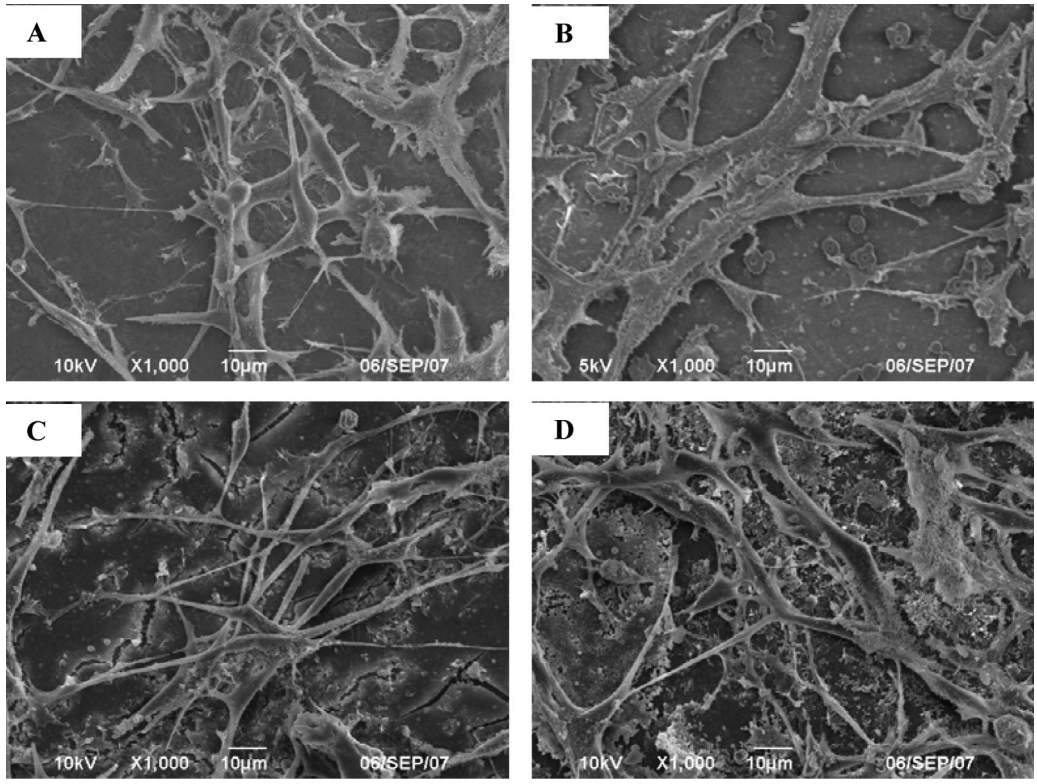

Figure 6. Scanning electron microscopy of PC 12 pheochromocytoma cells on PTFE (A), PDDTh (B), PTh (C), and PMTh (D) after 48 h of cell culture. All scale bars represent 10.0 μm.

(3-methylthiophene) film also gave a smooth globular structure with particle size of about 500 nm, but fiber-like structure was also present. Poly (3-dodecylthiophene) films from direct polymerization did not give the obvious structure, but instead gave a lamellar structure (Figure 1C). The films of poly (3-dodecylthiophene) through casting from the colloid of poly (3-dodecylthiophene) exhibited a much smoother, dense morphology with micro/nanostructure (Figure 1D). Though there have been many reports dealing with the synthesis of polythiophenes in the presence of surfactant, further study of the synthetic conditions for precisely controlling the morphology and properties of the result polymers was still needed. The mechanism by which the surfactant can precisely direct the structure of the films might relate to the ability of the surfactant to form "micelles" in the solutions, attach on the substrate surface, and function as templates. Compared to the polythiophene films exhibiting ribbon structures reported by Gök's group,[35] the morphology of polythiophene in the present study was significantly different, exhibiting quite uniform spherical particles. The reason for this may be due to the different concentrations of SDBS used, which can form different phases of micelles. A nitrogen atmosphere and one free of water were very important in this method, as otherwise uniform films would fail to form, and may even not deposit at all. Other concentrations of SDBS were also tested (0.2066 and 0.4445 g), and the resulted morphologies of the films were similar, but the conductivity was on the order of 10^5 Ω/square, lower than the film of Figure 1A. Experiments for PMTh with two other concentrations of SDBS were also carried out (0.1556 and 0.2066 g), but the morphology of the resulting films was more heterogeneous and the conductivity was much lower (on the order of 10^5 Ω/square). The fiberlike morphology observed on the PMTh films may be due to the lower oxidation of it (1.48 eV) compared to thiophene (1.6 eV). It is well-known that polymers with long and flexible main chains such as PDDTh are usually folded to form lamellae.[36] Films synthesized by direct polymerization failed to reveal the spherical morphology, but a soft, lamellae structure (Figure 1C). By trying various synthetic conditions of PDDTh, such as the concentration of SDBS (4, 7, 14, 18, and 35 mg), solvent (1 and 2 mL), and the concentration of PDDTh (5 and 10 mg), a smooth morphology with sparse, large spheres was achieved in the optimizing condition of 7 mg SDBS, 1 mL THF and 5 mg PDDTh by casting from the colloid of PDDTh (Figure 1D). This conductive and hydrophobic PDDTh film and its microstructure may be useful as coatings in many applications and further studies are underway.

3.2. Conductive Properties. The product of the oxidation polymerization in Scheme 1 should be p-doped polythiophene (doped Cl^- anions). The conductivity of these films was in the order of PTh > PMTh > PDDTh (Table 1). Compared to the polythiophene films reported by Gök's group, which exhibited very low conductivity, the conductivity of the PTh film in the present study exhibited a significantly increased value.[35] It is well-known that the conductivity of the conducting polymer depends on the counterions, the extent of doping, chain conformations and so on. The uniform and compact morphology of the PTh film may be one reason for the order of conductivity. The conductivity of the as-prepared films was larger than the reported.[31,35] The doped PDDTh would be dedoped due to the washing with water and methanol,[37] redoping with $FeCl_3$ is necessary.[38]

3.3. Protein Adsorption on the Synthesized Films. Protein adsorption and desorption on material surfaces play a key role in the biocompatibility of medical implants, biomineralization and protein separation. It is recognized that proteins can be adsorbed on the surfaces of biomaterials before cell attachment. Moreover, the amount, type, and conformation of the proteins are some of the main factors that affect the interaction between cells and the surface by supplying a structural support and message directing from the substrate to the cells.[39] FN is an

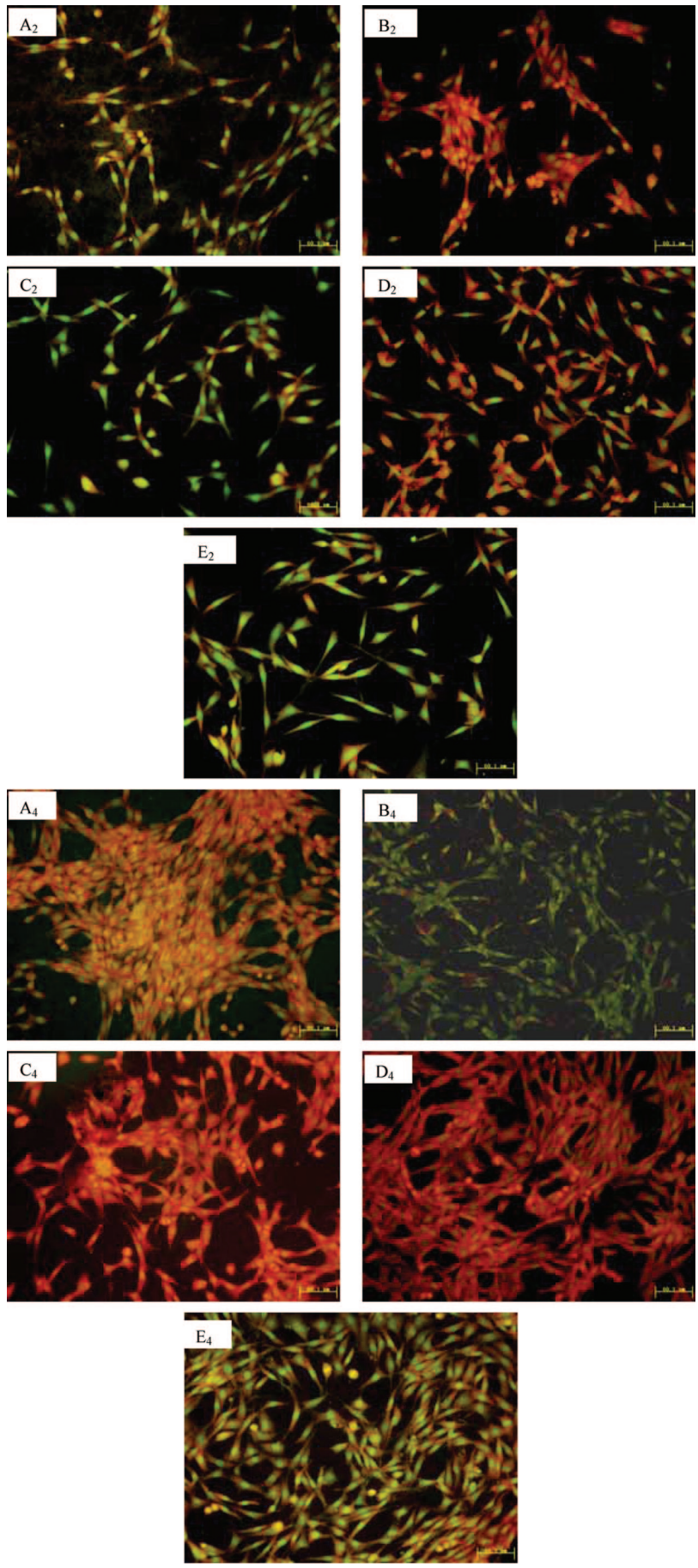

Figure 7. Morphologies and distributions of NIH 3T3 fibroblasts cultured on plate (A), PTFE (B), PDDTh (C), PTh (D), and PMTh (E) for 2 days (A_2-E_2) and 4 days (A_4-E_4). All scale bars represent 60.1 μm.

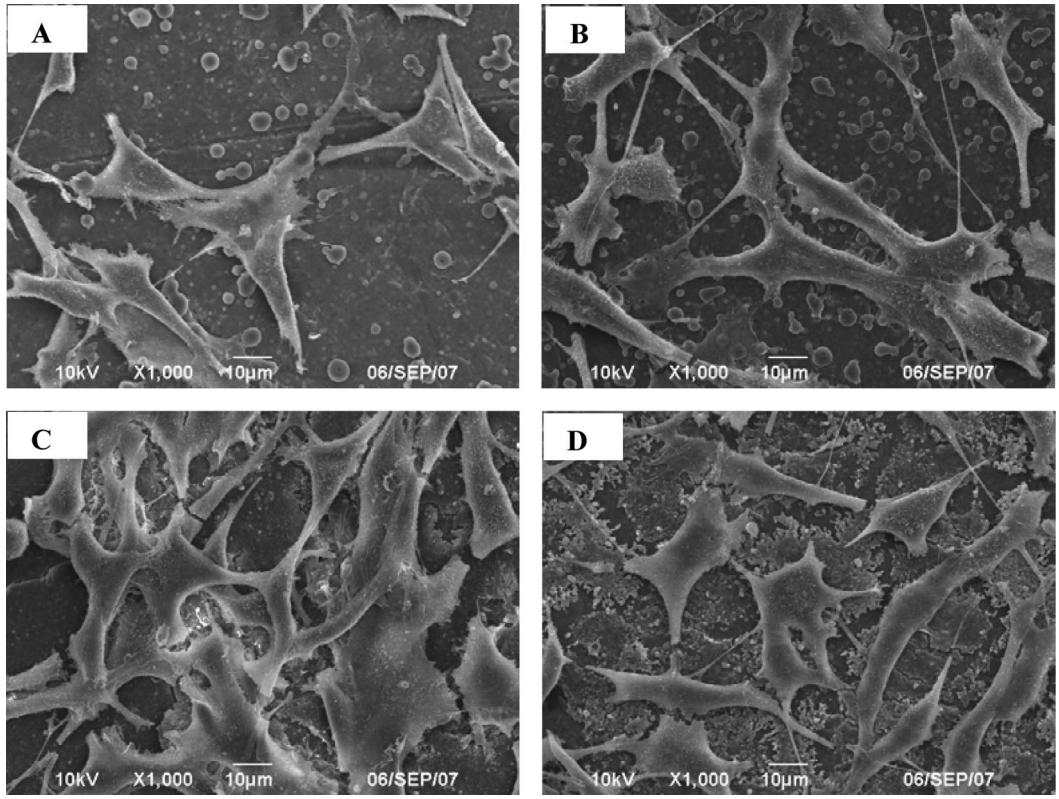

Figure 8. Scanning electron microscopy of NIH 3T3 fibroblasts on PTFE (A), PDDTh (B), PTh (C), and PMTh (D) after 72 h of cell culture. All scale bars represent 10.0 μm.

extracellular matrix protein known to promote cell attachment and spreading. Materials coated with FN are promising for a variety of cell-contacting applications. And a number of studies attest to the enhanced cell attachment and spreading of surfaces coated with FN, compared to identical materials in the absence of FN.[40] Schmidt et al. reported that increasing the adsorption of FN would increase neuronal attachment and neurite outgrowth.[41] Therefore, in the present study, the dynamic adsorption of FN and BSA on the synthesized films surfaces was further monitored. As shown in Figure 2, the amounts of both FN and BSA adsorption on the PDDTh film were higher than those on other films ($p < 0.05$, $n = 3$), though the adsorption of both proteins on all films increased gradually over 240 min. However, both the plate control and PTFE groups exhibited only a minor increase of protein adsorption in 240 min compared with the synthesized films (PDDTh, PTh and PMTh). An extensive amount of literatures has been reported on the factors mediating the interaction between the film surface and the protein, including surface charge, wettability, free energy, roughness, and chemistry.[39,42] In the present study, we prepared synthetic films with different length of alkyl side chains, which would result in a change in wettability. The water contact angles on different film surfaces were measured and are summarized in Table 2, which shows that the PDDTh film (with dodecyl side chain) was more hydrophobic than the PMTh (with methyl side chain) and PTh films. They showed 133.3 ± 4.03°, 115.5 ± 1.46°, and 75 ± 2.14° of water contact angles, respectively. As for the hydrophobic surface, water molecules bind to the hydrophobic film surfaces through Van der Waals forces, proteins can adsorb more easily than a hydrogen bond forming between hydrophilic surfaces and proteins.[43] Therefore, the increase in protein adsorption on the PDDTh film is attributed to the increasing surface hydrophobicity, which mainly resulted from the dodecyl side chain. On the other hand, Lee et al. reported that nanoscale roughness altered cellular adhesion by increasing the surface density of adsorbed FN.[44] As shown in Figure 1, the PTh film surface was composed of homogeneous, compact, spherical particles with an average diameter of 200−300 nm, while the PMTh film surface indicated a smooth globular structure with about 500 nm size. Moreover, there was no such structure in nanoscale on PDDTh film. That is to say, the PTh film surface could provide more effective adsorption sites to the protein than both the PMTh and PDDTh films. However, the difference of protein adsorption that could be explained by the morphologic characteristic of film surfaces between these films could not be observed in our study. But it is evident that hydrophobicity was the main reason for the observed adsorption properties of FN and BSA.

3.4. Cell Adhesion and Proliferation. The differences in physical properties among these films may affect their biological behaviors.[45] In order to clarify the influence of surfaces and assess the cell compatibility of the resulting polymer films, in vitro experiments were carried out to test the function of PC 12 pheochromocytoma cells and NIH 3T3 cells, such as attachment, spreading, and proliferation on these film surfaces. Figure 3A shows PC 12 pheochromocytoma cells adhesion on PTFE, PDDTh, PMTh, PTh, and plate control after two hours of cell seeding. On the basis of the succinic dehydrogenase activity analysis using the MTT method, the initial cell attachment numbers on PMTh and PTh substrates were significantly higher than on the plate control ($p < 0.05$, $n = 4$), but no significant difference could be observed among other groups. For NIH 3T3 cells on these films, as shown in Figure 3B, the adhesion of NIH 3T3 fibroblasts on both the plate control and PTh was better than on other surfaces; the percentage of the attached cells was only about 80%, 73%, and 67% on PTFE, PMTh, and PDDTh, respectively, compared to the plate control.

Figure 4A shows the proliferation of PC 12 pheochromocytoma cells on PTFE, PDDTh, PMTh, PTh, and the plate control after 1, 2, and 4 days of cell seeding. The initial cell proliferation (1 day) had no difference on these substrates. After 2 days of the cell seeding, the growth of cells on the PDDTh film surface was faster than for other groups, while the proliferations of PC 12 cells on the PTh, and PMTh groups showed no significant difference when compared with other groups. At the fourth day, the cell relative number in the plate group and the PDDTh group was higher than for other groups. In contrast, the proliferations of PC 12 cells on the PMTh and PTh groups showed no significant difference compared with that on the PTEF group. Moreover, there was no significant difference in the PDDTh group compared to the plate group. As for the proliferation of NIH 3T3 fibroblasts on different surfaces (as shown in Figure 4B), the result after 3 days of cell culture showed no difference between these groups. On the fourth day, however, the relative cell number in the plate group increased quickly, and to a higher extent than other groups. Meanwhile, the relative cell number on the PDDTh, PMTh and PTh film surfaces was not significantly different compared to that on the PTFE surface, which is known for its good biocompatibility.

In Figure 5, the morphology and distribution of PC 12 pheochromocytoma cells on different film surfaces was shown at different intervals after cell seeding, which were stained for 5 min with acridine orange in PBS buffer. There were no differences of cell morphologies on PDDTh, PMTh, and PTh compared with PTFE and the plate control after 1 day of cell culture (data not shown). At the second day, the PC 12 pheochromocytoma cells spread completely and the characteristic pseudopodia maintained physical contact with each other. Especially the cells in the PDDTh group were denser than in other groups, which showed the same trend as the MTT results (Figure $5A_2-E_2$). At the fourth day, the cells in the PDDTh and plate groups were denser than in the other three groups; also the cells in all the groups produced long neurites. Typical morphologies of PC 12 pheochromocytoma cells on different film surfaces were observed by SEM as shown in Figure 6. It is clear that PC-12 cells developed a spindle like morphology and extended much on PDDTh film compared with others (Figure 6B). Morphologies of NIH 3T3 cells and distribution were also visualized by a fluorescent staining method and SEM. As shown in Figure $7A_2-E_2$, there was no difference between PTFE, PDDTh, PMTh, PTh and the plate control after 2 days of cell culture. On the fourth day, confluent monolayer cells were formed in these groups (Figure $7A_4-E_4$), and the NIH 3T3 cells spread completely and exhibited their characteristic polygonal morphologies. Typical SEM photographs of NIH 3T3 fibroblasts on PTFE, PDDTh, PMTh, and PTh are shown in Figure 8, which indicates their spread-out morphology.

In order to be used in biomedical application such as electrode coatings, polymers must be biocompatible. In vitro biocompatibility tests are meant to simulate biological reactions of materials when they are placed on or into tissues of the body. The development of a cytotoxicity of neural probes and biosensors is often based on differentiated neuronal stem cells and PC 12 pheochromocytoma cells that may emulate components of the central nervous system. In the present study, PC 12 pheochromocytoma cells and NIH 3T3 fibroblasts were chosen as the models, and their functions, such as adhesion, spreading, and proliferation, were examined to analyze the interaction between both cells and the synthesized polythiophene films prepared by "in-situ" deposition. A great body of work has shown that the attachment, adhesion, and spreading of cells on polymeric materials depend largely on surface characteristics, such as hydrophilicity/hydrophobicity or surface free energy, chemistry, charge, roughness, and rigidity.[46,47] Chung et al. reported that the increased surface roughness of a surface at 10−100 nm scales could enhance adhesion and growth of human umbilical cord vein endothelial cells.[48] However, the present study indicates that the effect of the synthesized films on cell adhesion was cell-specificity. The attached cell numbers in both the PTh and PMTh groups were significantly enhanced compared to the plate control ($p < 0.05$) for PC 12 cells, while no such trend was observed for NIH 3T3 cells (Figure 3A and B). In the following 4 days of cell culture, a significant difference in NIH 3T3 cell proliferation could not be detected on the synthesized films when compared to that observed in the PTFE group. However, the relative numbers of PC 12 cells in the PDDTh group was 1.41 and 1.46 times than those in the PTFE group at the second and the fourth days of cell culture, respectively (Figure 4A). PC 12 cells have been known as inherently poorly adherent cells and require an adhesion-promoting protein to adhere and proliferate on substrates.[22] Therefore, behaviors of the synthesized polythiophene films in protein adsorption might be the main reason for the improvement of proliferation of PC 12 pheochromocytoma cells on PDDTh film.

4. Conclusions

In this work, high quality films of polythiophene with different length of alkyl side chains were successfully prepared by chemical in-situ deposition in the presence of a surfactant under a nitrogen atmosphere. The synthetic conditions were optimized, the morphologies of the films were homogeneous, and the conductivity was high enough for biomedical applications. We also performed a cell compatibility evaluation on the synthesized films by culturing two different cell lines on these surfaces, the results of which proved that the synthesized films were acceptable substrates for both cells, which could support cell attachment and proliferation. Especially a high hydrophobic polythiophene film, poly (3-dodecylthiophene) (PDDTh) exhibited high ability of protein adsorption and proliferation of PC 12 pheochromocytoma cells, implied as a good candidate for biomedical applications.

Acknowledgment. The authors gratefully acknowledge the financial support provided by the National Program on Key Basic Research Projects of China (973 Program, 2005CB724306) and the National Natural Science Foundation of China (30870635).

References and Notes

(1) Ahmad, N.; MacDiarmid, A. G. *Synth. Met.* **1996**, *78*, 103.
(2) Sivaraman, P.; Hande, V. R.; Mishra, V. S.; Rao, C. S.; Samui, A. B. *J. Power Sources* **2003**, *124*, 351.
(3) Huang, J.; Virji, S.; Weiller, B. H.; Kaner, P. B. *Chem.—Eur. J.* **2004**, *10*, 1314.
(4) Kaner, R. B. *Synth. Met.* **2001**, *125*, 65.
(5) Maziarz, E. P.; Lorenz, S. A.; White, T. P.; Wood, T. D. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 659.
(6) Joo, J.; Epstein, J. *Appl. Phys. Lett.* **1994**, *65*, 2278.
(7) Guimard, N. K.; Gomez, N.; Schmidt, C. E. *Prog. Polym. Sci.* **2007**, *32*, 876.
(8) Kotwal, A.; Schmidt, C. E. *Biomaterials* **2001**, *22*, 1055.
(9) Cui, X. Y.; Wiler, J.; Dzaman, M.; Altschuler, R. A.; Martin, D. C. *Biomaterials* **2003**, *24*, 777.
(10) Stanffer, W. R.; Cui, X. Y. *Biomaterials* **2006**, *27*, 2405.
(11) Li, M. Y.; Guo, Y.; Wei, Y.; MacDiarmid, A. G.; Lelkes, P. I. *Biomaterials* **2006**, *27*, 2705.
(12) Huang, L.; Hu, J.; Lang, L.; Wang, X.; Zhang, P. B.; Jing, X. *Biomaterials* **2007**, *28*, 1741.

(13) Bidez, P. R., III; Li, S.; MacDiarmid, A. G.; Venancio, E. C.; Wei, Y.; Lelkes, P. I. *J. Bomater. Sci. Polym. Edn.* **2006**, *17*, 199.
(14) Wangaman, M.; Sannigrahi, B.; Mcgeady, P.; Khan, I. M. *Eur. Polym. J.* **2003**, *39*, 1405.
(15) Cui, X. Y.; Martin, D. C. *Sens. Actuators B: Chem.* **2002**, *89*, 92.
(16) Xiao, Y. H.; Cui, X. Y.; Hancock, J. M.; Bouguettaya, M.; Reynolds, J. R.; Martin, D. C. *Sens. Actuators B: Chem.* **2004**, *99*, 437.
(17) Xiao, Y. H.; Cui, X. Y.; Martin, D. C. *J. Eletroanal. Chem.* **2004**, *573*, 43.
(18) Yang, J. H.; Kim, D. H.; Hendricks, J. L.; Leach, M.; Northey, R.; Martin, D. C. *Acta Biomater.* **2005**, *1*, 125.
(19) Richardson-Burns, S. M.; Hendricks, J. L.; Foster, B.; Povlich, L. K.; Kim, D. H.; Martin, D. C. *Biomaterials* **2007**, *28*, 1539.
(20) Richardson-Burns, S. M.; Hendricks, J. L.; Martin, D. C. *J. Neural Eng.* **2007**, *4*, L6.
(21) del Valle, L. J.; Aradilla, D.; Oliver, R.; Sepulcre, F.; Gamez, A.; Armelin, E.; Alemán, C.; Estrany, F. *Eur. Polym. J.* **2007**, *43*, 2342.
(22) Slaughter, G. E.; Bieberich, E.; Wnek, G. E.; Wynne, K. J.; Guiseppi-Elie, A. *Langmuir* **2004**, *20*, 7189.
(23) Widge, A. S.; Jeffries-EI, M.; Cui, X. Y.; Lagenaur, C. F.; Matsuoka, Y. *Biosens. Bioelctron.* **2007**, *22*, 1723.
(24) Jin, S.; Cong, S.; Xue, G.; Xiong, H.; Mansdorf, B.; Cheng, S. Z. D. *Adv. Mater.* **2002**, *14*, 1492.
(25) Roncali, J. *Chem. Rev.* **1992**, *92*, 711.
(26) Groenendaal, L.; Zotti, G.; Aubert, P. H.; Waybright, S. M.; Reynolds, J. R. *Adv. Mater.* **2003**, *15*, 855.
(27) Jensen, B. W.; Breiby, D. W.; West, K. *Synth. Met.* **2005**, *152*, 1.
(28) Jonas, F.; Morrison, J. T. *Synth. Met.* **1997**, *85*, 1397.
(29) Im, S. G.; Gleason, K. K. *Macromolecules* **2007**, *40*, 6552.
(30) Bloisi, F.; Cassinese, A.; Papa, R.; Vicari, L.; Califano, V. *Thin Solid Films* **2007**, *516*, 1594.
(31) Wang, J. G.; Neoh, K. G.; Kang, E. T. *Thin Solid Films* **2004**, *446*, 205.
(32) Hohnholz, D.; MacDiarmid, A. G.; Sarno, D. M.; Jones, W. E., Jr *Chem. Commun.* **2001**, 2444.
(33) Mazur, M. *Thin Solid Film* **2005**, *472*, 1.
(34) Daoud, W. A.; Xin, J. H. *J. Appl. Polym. Sci.* **2004**, *93*, 2131.
(35) Gök, A.; Omastová, M.; Yavuz, A. G. *Synth. Met.* **2007**, *157*, 23.
(36) Wang, W.; Toh, K. C.; Tjiu, C. W. *Macromol. Chem. Phys.* **2004**, *205*, 1269.
(37) Li, Y. F.; Qian, R. Y. *Synth. Met.* **1993**, *53*, 149.
(38) Li, D. F.; Wang, W.; Wang, H. J.; Jia, X. S.; Wang, J. Y. *Appl. Surf. Sci.* **2008**, *255*, 581.
(39) Shen, J. W.; Wu, T.; Wang, Q.; Pan, H. H. *Biomaterials* **2008**, *29*, 513.
(40) Wittmer, C. R.; Phelps, J. A.; Saltzman, W. M.; Van Tassel, P. R. *Biomaterials* **2007**, *28*, 851.
(41) Kotwal, A.; Schmidt, C. E. *Biomaterials* **2001**, *22*, 1055.
(42) Lu, J. R.; Zhao, X. B.; Yaseen, M. *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 60.
(43) Wang, H. J.; Liu, X. M.; Ji, L. W.; Ma, D. L.; Ren, Q. S.; Wang, J. Y. *J. Biomed. Mater. Res. B* **2006**, *79*, 411.
(44) Lee, M. H.; Ducheyne, P.; Lynch, L.; Boettiger, D.; Composto, R. J. *Biomaterials* **2005**, *27*, 1907.
(45) Niple, J. C.; Daigle, J. P.; Zaffarella, L. E.; Sullivan, T.; Kavet, R. *Bioeletromagnetics* **2004**, *25*, 369.
(46) Miller, D. C.; Thapa, A.; Haberstroh, K. M.; Webster, T. J. *Biomaterials* **2004**, *25*, 53.
(47) Hwang, J. J.; Lyer, S. N.; Li, L. S.; Claussen, R.; Harrington, D. A.; Stupp, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9662.
(48) Chung, T. W.; Liu, D. Z.; Wang, S. Y.; Wang, S. S. *Biomaterials* **2003**, *24*, 4655.

JP8041704