Characterization of Nanostructure and Cell Compatibility of Polyaniline Films with Different Dopant Acids

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Polyaniline (PANi) films were prepared by direct polymerizing deposition with four different kinds of acids as dopants or were prepared by a casting method on the surface of a polytetrafluoroethylene substrate. The properties of PANi films were characterized using atomic force microscopy, electrical conductivity measurements, and water contact angle measurements. Unlike the casting PANi film, experimental results indicated that the synthesized PANi films had a similar nanostructure as that of average nanoparticles (approximate diameter of 30−50 nm). To investigate the potential usefulness of PANi films in biomedical applications, we also studied their biocompatibility through the adhesion and proliferation properties of PC-12 pheochromocytoma cells. All the films were found to be biocompatible and allowed cell attachment and proliferation. However, the synthesized films have a much higher ability for cell adhesion than the casting film. After 4 days of culture on different PANi films, the cells formed more confluent monolayers on the synthesized PANi films than on the casting films. These results demonstrate that the PANi films could be used to culture neurotic cells and that their surface architecture on the nanoscale may affect cell function such as attachment and proliferation.

Introduction

Since their discovery about 30 years ago, conducting polymers have found an increasing number of applications in various areas including battery manufacturing, anticorrosion coating, and light-emitting diodes.[1,2] More recently, there has been a growing interest in diverse biomedical applications, including tissue engineering, drug delivery, and a variety of sensors and actuators due to the polymers' combination of useful mechanical, optical, and electronic properties. Some conducting polymers, such as polypyrrole (PPy), poly(3,4-ethylenedioxythiophene) (PEDOT), and PEDOT derivatives, which are well-characterized in terms of their chemical and physical properties, have gained attention as electroactive substrates for the culture of electrically excitable cells, such as neuronal or muscle cells.[3−5]

Schmidt and co-workers functionalized the surface of chlorine-doped PPy to anchor peptide molecules that promote nerve regeneration, blood vessel growth, or other biological processes.[6] Langer et al. examined the biocompatibility of PPy through culturing dissociated primary cerebral cortical cells on PPy samples that had been doped with polystyrene sulfonate or sodium dodecylbenzenesulfonate and observed a neural network growth on the PPy surface.[7] Martin and Cui prepared biological active coatings by the electrochemical deposition of conducting PEDOT on microfabricated neural electrode sites used for long-term implantation.[8] Additionally, several other surface modifications of neural recording electrodes with conducting polymers have been studied both in vitro and in vivo.[9,10]

By comparison, it is only quite recently that the tunable electroactivity of PANi has been explored in the realm of diverse biomedical applications.[11] Mattioli-Belmonte et al. were the first to demonstrate that PANi is biocompatible in vitro and in long-term animal studies in vivo.[12] Ruckenstein and Li reported that a PEO-grafted PANi surface could decrease the amount of bovine serum albumin protein adsorption and human blood plasma platelet adhesion on the PANi film and that the films possessed a high biocompatibility.[13] Lelkes et al. also demonstrated the potential for using polyaniline as an electroactive scaffold for cardiac and/or neural tissue engineering applications.[14] Currently, the same group also reported that a PANi−gelatin blend of nanofibers might provide a novel conductive material well-suited as biocompatible scaffolds for tissue engineering.[15]

In the past decade, all kinds of PANi nanostructures have been created by nanoporous membranes,[16] template-guided polymerization within channels of zeolites,[17] or through interfacial polymerization.[18] However, the previously mentioned polymerization routes could not prepare PANi films directly. Electrochemical polymerization methods can be used to produce conducting polymer films,[19] although these methods require a special reaction apparatus. Using dipping polymerization to prepare conducting and transparent polymer films is a simple and straightforward deposition process that does not require special instruments. It is also a potentially inexpensive method to produce smooth and continuous conductive PANi films on a variety of different substrate materials with reproducible thicknesses.[20,21] Potentially, this method may be applied to the modification of neural probes to minimize the effect of

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TABLE 1: Conductivity and Diameter Size of PANi Films Deposited on PTFE Substrates

	P-PANi	H-PANi	MA-PANi	C-PANi	casting PANi
surface resistivity (Ω/square, $n = 6$)	5.0×10^4	1.0×10^5	4.0×10^6	4.0×10^5	78.90
thickness of film (μm, $n = 3$)	0.20	0.17	0.15	0.20	3.49
conductivity (S cm^{-1}, $n = 3$)	1.00	0.60	0.02	0.12	36.49
av diameter ($n \geq 40$)[a]	41.92 ± 10.23	34.71 ± 6.01	37.01 ± 6.06	38.09 ± 6.43	*c*
av diameter ($n = 40$)[b]	51.55 ± 8.82[d]	49.44 ± 10.65[d]	46.38 ± 7.51[d]	66.74 ± 5.70[d]	42.48 ± 15.69

[a] Size of nanoparticles in PANi films before immersion into DMEM. Values are expressed as means $\pm$ SD. [b] Size of nanoparticles in PANi films after immersion into DMEM for 4 days. Values are expressed as means $\pm$ SD. [c] No noticeable nanoparticles. [d] Denotes statistically significant difference between before and after immersion into DMEM ($p < 0.01$).

TABLE 2: Surface Roughness Characteristics Measured by AFM ($n = 5$) and Water Contact Angles of PANi Films ($n = 3$)

sample	R_a (nm) mean $\pm$ *SD*	R_{max} (nm) mean $\pm$ *SD*	rms (nm) mean $\pm$ SD	angle (deg)
P-PANi	3.7 ± 0.5	16.5 ± 1.7	4.9 ± 0.7	95.38 ± 2.14
H-PANi	5.3 ± 0.8	25.8 ± 7.8	6.6 ± 1.2	96.42 ± 1.46
MA-PANi	3.3 ± 0.2	12.2 ± 1.6	4.4 ± 0.9	98.65 ± 4.03
C-PANi	6.8 ± 0.3	29.1 ± 4.2	27.1 ± 1.8	102.95 ± 4.00
casting PANi film	5.9 ± 0.4	20.8 ± 3.3	8.5 ± 2.7	73.7 ± 13.13[a]

[a] Represents significant difference in contact angle of casting PANi film with other groups ($p < 0.05$).

interfering reactions and to promote better integration between the central nervous system and micromachined neural prosthetic devices.[8]

In this paper, conducting PANi films were prepared through dipping and interfacial polymerization on the surface of a polytetrafluoroethylene (PTFE) substrate using four kinds of different dopant acids (named as synthesized PANi films), including perchloric, hydrochloric, malic, and citric acid (doped polyaniline films were named as P-PANi, H-PANi, MA-PANi, and C-PANi, respectively). As a comparison, we also casted a thin PANi film from a saturated solution of PANi in xylene (named the casting PANi film).

To investigate the application of PANi films in biomedical fields, we also studied the attachment and proliferation of PC-12 pheochromocytoma cells on film surfaces. The microstructure and properties of PANi films were characterized by atomic force microscopy (AFM) and water contact angle measurements.

Materials and Methods

Materials. Polyaniline (PANi, emeraldine base, in xylene) and aniline were purchased from Sigma-Aldrich. Different dopant acids (including perchloric, hydrochloric, malic, and citric acid), methylene chloride, and ammonium peroxydisulfate (APS) were of analytical grade and used as received without further treatment. All cell culture media and supplements were from Gibco (Langley, OK). Hoechst 33258 dye and salmon sperm DNA were purchased from Sigma-Aldrich. Acridine orange fluorescent dye was purchased from the Shanghai Chemical Reagent Corporation.

Synthesis and Processing of PANi Films. The polymerization reaction was carried out in a 120 mL glass vial. A total of 8 mmol of aniline was dissolved in the methylene chloride phase (50 mL), while ammonium peroxydisulfate (4 mmol) was dissolved in 50 mL of a 1 M dopant acid solution. Both solutions were stirred and cooled in an ice bath. While stirring, the aniline solution was added to the acid solution within 2 min. The agitation was stopped, and the PTFE substrate, which had been thoroughly rinsed before being used, was inserted into the solution for about 2 h. Four dopant acids, including perchloric, hydrochloric, malic, and citric acid, were used. In all reactions, the aniline/ammonium peroxydisulfate molar ratio was kept at 2:1.

The as-prepared polyaniline transparent films on the PTFE substrate surface were processed by being dipped in a 1 M hydrochloric acid solution for about 30 min, followed by several washes with deionized water. Finally, the film was dried under an infrared lamp. The casting thin film was prepared from a saturated solution of PANi in xylene. This solution was poured on the PTFE surface, forming a single thin layer, and dried in a vacuum oven at 37 °C under 250 mmHg.

Conductivity Analysis. Film thickness was measured using an Alpha-Step IQ surface profiler (KLA-Tencor Corporation). Surface conductivities of PANi films were measured using a four-probe technique with a low-impedance instrument (MCP-T610, Mitsubishi Chemical Corporation).

Surface Characteristics. Surface morphology and polymer particle structure were determined as roughness and particle diameter using an AFM (Nanoscope IIIa, Digital Instruments) at ambient temperature in noncontact mode with a conical silicon high-resonance frequency probe. The roughness parameter for the surface, R_a, which is the centerline average or the distance between the highest and the lowest point of the surface irregularities, was calculated using Nanoscope III software (version 5.30r3sr3). At the same time, the root mean square (rms) and maximum roughness height (R_{max}) were also calculated as typical height parameters. The vesicle size was randomly measured in each image with Image-Pro Discovery version 4.5 (Media Cybernetics Inc.).

Aqueous Contact Angle Measurements. Wettabilities of the synthesized and casting PANi films were evaluated as static water contact angles using a contact angle goniometer (JC2000A).[22] Briefly, a droplet of water (0.5 μL, ultrapure grade) was put on the surfaces of different materials, and the images were photographed, with the temperature and moisture kept 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.

Cell Culture. PC-12 pheochromocytoma cells were used to study cell adhesion, spreading, and proliferation on the PANi film surfaces. The 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 (Sigma), 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 2 days. Five kinds of synthesized and casting PANi films were used

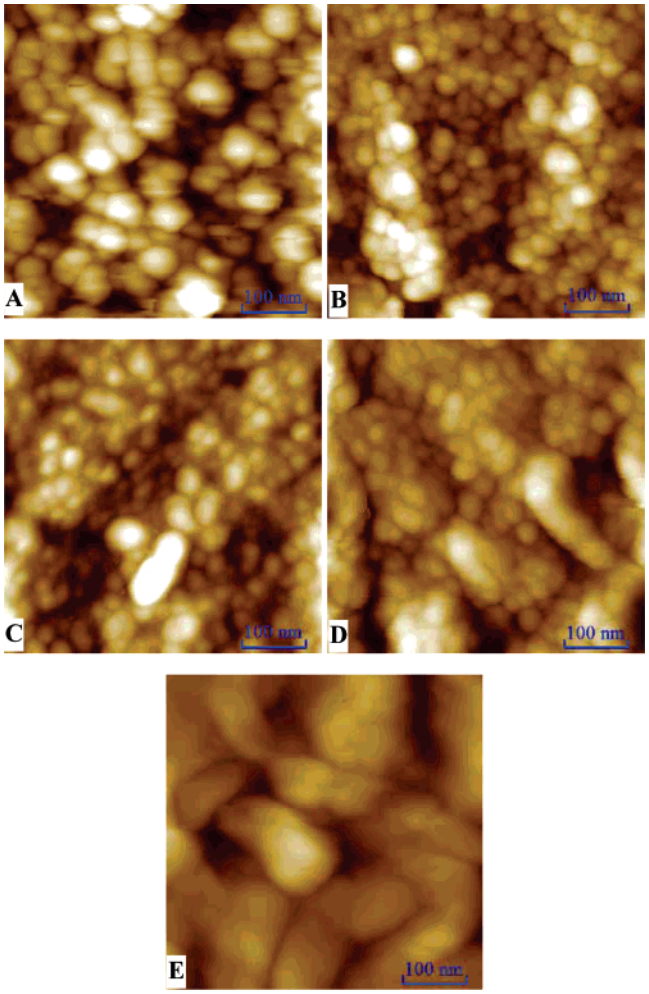

Figure 1. AFM images of PANi films prior to DMEM immersion. (A) P-PANi film, (B) H-PANi film, (C) MA-PANi film, (D) C-PANi film, and (E) casting PANi film.

for this study, and both PTFE substrate and tissue culture polystyrene (plate, flat bottom with lid, Corning Inc.) were also chosen as the controls. When the cells became almost confluent after 5 days, they were released by treatment with 0.25% trypsin for 3 min at 37 °C. Prior to cell seeding, the different substrates were placed into a 96-well culture plate and sterilized by UV irradiation for 20 min and then were equilibrated with pre-warmed (37 °C) HG-DMEM medium for 2 h. After removing the medium from the wells by pippetting, the cells were counted and diluted to 10^4 cells/cm^2, and 200 μL of the cell suspension was poured onto each substrate.

Cell Adhesion and Proliferation. Cell adhesion and proliferation were measured by the fluorometric quantification of cellular DNA according to the method reported by Rao and Otto and Takahashi et al.[23,24] Briefly, after 1 h, 1 day, 2 days, and 4 days of cell seeding, the wells were washed with PBS, and then the cells on the substrates were lysed by immersion in Tris-HCl (pH 8.0) containing Triton-X 100, EDTA at 37 °C for 12 h with occasional mixing. The cell lysate was mixed with 1 μg/mL dye solution (Hoechst 33258 dye). Then, the fluorescence intensity of the mixed solution was measured in a fluorescence spectrometer (FL-2000, Hitachi) at excitation and emission wavelengths of 355 and 460 nm, respectively. The standard curve was performed with salmon sperm DNA.

Cell Morphology Analysis. At the prescribed time points, all substrates were rinsed in PBS, and the cells that were 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).

Other films were examined by scanning electron microscopy (SEM, S-450, Hitachi) after being cultured for 2 days. Briefly, after being washed with PBS (pH 7.2), the cells that were attached on the films were fixed by immersing the materials into a 2.5% solution of glutaraldehyde in PBS and allowing them to stand at 4 °C for more than 2 h. Finally, standard dehydration in an ethanol graded series and critical point drying were performed. The samples were mounted on stubs, coated with gold in a vacuum, and examined with SEM.

Statistical Analysis. The number of independent replica was listed individually for each experiment. Where applicable, all data were mean ± SD. The analysis of data for cell adhesion and proliferation was performed by a Student's *t*-test, and the other data were analyzed by one-way factorial ANOVA and multiple comparisons (Fisher's method as Post-Hoc test, $p < 0.05$).

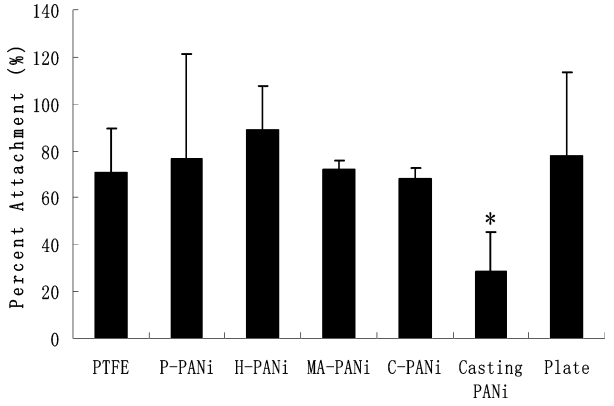

Figure 2. PC-12 pheochromocytoma cells adhered on PTFE, P-PANi, H-PANi, MA-PANi, C-PANi, casting PANi film, and the plate control. Cells (10^4 cells/cm^2) were allowed to attach for 1 h. Cell numbers were assessed by measuring Hoechst 33258 dye fluorescence. The data are expressed as means ± SD ($n = 3$). Symbols indicate the significant difference as compared to other groups ($p < 0.05$).

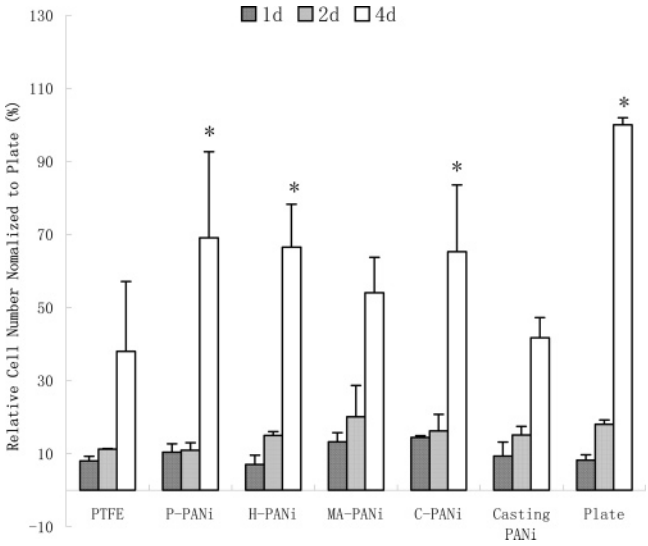

Figure 3. Proliferation of PC-12 pheochromocytoma cells on PTFE, P-PANi, H-PANi, MA-PANi, C-PANi, casting PANi film, and the plate control. Cells were seeded at 10^4 cells/cm^2 and allowed to proliferate for 4 days. The data are expressed as means ± SD ($n = 3$). Symbols indicate the significant difference as compared to the casting PANi film ($p < 0.05$).

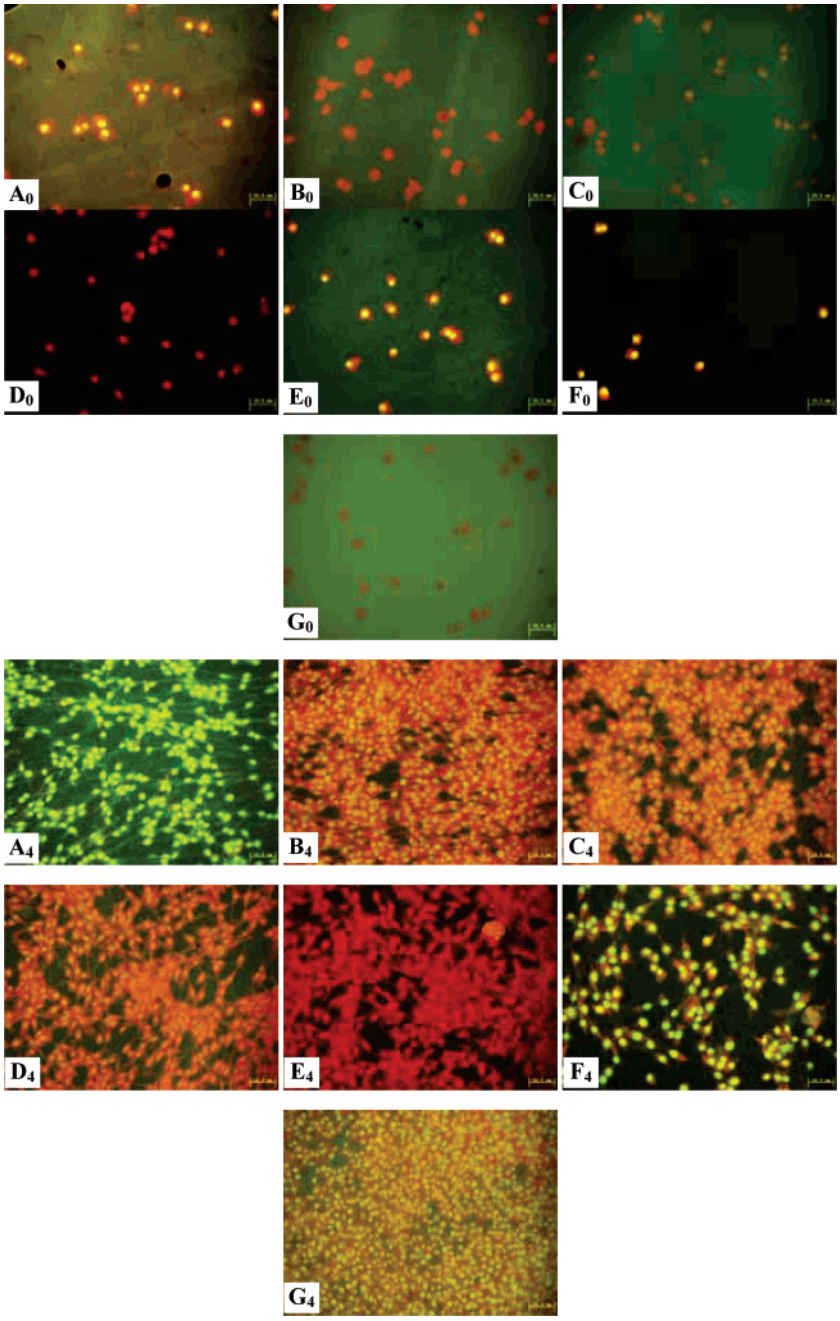

Figure 4. Biocompatibility of PC-12 pheochromocytoma cells cultured on PTFE (A_0-A_4), P-PANi (B_0-B_4), H-PANi (C_0-C_4), MA-PANi (D_0-D_4), C-PANi (E_0-E_4), casting PANi film (F_0-F_4), and the plate (G_0-G_4) for 0 days (A_0-G_0) and 4 days (A_4-G_4). All scalebars represent 39.9 μm.

Results

Conductive Properties. The electrical conductivity of synthesized PANi films was different when doped with four different acids, consistent with former experimental results.[18] When using strong mineral acids (such as perchloric and hydrochloric acid) as the dopants, the prepared polyaniline had a higher conductivity than when using medium or weak acids (such as citric and malic acid) as the dopants. The detailed results are shown in Table 1. By comparison, the casting PANi film had a higher conductivity (36.43 S/cm), which corresponds to the value reported by others.[1,21]

Morphology of PANi Films. AFM results indicated that the synthesized PANi films were composed of nanoparticles with an average size of about 30−50 nm (Figure 1 A−D and Table 1). By contrast, the casting film surface had no such similar structure as did the synthesized PANi films but contained much larger particles (Figure 1E). The differences in roughness (R_a, rms, and R_{max}) between the synthesized films and the casting film were not distinct despite there being some variability between samples. This variance was likely due to the uneven nature of the deposition.

Wettability of PANi Films. The prepared PANi films had different wettabilities, as analyzed by air−water contact angle measurements (Table 2).[22] The experimental results indicated that the synthesized PANi films had a higher hydrophobicity, whereas the casting PANi film had a more hydrophilic surface.

Cell Adhesion and Proliferation. Figure 2 shows PC-12 pheochromocytoma cell adhesion on PTFE, P-PANi, H-PANi, MA-PANi, C-PANi, casting PANi film, and the plate control after 1 h of cell seeding. On the basis of DNA measurements,[23,24] the initial cell attachment showed no difference between the different substrates except the casting PANi film, on which the

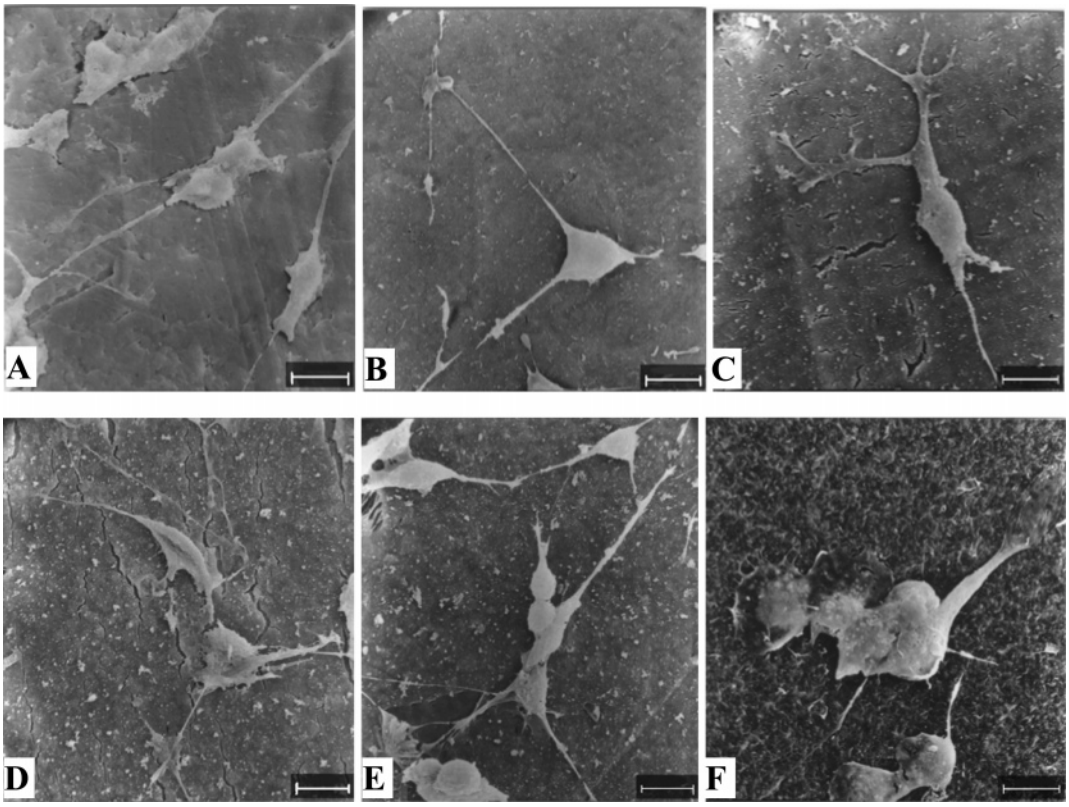

Figure 5. Scanning electron microscopy images of PC-12 pheochromocytoma cells on PTFE (A), P-PANi (B), H-PANi (C), MA-PANi (D), C-PANi (E), and casting PANi film (F). PC-12 pheochromocytoma cells were cultured in HG-DMEM for 48 h. All scalebars represent 15 μm.

PC-12 pheochromocytoma cell number was significantly lower and the percentage of attached cells was only about 30%, while cell attachment on the other surfaces was much higher, amounting to more than 70%.

Figure 3 shows PC-12 pheochromocytoma cell proliferation on PTFE, P-PANi, H-PANi, MA-PANi, C-PANi, casting PANi film, and the plate control after 1, 2, and 4 days of cell seeding. The initial cell proliferations (day 1) were significantly faster for the MA-PANi and C-PANi groups than for PTFE ($p < 0.05$): their relative cell numbers normalized to the plate were 13.2 ± 2.5 and 14.4 ± 0.5%, respectively. There were no differences among PTFE, P-PANi, H-PANi, casting PANi, and the plate groups: the cell numbers were 8.0 ± 1.3, 10.4 ± 2.3, 7.0 ± 2.6, 9.3 ± 3.8, and 8.2 ± 1.5%, respectively. After 2 days of cell seeding, there were increases in DNA content in H-PANi (15.0 ± 1.1%), casting PANi (15.1 ± 2.4%), and the plate groups (18.0 ± 1.2%) as compared to the PTFE group (11.2 ± 0.2%). On the contrary, the proliferation of PC-12 cells on MA-PANi, P-PANi, and C-PANi was slow and showed no significant differences as compared to others. On the fourth day, the DNA content in each group increased quickly, especially in the plate group. The relative cell number on the casting PANi film normalized to the plate control was 41.7 ± 5.5%, which was significantly decreased as compared to the synthesized PANi film groups, except for the MA-PANi film, and there was no difference when compared to PTFE.

Cell Morphology. Figure 4 shows the morphologies of PC-12 pheochromocytoma cells on PTFE, synthesized PANi films, casting PANi film, and the plate control, which were stained for 5 min by Acridine orange in PBS buffer. After 1 h of cell seeding (Figure 4, A_0-G_0), denser and greater PC-12 pheochromocytoma cell spreading on P-PANi, H-PANi, MA-PANi, and the plate control was observed, and the cells developed characteristic pseudopodia, while the cells on other substrates still kept a round shape at the same time. Especially, little cell adhesion was observed on the casting PANi film surface. Then, the PC-12 pheochromocytoma cells spread completely, and the characteristic pseudopodia maintained physical contact with each other. The pseudopodia of the cells on the casting PANi film surface were shorter, as verified by SEM images (Figure 5). On the fourth day, the cells on the synthesized PANi films as well as on the plate control surface were the most dense, while the least dense cells were on the casting PANi film group (Figure 4, A_4-G_4).

Discussion

It has previously been reported that traditional oxidative chemical polymerization using common mineral acids can yield granular PANi, about 50−100 nm in size.[1,21] In our experiment, much smaller and rather uniform nanoparticles were observed on the PTFE substrate as compared to those reported by MacDiarmid et al.,[21] despite the acid dopants that were used (Figure 1A−D and Table 1). Hong and Park reported that the degree of protonation of the monomers and the main reactions taking place during an early stage of the polymerization reaction are important factors determining the chemical structures as well as the conductivities and morphologies of electrochemically prepared PANi films.[25] Huang and Kaner suggested that the diameters of the resulting nanofibers are affected by the dopants used in the polymerization (acids including hydrochloric, sulfuric, nitric, camphorsulfonicacid, and 4-toluene sulfonicacids) and yield average diameters between 30 and 50 nm (those synthesized in perchloric acid are centered around 120 nm) when interfacial polymerization is carried out.[18] Moreover, Huang and Kaner indicated that polyaniline preferentially formed as nanofibers even in aqueous solution and that later stages of secondary

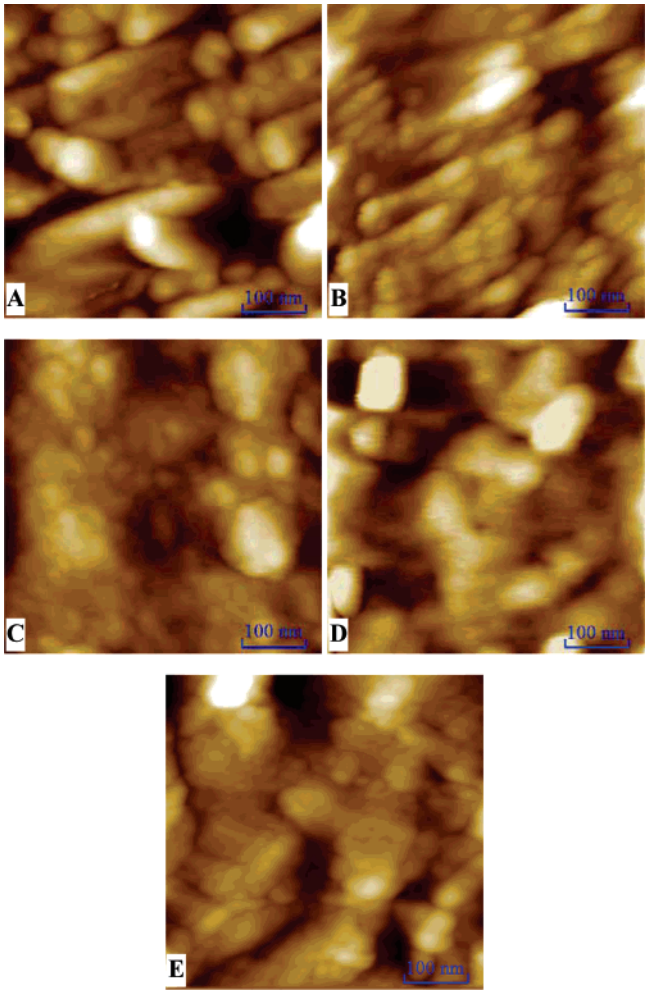

Figure 6. AFM images of PANi films following DMEM immersion for 4 days. (A) P-PANi film, (B) H-PANi film, (C) MA-PANi film, (D) C-PANi film, and (E) casting PANi film.

growth led to the large agglomerates containing irregularly shaped particles and nanofibers.[26] In our system, the organic aniline solution was added into the aqueous oxidant solution quickly while stirring to form an emulsion before the PTFE substrate was inserted into the reaction system. We suggested that the polymerization in the inhomogenous solution occurred at the interface of the organic/aqueous phase to form particles in small sizes at this stage, which eventually diffuse and stick to the PTFE substrate surface without further agglomeration. It was this modified synthetic method that similar sizes of PANi particles could be obtained even if different dopant acids were used. Although the process is similar to classical chemical polymerization, the existence of an organic phase suppresses the secondary growth of larger and irregular particles. In this case, the reaction time is important for the control of particle size. As such, the film thickness and particle size can be controlled by a combination of dipping polymerization. As compared to synthesized films, the casting film surface has no similar structure (Figure 1E) to the synthesized PANi films (Figure 1A−D).

The electroconductivities of PANi films using different doping acids were in the order of P-PANi > H-PANi > C-PANi > MA-PANi (Table 1). It has been known that the order of magnitude variation of the conductivity is observed as a function of the pH value for one acid intercalated polyaniline. The protonation degree modifies both the electronic and the proton conductivities.[26] With the passage of a constant electrical current through the conductive film, an electrical field is generated, which may induce redistribution of the charged proteins. As a result, protein adsorption behavior can be expected to depend on the electrostatic interaction between the negatively charged proteins and the electrical field generated on the conductive film during electrical stimulation.[27]

For any polymers to be useful in tissue engineering, they must be biocompatible. Some methods have been used to improve biocompatibility by modification of the surface of biomaterials, which first come into contact with the biological surroundings. These methods include plasma-treatment techniques,[28] blending with other macromolecules,[29] immobilizing small or large molecules on the surface,[30] loading some active factors or drugs,[31] etc. In the present study, as mentioned previously, we obtained two kinds of PANi films with different micromorphologies. To evaluate their biocompatibility, in vitro experiments were carried out to test PC-12 pheochromocytoma cells, such as attachment, spreading, and proliferation on these film surfaces as assessed by DNA analysis with Hoechst 33258 fluorescent dye and microscopic techniques.

First, cell adhesion was evaluated on the polymers of interest after 1 h of cell seeding. PC-12 pheochromocytoma cell adhesion was significantly ($p < 0.05$) enhanced on synthesized PANi film surfaces as compared to cells grown on the casting PANi film surface (Figure 2), while no significant differences were seen among other groups. Generally, the biocompatibility of materials was very closely related to cell behavior on contact with the films and particularly to cell adhesion on their surfaces. It is recognized that the attachment, adhesion, and spreading of different types of cells on polymeric materials largely depend on surface characteristics such as hydrophilicity/hydrophobicity or surface free energy, chemistry, charge, roughness, and rigidity.[32] Some works have indicated that cell attachment and proliferation are influenced by the surface roughness and topography.[33] Chung et al. reported that an increased surface roughness at 10−100 nm scales could enhance the adhesion and growth of human umbilical cord vein endothelial cells.[34] Our previous study also reported this possibility.[35] In the present study, as shown in Figure 1A−D, the synthesized PANi films were composed of nanoparticles 30−50 nm in size, while the casting PANi film had no such structure on the nanoscale (Figure 1E and Table 1). As compared to the casting PANi film, the synthesized PANi film surfaces had much more excellent cell behavior, such as attachment and proliferation. We hypothesized that this difference in nanoscale morphology might lead to synthesized PANi films having a much higher cell adhesion and more confluent cell numbers as compared to the casting PANi film. However, concerning the roughness, there was no significant relationship between the synthesized PANi films and the casting PANi film as indicated in Figure 1 and Table 2. Murphy et al. found that PC-12 cells plated on nano- and micropatterned surfaces extended neurites along grooves and ridges, and they demonstrated contact guidance to feature sizes as small as 70 nm.[36] The nanoscale structure could provide special nucleation sites and nucleation centers resulting in a certain degree of order of the protein adsorbed at or near it.[37,38] As a result, a high nanostructure density could have much more effective adsorption sites that may also modify protein adsorption and activity since the adsorption site geometry can alter the conformation and thereby the biological activity.[39,40] Moreover, hydrophobicity may also play an important role in biocompatibility.[41,42] Lee et al. reported that 55° was suitable for serum protein adsorption and that the amount of adsorbed nerve growth factor increased with an increase of the water

contact angle from 45 to 86°.[43] In contrast, Ueda-Yukoshi and Matsuda prepared gradients on poly(vinylene carbonate) by progressive wet chemical reactions and found optimal adhesion and growth of bovine aortic endothelial cells on the hydrophobic side of their wettability gradients.[44] Higher water contact angles of synthesized PANi films were obtained in our experiment (nearly 100°), while the value for the casting PANi film was quite small (74°).

We also studied the microstructure change of PANi films after immersion in DMEM for 4 days. The images in Figure 6 indicate that there was an increasing tendency in particle size for the synthesized PANi films ($p < 0.01$). We can explain that the change in size after immersion is due to the swelling of the PANi film.[45] According to this experimental result, it is possible that the effect of the PANi film surface microstructure on PC-12 pheochromocytoma cell behavior in later periods was relatively minor.

Conclusion

We selected two different ways to prepare PANi films. The synthesized PANi films doped with four kinds of different acids showed a similar nanostructure with an average nanoparticle diameter of about 30−50 nm, while the casting PANi film did not have this similar microstructure. This was also verified by AFM (Figure 1), which showed differences in their surface morphologies. We also studied the biocompatibility of these PANi films through the attachment and proliferation of PC-12 pheochromocytoma cells. The experimental results indicated that all of the films prepared are biocompatible and allow for cell attachment and proliferation, although some are more biocompatible than others. The synthesized films had a much higher attachment rate than the casting film did. Our findings imply that the existence of nanoscale topography on PANi films may contribute to the change in hydrophobicity of the surface, which acts cooperatively to promote the attachment and proliferation of PC-12 pheochromocytoma cells on PANi films.

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