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Effect of Immobilized Nerve Growth Factor on Conductive Polymers: Electrical Properties and Cellular Response**

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The use of biologically active dopants in conductive polymers allows the polymer to be tailored for specific applications. The incorporation of nerve growth factor (NGF) as a co-dopant in the electrochemical deposition of conductive polymers is evaluated for its ability to elicit specific biological interactions with neurons. The electrochemical properties of the NGF-modified conducting polymers are studied by impedance spectroscopy and cyclic voltammetry. Impedance measurements at the neurobiologically important frequency of 1 kHz reveal that the minimum impedance of the NGF-modified polypyrrole (PPy) film, 15 kΩ, is lower than the minimum impedance of peptide-modified PPy film (360 kΩ). Similar results are found with NGF-modified poly(3,4-ethylene dioxythiophene) (PEDOT). The microstructure of the conductive polymer films is characterized by optical microscopy and electron microscopy and indicates that the NGF-functionalized polymer surface topology is similar to that of the unmodified polymer film. Optical and fluorescence microscopy reveal that PC-12 (rat pheochromacytoma) cells adhered to the NGF-modified substrate and extended neurites on both PPy and PEDOT, indicating that the NGF in the polymer film is biologically active. Taken together these data indicate that the incorporation of NGF can modify the biological interactions of the electrode without compromising the conductive properties or the morphology of the polymeric film.

1. Introduction

Microfabricated neural probes, fabricated with silicon-processing technology, facilitate the stimulation and recording of electrical activity from the peripheral and central nervous system. These probes have long-term inadequacies that limit their ability to measure local neuron activity. Glial encapsulation of the implanted electrodes is perhaps the single most detrimental factor hindering chronic neural communication. The cellular encapsulation results from inflammation surrounding the electrode.[1–4] Fibrous tissue encapsulation, the final stage of wound healing in response to implanted foreign materials, consists of an inner layer of active macrophages surrounded by a concentric layer of fibrous tissue and an outer layer of astrocytes.[5] It is our goal to improve the neural recording abilities by minimizing the host response and by promoting better integration between the electrode and the neurons.[3,6]

In order to confer the useful electrical and optical properties of inorganic metals and semiconductors upon such probes, considerable efforts have been dedicated to a new class of polymers, known as inherently conductive polymers or electroactive conducting polymers. A key requirement for a polymer to become intrinsically conductive is the molecular orbital overlap to allow formation of a delocalized molecular wave function. Oxidative electrochemical polymerization is the preferred technique for synthesis of conducting polymers. Electrochemical polymerization of conducting polymers is generally performed by application of constant current (galvanostatic), constant potential (potentiostatic), or potential scanning or sweeping methods. Conducting polymers have attracted much interest as suitable immobilization matrices because the electrochemical incorporation of biospecies within them permits the localization of these molecules on electrodes of any size or geometry. Conducting electroactive polymers, such as polypyrrole, polyaniline, and polythiophene, have been used to modify bioelectronics[1,7–15] and biosensors by immobilizing a biological element,[16] for example enzymes,[17–19] antibodies,[20,21] or DNA.[22] This incorporation is also appropriate for the fabrication of multianalyte sen-

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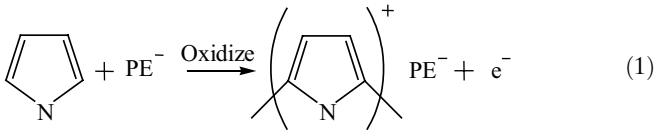
sors. A number of techniques, such as physical adsorption,[23] entrapment,[24] crosslinking,[25] and covalent bonding,[26] have been used to immobilize biological molecules in conducting polymers.

Surface modification of neural electrodes with conducting polymer films is intended to improve probe sensitivity while suppressing detrimental immune responses and promoting neural tissue regeneration by angiogenesis, neurite extension, and synapse formation.[1] Of these conductive polymers, polypyrrole (PPy) has been widely studied due to its well-known aqueous electrochemistry, high conductivity, and apparent compatibility with mammalian cells.[27,28] Poly(styrene sulfonate) (PSS) was chosen as a relatively inert dopant because of its stability once deposited and biocompatibility upon implantation.[7] The use of nerve growth factor (NGF) as a neurotrophic co-dopant allows the fabrication of microelectrodes that may induce specific cell responses in vivo. The conductive monomer is most efficiently deposited in the presence of an equal amount of dopant. The amount of NGF required to elicit phenotypical changes in the cells (> 20 ng mL^{-1}) is much smaller than the concentration of co-dopant required, so both NGF and PSS were deposited simultaneously from solution with the monomer. It has been reported that biomolecules can be entrapped in the PPy film only if the biomolecules are present in the vicinity of the growing films.[29] As a consequence, NGF, which carries a net positive charge at the pH used for synthesis (pH 7), could be electrochemically entrapped into the composite as a complex with the polyelectroyte.[30,31]

When a polyelectrolyte with a negative charge serves as the counterion, the polymerization process can be represented as follows:



(1)

where PE$^-$ stands for the polyelectrolyte (PSS in this case). Based on this mechanism, bioactive-polymer-composite surfaces have been created by incorporating species such as heparin,[32] poly(hyaluronic acid),[33] and red blood cells.[30,34] Such ion-gated, conducting-polymer membranes can be exploited for controlled drug release.[35–37]

NGF is a water-soluble protein involved in neural development. NGF promotes the survival and differentiation of sensory and sympathetic neurons. The regulation of NGF is controlled by glial and inflammatory cells in response to injury or damage to components of the central nervous system.[38–40] A biomaterial possessing both electrical and biological capabilities would be useful for neural-electrode devices, as well as for directing neuronal repair and guiding neural-tissue regeneration in nerve guides.

The use of cultured rat pheochromocytoma (PC-12) cells to detect NGF is a widely used cell-based model system for indicating the presence of NGF due to the marked phenotypical PC-12 cell differentiation and neurite extension that occur in response to critical concentrations of the neurotrophin (20–100 ng mL^{-1}).[41]

Our objectives for this study were to synthesize and characterize conductive polymer films with NGF as a co-dopant and to investigate their use as suitable materials for neural microelectrode devices. PPy/PSS/NGF and PEDOT/PBS/NGF (PBS: phosphate-buffered saline; PEDOT: poly(3,4-ethylene dioxythiophene)) electrode coatings were subjected to in vitro PC-12 cell culture and their electrical properties were characterized by impedance spectroscopy and cyclic voltammetry. For the in vitro tests, we also used collagen (rat-tail collagen type I) as a co-dopant in the polymer-electrode deposition. Collagen is a cell-adhesion molecule that promotes the attachment of PC-12 cells to substrates. Attachment and differentiation of PC-12 cells on the composite conducting-polymer films demonstrates the successful incorporation of collagen and NGF into the films by electrochemical deposition as well as the delivery of the bioactive proteins to the cells.

2. Results and Discussion

Figure 1 shows the change in impedance as a function of polymerization time. At a very early stage (0.18 μC) the impedance started to drop into the lower frequency range (≲ 1500 Hz), presumably owing to the limited ionic mobility in the thin layers of the conducting polymer. At 3.8 μC, the polymerized film showed a minimum impedance (of 15 kΩ) and then started to increase at 10.08 μC. These observations are consistent with previous studies that have shown a correlation between the morphology of the conducting polymer film and impedance.[42] This behavior has been correlated with the roughening of the film in the early stages of deposition, leading to a decrease in impedance because of the increase in effective surface area. The impedance later increases as the polymer film becomes fully dense and thickens.

The impedance behavior of PPy/PSS/NGF films at 1 kHz as a function of deposition charge for different deposition currents and deposition times is shown in Figure 2. Both changing deposition time and deposition current show similar trends in the impedance drop, indicating that the impedance changes with thickness depended primarily on the total charge of deposition (the product of time and current), although the imped-

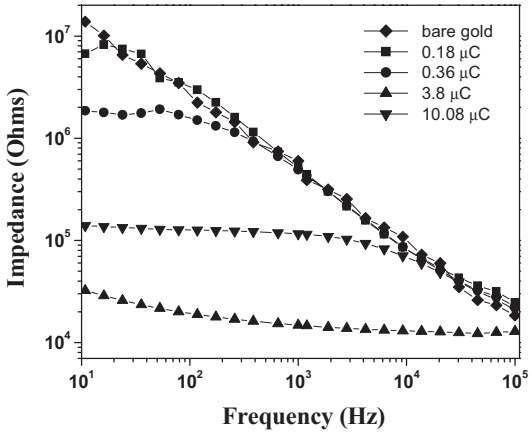


Figure 1. Impedance spectroscopy of gold sites with and without PPy/PSS/NGF deposition as a function of frequency.

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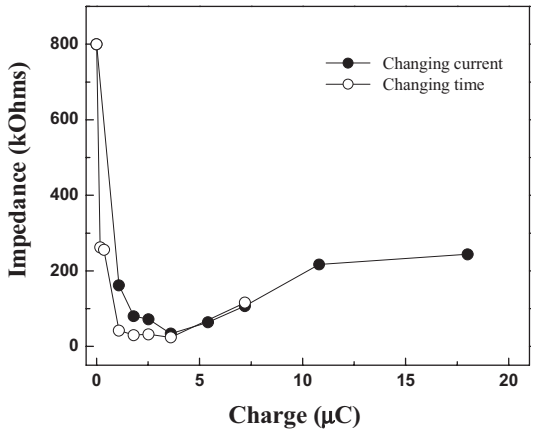

Figure 2. The impedance behavior of PPy/PSS/NGF films at 1 kHz as a function of deposition charge with changing current or deposition time.

ance of the conducting polymer created by changing the deposition time had a faster initial drop. The impedance dropped dramatically right after applying charge to the monomer solution, and the minimum impedance was reached for a range of total charge of 1.8–3.6 μC. With further charge, the impedance increased slowly. Although the effect of current density, tested at constant charge, did not show any significant influence, it was observed that at higher current densities there were a limited number of PC-12 cells with neurite extensions. This may be because the amount of NGF incorporated was smaller than with lower current densities due to diffusion of the NGF,[21] or that most of the incorporated NGF was denatured with excessive current. Figure 3 shows the impedance behaviors of the PPy with different dopants, such as PSS, silklike protein with fibronectin functionality (SLPF), and NGF. Impedance measurements at the neurobiologically important frequency of 1 kHz revealed that the minimum impedance of the PPy/PSS/NGF film, 15 kΩ, was lower than the minimum impedance of a peptide-modified PPy film (360 kΩ).

The voltammetric behavior of the PPy/PSS/NGF film was studied as a function of the applied charge used for polymerization. The I_{pa} (anodic peak current) was estimated to be 0.57 nA when applying 0.18 μC to the electrode. By increasing the applied charge, the I_{pa} increased up to 0.79 μA (Fig. 4B). The anodic peak potentials (E_{pa}) shifted to more positive values with increasing applied charge (i.e., more deposition of PPy and NGF; Fig. 4A). At 0.18 μC of applied charge, the E_{pa} was –0.33 V (versus a saturated calomel electrode, SCE), while at 10.8 μC of applied charge, the E_{pa} was –0.015 V, as shown in Figure 4C. The positive shift in E_{pa} with increasing charge indicates binding of the NGF to the PPy. The presence of biomolecules incorporated into conducting polymers can cause a shift in redox potential, and this shift can be used for indirect detection of biomolecules in the conducting-polymer films. Biomolecules in the conducting polymers can also increase the resistance of the conducting polymers. The shift of E_{pa} was consistent with the result of voltammetric behavior with PPy/anti-HSA (HSA: human serum albumin).[21] There was an observed increase in the oxidation peak current with increasing applied charge (Fig. 4B and D), demonstrating the more capacitive properties of this composite. Since the voltage was swept at a constant scanning rate (100 mV s^{-1}), the cyclic voltammetry (CV) curve could be converted linearly into $I(t)$ (t = time). The integration of $I(t)$ within the cycled region gave the charge capacity of the film, which was determined by how many mobile charges were moved in and out of the film during the cycle. The number of mobile charges is determined by either the doping level or interfacial area between the conducting polymer and the electrolyte in a solution. In this case, higher doping level in the conducting polymer with increasing polymerization time (applied charge) increased the charge capacity of the film (Fig. 4D).

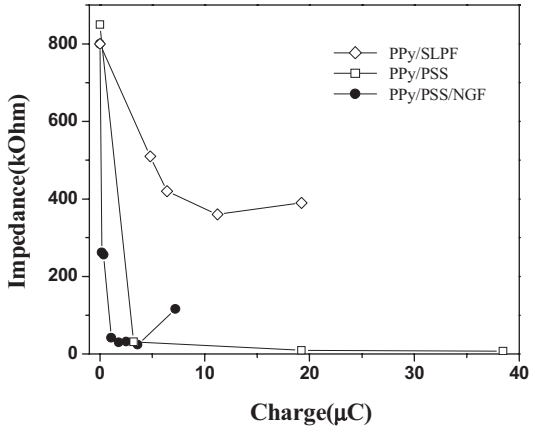

Figure 3. The impedance behavior at 1 kHz of a composite film with various dopants. PPy/PSS and PPy/SLPF data are from Cui et al. [1].

The scanning electron microscopy (SEM) images in Figure 5 show PPy/PSS/NGF composites on the gold electrode sites with different deposition charge. The surface morphology of the polymer film has a rough, cauliflower-like appearance with charge-dependent feature sizes. The PPy deposited with the lowest charge shows sub-micrometer roughness, while higher charges yielded micrometer-sized clumps of conducting polymer. Figure 5A, C, and E shows the overall electrode coating. Figure 5B, D, and F reveals the microscale surface morphology.

Fourier transform IR (FTIR) analysis was performed to help detect the presence of the NGF protein in the composites. Although the size of peak indicating the presence of NGF was not clear presumably due to the small amount of NGF incorporated into these conducting polymers, there were reproducible peaks (data not shown). The characteristic peaks caused by incorporation of NGF are observed at 1730 cm^{-1} in the spectrum, while previous observation of peptides in our laboratory[1] showed peaks at 1452, 1547, and 1650 cm^{-1}.

Characterization of the proteins in the composites is important to determine whether the protein remains in a bioactive state or is denatured by the electrical properties applied or the local pH changes that occur during reduction of the conducting polymer. To confirm the bioactivity of the incorporated NGF in the composite, three samples were studied: 1) PPy composites that were electrochemically polymerized from a monomer solution containing 12.7 μL of Py monomer, 1 mL of collagen type I, and 10 μg of NGF 2.5S (PPy/collagen/NGF); 2) PPy composites

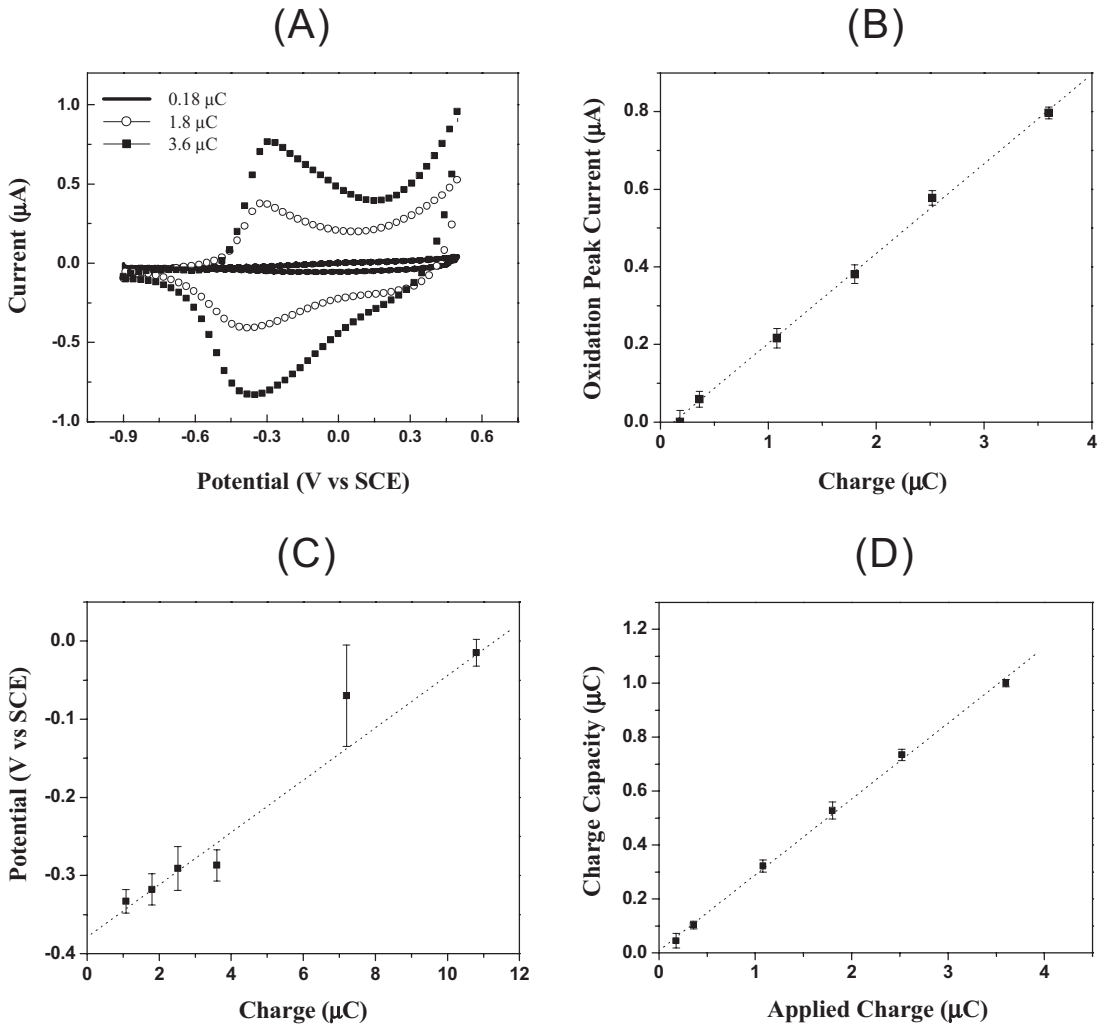

Figure 4. A) Cyclic voltammogram, B) oxidation peak current, C) oxidation peak potential, and D) charge capacity as a function of applied deposition charge.

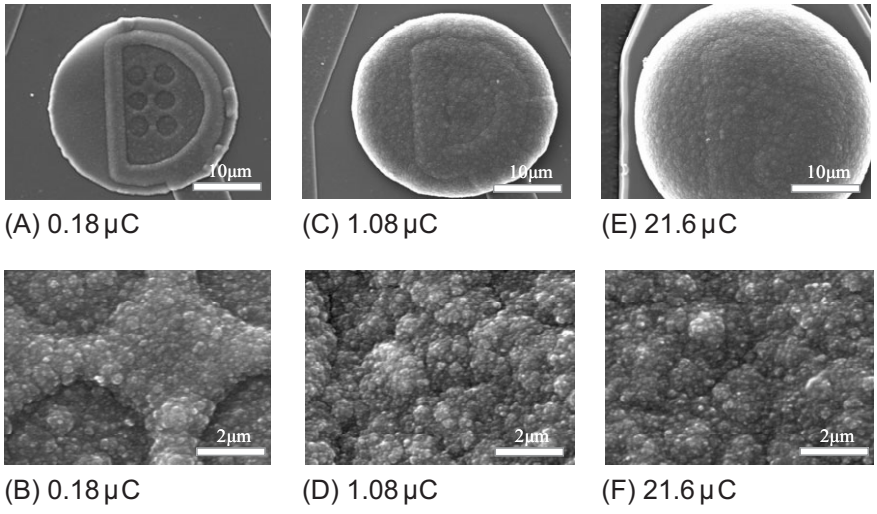

Figure 5. SEM images of PPy/NGF/PSS composites as a function of applied charge. A,C,E) Low resolution, B,D,F) high resolution.

that were electrochemically polymerized from a monomer solution containing 12.7 μL of Py monomer and 1 mL of collagen (PPy/collagen); 3) PPy composites that were electrochemically polymerized from a monomer solution containing 12.7 μL of Py monomer, 20 mg of PSS, and 10 μg of NGF 2.5S (PPy/PSS/NGF).

Seeding and culturing of PC-12 nerve cells on these three sets of composite conductive polymer substrates demonstrated the effects of the incorporated biomolecules. Attachment and differentiation of PC-12 cells, as shown by neurite extension and morphological variation of a significant fraction of the cells, was seen in the presence of NGF in the polymer films.

Optical microscopy images of PC-12 cells cultured for 48 h on the conducting

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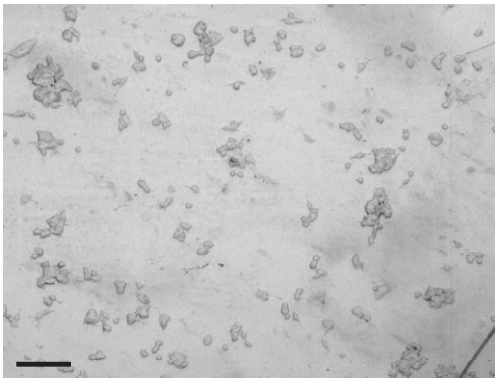
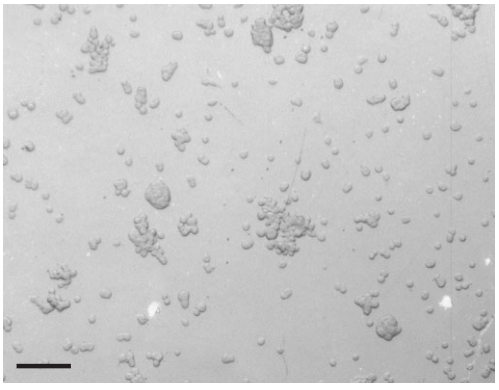
polymers with different dopants are shown in Figure 6. PPy/PSS/NGF composites showed few attached PC-12 cells, demonstrating the requirement for this cell type to have a suitable cell-adhesion protein available. PPy/collagen has significantly more cells adhered to the conductive polymer than the composite without collagen, but shows few differentiated cells (Fig. 6B). The subsequent addition of NGF to the media (100 ng mL^{-1}) produces flattened cells with extended neurites (data not shown). Composite samples containing both collagen and NGF as co-dopants in the electrochemical deposition yield a significant population of differentiated cells, indicating successful delivery of both biomolecules in sufficient quantities to make cells differentiated (Fig. 6A). PC-12 cell adhesion and neurite extension on PPy/collagen and PPy/collagen/NGF composite films are quantified in Figure 6C. About 24.3 % of PC-12 cells were differentiated responding to NGF incorporation on the substrates, while 2 % of cells were differentiated without NGF. Considering that clusters of PC-12 cells (Fig. 6A) do not express neurite extension, unlike non-clustered cells, about 62 % of non-clustered cells expressed neurite extension and responded to the NGF, consistent with result found in PEDOT/NGF composites, as described below.



(A)



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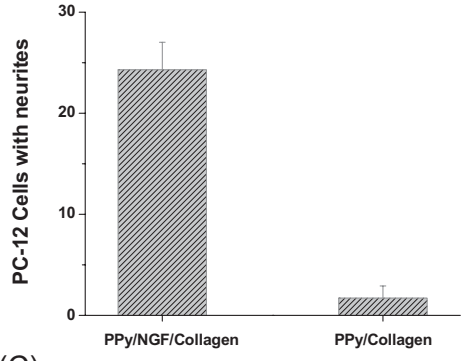

(C)

Figure 6. Optical images of PC-12 cells A) on the PPy/NGF/collagen substrates and B) on the PPy/collagen substrates. C) Bar graph showing quantification of data represented in (A,B). Percentage of cells with neurites were quantified. (Scale bars = 100 μm, * is $p < 0.01$, Student t-test, error bars are standard errors in the mean.)

By using the redox properties of the composite film, the release capability of a model protein, bovine serum albumin (BSA), from the PPy matrix also was studied. BSA bound successfully to the matrix of PPy by copolymerization, but due to its large size and folded nature, it was incapable of diffusing out through the PPy matrix (data not shown).[35,37]

Recent studies in our laboratory have focused on the conductive polymer PEDOT, owing to its excellent electrical and mechanical stability as well as its growing popularity in the field of polymer electronics.[19] Similar to results with PPy, we found that both NGF and collagen could be incorporated into PEDOT films by electrochemical deposition from EDOT monomer solutions containing the proteins. For PEDOT studies, rather than using the microelectromechanical system (MEMS) neural probe electrodes, we used 6 mm diameter Au/Pd electrodes specially developed in house for experiments involving cultured cells.

Figure 7A shows the impedance spectra for the bare Au/Pd electrode and Au/Pd electrodes coated with electrochemically polymerized PEDOT/PBS/collagen and PEDOT/PBS/NGF (both 300 μC deposition charge). Due to their comparably larger diameter, Au/Pd electrodes have lower bare-electrode impedance than the 40 μm diameter MEMS neural probe electrodes, yet the characteristic drop in impedance associated with coating by a conductive polymer is still evident. Likewise, CV studies comparing bare electrodes and electrodes coated with PEDOT/protein composite films reveal increased charge capacity and redox behaviour, consistent with results obtained with PPy/protein composites (Fig. 7B).

We next cultured PC-12 cells on electrochemically deposited PEDOT films (300 μC deposition charge each), polymerized from a variety of EDOT monomer solutions, specifically EDOT in PBS, pH 7.2, EDOT in PBS and 1 mg mL^{-1} rat-tail collagen type I, and EDOT in PBS with 10 μg mL^{-1} 2.5S NGF. In order to better visualize any changes in cellular morphology associated with NGF exposure, the actin cytoskeleton of the cells was stained with phalloidin–Oregon Green. Consistent with our findings for PPy/PSS/NGF composite films, PC-12 cells cultured on PEDOT/PBS/NGF composite films show evidence of differentiation and neurite outgrowth that is typically associated with exposure to NGF (compare Fig. 8A–C). Figure 8A is an image of PC-12 cells seeded on control

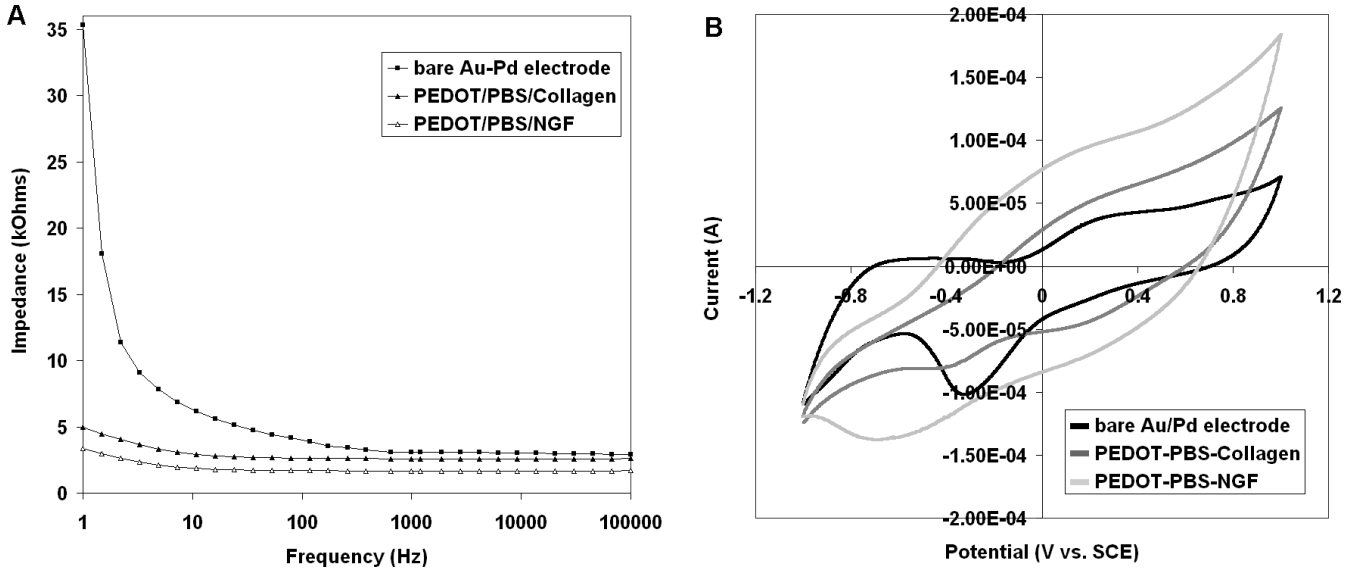

Figure 7. Impedance spectroscopy and cyclic voltammetry for PEDOT/protein composite films. A) The electrical impedance of PEDOT/PBS/collagen and PEDOT/PBS/NGF coatings (300 μC) on "in house" fabricated 6 mm diameter Au/Pd electrodes reveals the lowering of impedance typical of conductive polymer films. B) Cyclic voltammograms of PEDOT/PBS/collagen and PEDOT/PBS/NGF coatings (300 μC) on Au/Pd electrodes show increased charge capacity and altered redox behavior for electrodes with conductive polymer/protein composite coatings as compared to bare electrodes.

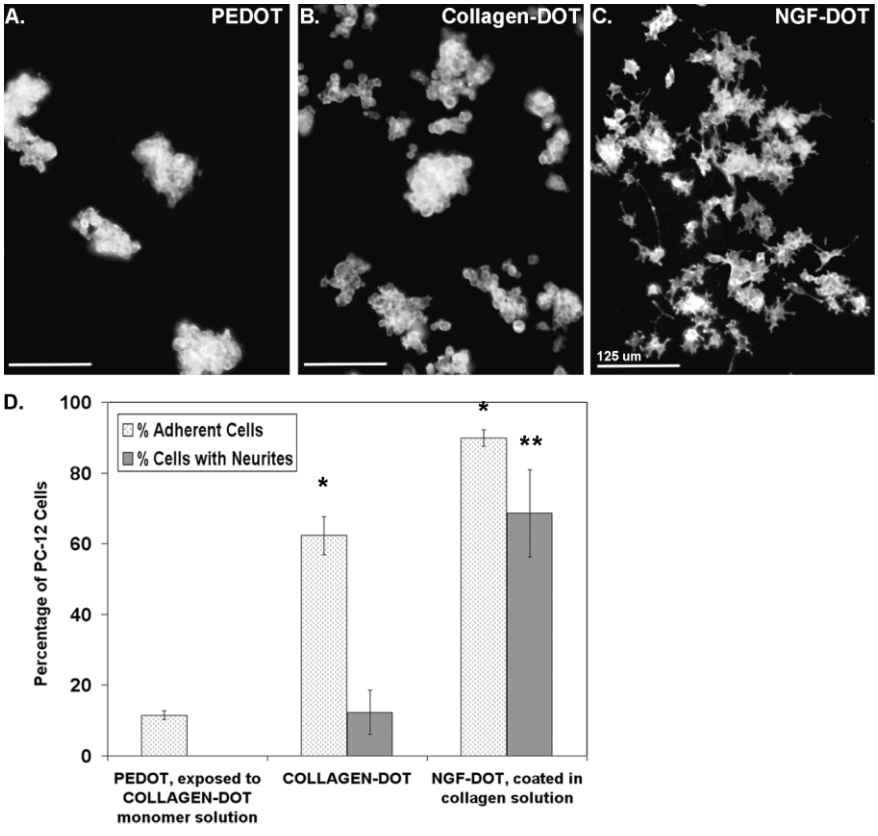

Figure 8. A) PC-12 cells seeded on control PEDOT as a control, B) PC-12 cells seeded on PEDOT/collagen, C) PC-12 cells seeded on PEDOT/NGF. D) Bar graph showing quantification of data represented in (A–C), percentage of cells adhered to each substrate and percentage of cells with neurites were quantified (* is $p < 0.01$ for % adherent cells PEDOT versus PEDOT/collagen and PEDOT/NGF; ** is $p < 0.01$ for % cells with neurites PEDOT/collagen versus PEDOT/NGF; Student's t-test, error bars are standard errors in the mean).

PEDOT (monomer: EDOT, PBS) that was exposed to collagen (1 mg mL^{-1} in PBS) solution for 5 min, as a control for exposure to collagen in the monomer solution during the polymerization procedure. Figure 8B shows PC-12 cells seeded on PEDOT/collagen (monomer: EDOT, PBS, 1 mg mL^{-1} collagen), and Figure 8C is PC-12 cells seeded on PEDOT/NGF (monomer: EDOT, PBS, 10 μg mL^{-1} 2.5S NGF) that was then coated in collagen (1 mg mL^{-1} in PBS for 30 min) to promote PC-12 cell adhesion to the polymer substrate. Cells were incubated with each polymer substrate for 48 h prior to imaging. Figure 8D is a bar graph showing quantification of data represented in Figure 8A–C. The percentage of cells adhered to each substrate and the percentage of cells with neurites were quantified using Student's t-test.

These results indicate that the NGF incorporated in the PEDOT film is not only bioactive but accessible to the cells. Because of their need for a substrate coated with collagen or a similar extracellular matrix-adhesion molecule, the PC-12 cells do not adhere well to PEDOT/PBS films (see Fig. 8A). However, PC-12 cells adhere to PEDOT/protein composite films prepared with collagen, which suggests that, like NGF, collagen is incorporated into the PEDOT film and is bioactive and

accessible to the cells. PC-12 cell adhesion and neurite extension on PEDOT/PBS, PEDOT/PBS/collagen, and PEDOT/PBS/NGF composite films is quantified in Figure 8D.

3. Conclusions

Biomolecule entrapment within electrochemically polymerized conducting polymers provides a versatile and powerful strategy to fabricate electrically active biomaterials that have enhanced cellular attachment. NGF can be incorporated via electrochemical polymerization into PPy and PEDOT films on microelectrode arrays and in-house fabricated Au/Pd electrodes, respectively. PPy/NGF coatings decreased the impedance amplitude by almost two orders of magnitude as compared with controls and revealed a minimum impedance of 15 kΩ, lower than the minimum impedance of peptide-modified PPy (360 kΩ). The impedance of PPy/NGF-deposited electrode sites dropped dramatically immediately after applying charge to the monomer solution—with further charge the impedance increased slowly. Similar results were found for PEDOT/NGF. At higher current density, the amount of NGF incorporated was smaller than at lower current densities due to either slow diffusion or denaturing of the incorporated NGF with excessive current.

Both PPy/NGF and PEDOT/NGF films yield a significant population of differentiated PC-12 cells, indicating successful delivery of NGF in sufficient quantities to initiate the differentiation. Exposure of PC-12 cells to PPy/NGF/PSS and PEDOT/NGF films for 48 h resulted in 24.3 and 65 % of cells developing extended neurites, respectively. It could be estimated that the amount of incorporated NGF is relatively small, based on the morphology of PPy/NGF films, which shows a rough, cauliflower-like appearance that is characteristic of the unmodified PPy conducting polymer controls. The morphology of PPy conducting polymer can be changed to more hairy structures when it is incorporated with proteins, which are probably related to the formation of phase-separated domains, as seen in previous data.[1] Also, the NGF may limit cellular response to the surface layers only because of restricted diffusion. Therefore, further studies to assess and compare the amount of entrapped active NGF in PEDOT and PPy composite films should be performed. In addition, future studies will be performed to determine whether the amount of NGF incorporated into the conducting polymer films can be increased using adsorption techniques (either physical adsorption or with an external bias potential) after polymerization. Bioactive, electrically conductive polymer–protein complexes remain attractive candidates for coating neural electrodes to overcome the current obstacles of chronic applications.

4. Experimental

Materials: The pyrrole monomer and PSS were purchased and used as received from Aldrich. The EDOT monomer was purchased from H. C. Stark. NGF (both 7S and 2.5S, Murine) was purchased from Invitrogen (Carlsbad, CA). Collagen type I (rat tail, BD Science) was purchased from Sigma. Gold-coated silicon wafers were obtained from the Center for Neural Communication Technology (CNCT) at the University of Michigan. Dulbecco's Modified Eagle Media (DMEM), horse serum (heat inactivated), fetal bovine serum, antibiotic-antimycotic (containing penicillin, streptomycin, amphotericin), and trypsin-EDTA (EDTA: ethylenediaminetetraacetic acid; 0.05 %, 0.5 mM respectively) were purchased from Invitrogen. Formaldehyde (16 %) was purchased from Ted Pella.

Material Preparation: Microfabricated silicon probes were provided by the CNCT. Conductive polymer/protein composite films were polymerized galvanostatically on the electrode sites (1250 μm^2 in area) of neural probes (PPy), on gold-coated silicon wafers (5 mm × 5 mm × 0.5 mm) for in vitro testing (Ppy), and on 6 mm diameter in-house fabricated Au/Pd electrodes. The electrochemical apparatus was an Autolab potentiostat/galvanostat model PGSTAT12. The reaction was performed in a three-electrode cell. A platinum foil was used as the counter electrode and an SCE was used as the reference electrode. A site on the probe was used as the working electrode. The Py monomer solution (1 mL) containing 0.2 M Py monomer, 0.1 M PSS, and 100 μg of NGF (7S) was purged with N_2 for approximately 10 min to prevent oxidation of the monomer before use. The EDOT monomer solution contained 0.01 M EDOT, 0.1 M PBS, and in some cases 1 mg mL^{-1} rat-tail collagen type I (Sigma/Aldrich) or 100 μg mL^{-1} 2.5S NGF (Promega, Madison, WI). In galvanostatic mode, current densities of 0.5–5 mA cm^{-2} were used. The thickness of deposited material was controlled by varying the deposition time and applied current.

Microfocusing FTIR: To characterize the chemical composition of the PPy/PSS/NGF composite, reflective FTIR spectroscopy was performed using a Perkin–Elmer Spectrum GX attached to a Perkin–Elmer Multiscope, and equipped with a liquid-nitrogen-cooled mercury–cadmium telluride (MCT) detector. The microscope accessory enabled the focusing of the IR beam on a small area of the sample. A probe sample was placed on a gold mirror, from which the background reflection signal was collected. The samples were scanned 100 times in the wave-range of 4000 to 750 cm^{-1}. Perkin–Elmer's Spectrum Software Version 3.01 was used to generate the FTIR spectra.

Impedance Spectroscopy Measurements: An Autolab FRA2 was used to obtain impedance spectra of the electrode sites. The apparatus was comprised of an Autolab model PGSTAT 12 potentiostat/galvanostat and an Autolab FRA2 module. A solution of 0.1 M phosphate buffer solution (pH 7) was used as the electrolyte in a three-electrode cell. The electrode setup was the same as that used in the electrochemical deposition. An AC sinusoidal signal of 5 mV amplitude was used, and the DC potential was set to 0 V. The value of the impedance was determined over a range of 10–100 000 Hz.

Cyclic Voltammetry: CV was performed using the Autolab potentiostat/galvanostat model PGSTAT12 with GPES software package. The three-electrode cell setup was the same as that used for impedance spectroscopy. A scan rate of 100 mV s^{-1} was used and the potential on the working electrode was swept between –0.9 to 0.5 V versus SCE for PPy films and –0.9 to 0.9 V versus SCE for PEDOT films. These limits were selected to include the reversible redox reaction while avoiding over-oxidation. Before each CV curve was recorded, several cycles were swept to ensure that the film had reached a stable equilibrium state with the electrolytic solution.

In Vitro Cell Tests: Rat Pheochromocytoma (PC-12) cell lines were used between passage 10 and 15 as in vitro indicators of the presence of active NGF. The neural cells respond by phenotype differentiation when subjected to critical levels of NGF [43]. Cells were maintained at 37 °C in a humid 5 % CO_2 incubator in media containing DMEM, 5 % fetal bovine serum, 10 % horse serum, 1 % L-glutamine (200 mM), 1 % antibiotic–antimicotic and passaged using a solution of 0.25 % trypsin and 1 mM EDTA. Differentiation media contains DMEM, 1 % fetal bovine serum, 2 % horse serum, 1 % L-glutamine.

After electrochemical deposition of PPy/PSS/NGF on silicon wafers (5 mm × 5 mm × 0.5 mm) or PEDOT/PBS/NGF on Au/Pd electrodes, unbound protein was removed by soaking in deionized water for 24 h. The composites were then placed in 12-well culture dishes (polystyrene Corning) and sterilized by exposure to ultraviolet light for 30 min each

side in a laminar flow hood. Differentiation media was then added to the culture dishes.

PPy/PSS and PPy/collagen and PEDOT/PBS and PEDOT/PBS/collagen composites were used as biomolecule-absent controls. These electrochemically deposited films were produced, soaked, and sterilized in the same manner as other electrodes. Cells were prepared for imaging by first rinsing with PBS to remove residual media. Cells are fixed while on the electrodes with 4 % paraformaldehyde in PBS for 30 min. In PEDOT experiments, after fixation, cells were incubated overnight at 4 °C with phalloidin–Oregon Green (1:300 dilution; Molecular Probes/Invitrogen) to stain the actin cytoskeleton to allow for easy visualization of any changes in cellular morphology associated with differentiation or neurite extension. Optical and fluorescence microscopy was performed using a Nikon Optiphot-2 POL with a Spot (Diagnostic instruments Inc.) red–green–blue (RGB) digital camera and an Olympus BX-51 with mercury arc lamp and an Olympus charge-coupled device (CCD) camera.

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