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Electrically Controlled Drug Delivery from Biotin-Doped Conductive Polypyrrole**

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An externally controlled, polymeric drug-delivery system potentially allows for release profiles that can be tailored to match physiologic processes.[1] Current implantable electronic delivery systems are not biodegradable and often require additional components, while extended- or controllable-release polymeric systems that have been used do not allow for switchable release profiles.[2,3] Conducting polymers (e.g., polypyrrole (PPy)) offer the possibility of controllable drug administration through electrical stimulation.[4] However, the use of conductive polymers in delivery systems has been restricted due to limitations in the choice of dopant and the molecular weight of the delivered drug. To circumvent these barriers, we have developed a method for attaching molecules to the surface of PPy through biotin–streptavidin coupling. After attachment of the desired molecule to the biotin dopant, drug release is triggered through electrical stimulation. This method provides a novel platform for controlled drug delivery from a conductive polymer substrate.

Because of PPy's beneficial chemical properties and ease of preparation, it is often chosen for biological applications.[5–7] PPy's favorable biocompatibility also makes it an ideal electroactive polymer for drug-delivery applications.[8–13] Additionally, the fact that PPy can be made in degradable forms enhances its value for these biomedical applications. In previous approaches to deliver drugs from PPy, the molecule was either incorporated into the PPy film or transported through a voltage-controlled polymer membrane.[4,14,15] These factors limit the range of drugs that can be administered from PPy. Other methods have used PPy as an active element to trigger release from other polymers, such as degradable hydrogels.[16] Our approach relies on direct release from PPy and can be utilized with a broad range of payloads, virtually independent of molecular weight and charge. We have shown that biotin can be used as a co-dopant in the PPy film, which allows great flexibility in designing a release system, as this system has much more uniform release kinetics, especially when considered across a broad range of charge or size. Biotin is negatively charged in aqueous solutions,[17] making it possible to use biotin as a counteranion for PPy formation. Other groups have chemically modified the PPy monomer to attach a biotin molecule, making it a part of the PPy backbone.[18–20] This approach is ideal for sensor applications,[21,22] which benefit from this strong covalent attachment, but we have shown that a weaker electrostatic attachment provides a novel approach for drug delivery. By incorporating biotin as a dopant, electrical stimulation results in reduction of the PPy backbone, which is believed to trigger the release of the biotin and the attached payload.

Stability of the dopant in PPy is essential for controlled drug delivery as well as other applications. To illustrate the stability, fluorescently tagged streptavidin was used to verify the incorporation of biotin into synthesized PPy films over a two week time course. After deposition, the biotin-doped PPy was stored in phosphate-buffered saline (PBS) for 14 days. At each end point, fluorescently tagged streptavidin was incubated with thorough rinsing (Fig. 1). As shown by the areas of the fluorescent intensity of each sample, the amount of biotin in the PPy remained constant without a statistically significant change over time ($p < .05$). These results indicate that biotin is not released from the PPy film without activation, which would provide minimal passive drug release.

For drug delivery, biotin-doped PPy film was electrodeposited in the same manner as above, and then incubated with streptavidin to form a surface capable of attaching any biotin-labeled compound (Fig. 2a). Because streptavidin has four biotin binding sites, it can adhere to the biotin dopant found at the polymer's surface and still have open sites for the addition of a biotinylated drug. In this study, nerve growth factor (NGF) was used to illustrate controlled drug delivery for neurological applications. NGF is a member of the neurotrophin family that influences neural growth, differentiation, survival,

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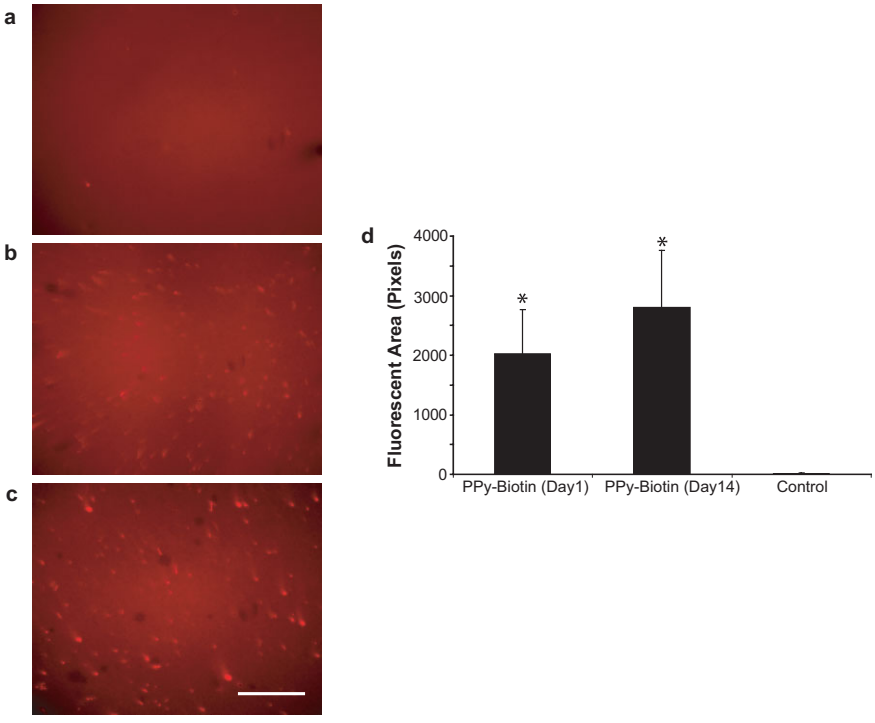

Figure 1. Stability study of biotin-doped PPy surface using fluorescently tagged streptavidin to indicate biotin incorporation in PPy film. a) PPy without biotin but with tag introduced. b) One-day time point with tagged streptavidin attached to biotin in the PPy. c) Two-week time point showing biotin remaining in the PPy surface. d) Area of fluorescence above background intensity at 1 day and 14 days after plating compared to the control sample without biotin. There was no statistical difference ($p < .05$) between the samples containing biotin at either time point. The asterisks indicate significant differences versus the control sample without biotin ($p < .05$). Scale bar: 500 μm.

and death in the central and peripheral nervous systems.[23] NGF has many possible applications because of its ability to stimulate nerve growth as well as its involvement in Alzheimer's and other neurodegenerative diseases;[24–26] NGF can also be biotinylated,[27] which is necessary for this method.

The PPy film was washed repeatedly (3× with PBS) to ensure removal of all NGF that simply adsorbed to the surface instead of binding to the streptavidin. Samples were placed in PBS, and release of the NGF complex was triggered by applying a potential between a reference electrode and the PPy for either 30 or 150 s (Fig. 2a). The supernatant was removed and fresh solution was added. After 5 min, the supernatant was replaced and the samples were stimulated again. The supernatant was again replaced and the films were incubated for an additional 5 min. An enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) was used to quantify the release of NGF at each time point. Control samples were also tested in two different conditions: one group included NGF but was not stimulated, and the other was exposed to NGF but the polymer did not contain the original biotin dopant. For the control group with NGF but without stimulation, there was minimal NGF release, which we attributed to ion exchange. Additionally, in the control materials produced without biotin as a dopant, there was essentially no NGF release. As seen in Figure 2b, the first stimulation of 3 V for 30 s resulted in greater NGF release than with unstimulated controls. When the stimulation was extended to 150 s, there was a statistically significant ($p < .05$) increase in the amount of NGF release over all of the controls. This stimulation released nearly all of the NGF on the PPy surface, and the second stimulation resulted in a small release that was not statistically significant. The stimulation parameters, such as shape of stimulus, frequency, and duration, could be explored to tailor release profiles. Based on the 150 s pulse, 22 ng cm^{-2} of NGF was released from the PPy surface. This projected area does not account for the substantial roughness of the PPy surface. It is expected that small conductivity changes in the polymer occur after the release of the biotin complex; the low levels of dodecylbenzenesulfonic acid (DBSA) incorporated as dopant also stabilizes the conductivity of the polymer. With pulse stimulus patterns, voltages higher than 3 V resulted in hydrolysis and ineffective delivery of NGF, while lower voltages resulted in statistically insignificant amounts of released NGF compared to controls (less than 3 ng cm^{-2}).

Another important aspect of a drug-delivery system is to maintain the chemical integrity of the drug before and during release. The activity of the released NGF was assayed after release as a way to determine if any of the process or release steps inactivated or degraded the NGF. PC-12 cells are known to express functional TrkA receptors that bind to NGF and cause the extension of neurites,[28,29] and thus can be used to assay the functional activity of NGF. After release, biotinylated NGF (5 ng mL^{-1}) was introduced to PC-12 cells, and the degree of neurite extension was compared to both unmodified NGF (5 ng mL^{-1}) and biotinylated NGF not subjected to the surface attachment and release steps (5 ng mL^{-1}). As seen in Figure 3, the stimulated, released NGF remained active and caused neurite outgrowth with no statistical difference when compared to unstimulated, biotinylated-NGF (positive control) or the unmodified NGF (positive control). No outgrowth was seen from any of the PC-12 cells exposed to media alone (negative control), and cells exposed to all three types of NGF had statistically significant outgrowth when compared to the negative control ($p < .01$). These results indicate that the NGF incorporated in PPy and released from the system remains intact and functional.

Electrically triggered drug release from PPy provides a new platform for controlled drug delivery. Although we used the delivery of NGF as a model system, a wide range of com-

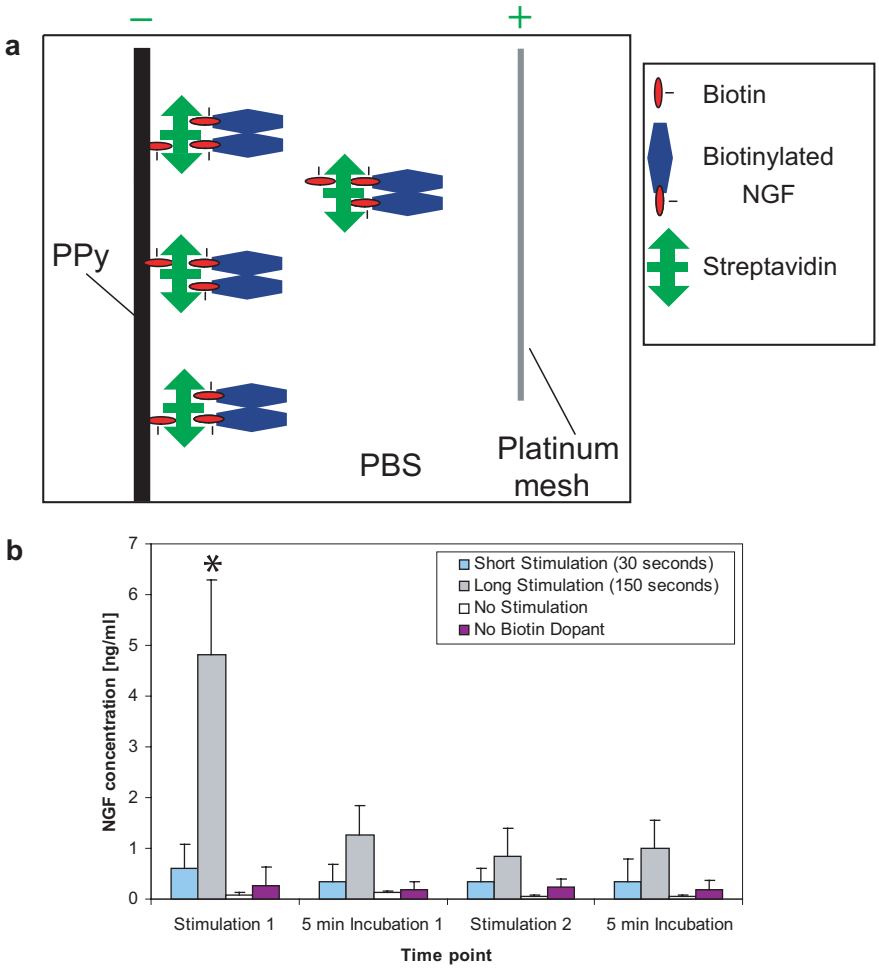

Figure 2. Stimulated nerve growth factor (NGF) release. a) Voltage applied across the polymer in PBS solution causes the release of the biotin from the PPy surface. b) A 3 V stimulation of the PPy showed an increase in the amount of NGF released from the surface of the conductive polymer. Short stimulations of 30 s did not result in as much release as long stimulations of 150 s. The asterisk indicates a statistical difference versus short stimulation, no stimulation, and no biotin dopant ($p < .05$).

pounds, biomolecules, and drugs could be released with this system. Multiple compounds could be released by selectively attaching compounds to an array of PPy electrodes; the same approach could also be used to further tailor release profiles. Additionally, the conductive properties of PPy open the door for remotely controlling an entirely polymeric drug-delivery device—the charge to drive release could be harnessed from an applied external field. This strategy is all the more compelling when used with degradable conductive polymers.[30]

The incorporation of biotin as a dopant also provides a new method to selectively tune the surface properties of a PPy film through the attachment of hydrophilic and hydrophobic moieties. Because PPy is frequently used to improve tissue interactions, the additional ability to control both release and local surface chemistry provides a means for flexible control of the tissue/PPy interface.

Experimental

Electrodeposition: PPy was deposited by electro-oxidation onto micropatterned 5 mm × 8 mm gold templates (300 nm of gold with 20 nm of titanium for adhesion) on a <100> silicon substrate [10]. The dies were cleaned by washing with acetone, isopropyl alcohol, and water before use. Each of the compositions of PPy were electrodeposited onto the gold surface using a constant-current power supply (HP 6614C). A current density of 2 mA cm^{-2} was applied between the gold template and a platinum wire-mesh reference electrode maintained at 4 °C and perfused with N_2 throughout the process. For the biotin-doped PPy, the solution consisted of 0.1 M pyrrole (Aldrich), 0.02 M sodium dodecylbenzenesulfonate (NaDBS) (Aldrich), and 8.2 mM biotin (Molecular Probes) in an aqueous solution. The biotin-doped PPy solution was saturated with biotin to incorporate higher levels of biotin into the polymer. NaDBS in the deposition solution was maintained at levels tenfold lower than commonly used [31] to favor the incorporation of biotin. For the control samples without biotin, the solution consisted of only 0.1 M pyrrole and 0.02 M NaDBS.

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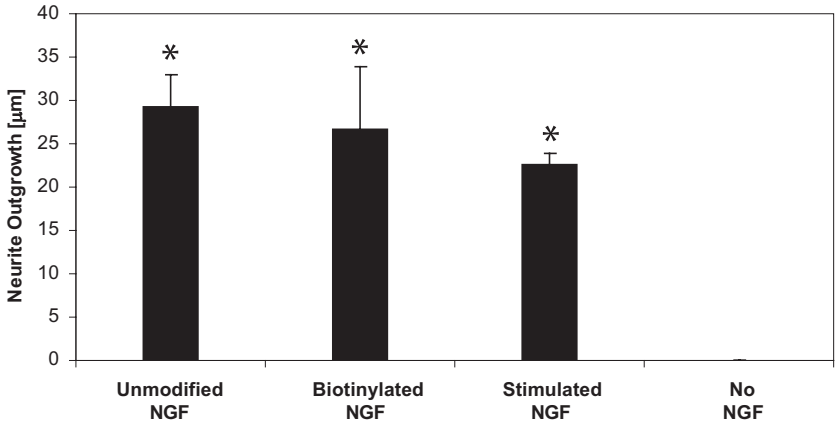

Figure 3. Average neurite outgrowth of PC-12 cells after the addition of NGF. Unmodified NGF, biotinylated NGF, and stimulated biotinylated NGF all resulted in similar neurite outgrowth in PC-12 cells. Cells that were just exposed to media showed no outgrowth. The asterisks indicate statistically significant differences ($p < .01$) versus cells with no NGF.

Stability Studies: The biotin-doped PPy samples were stored in PBS at 4 °C until the specified time point. Streptavidin tagged with rhodamine (Molecular Probes) at a concentration of 0.1 mg mL^{-1} was added to the sample at 1 or 14 days and incubated for 15 min. Negative control samples were made from PPy without biotin and were incubated with streptavidin tagged with rhodamine, just as the other samples were. All samples (three for each time point) were imaged at 20× (numerical aperture $NA = 0.5$) magnification using a Zeiss Axiovert microscope and Hamamatsu camera. ImageJ software was used to quantify the average pixel intensity and for image processing. The average fluorescent intensity of the control samples was subtracted from each image, and the area of fluorescence above the background level was calculated for each sample.

Drug-Release Studies: 0.2 mg mL^{-1} of unmodified streptavidin (Molecular Probes) in PBS was incubated for 15 min at 24 °C with gentle agitation on each PPy sample in a twelve-well plate. After washing the sample once with PBS, 16 μg mL^{-1} of biotinylated NGF was added and incubated for 15 min at 24 °C with gentle agitation. The samples were then washed three times with PBS and incubated overnight in PBS at 4 °C. The supernatant was removed and fresh PBS added before release studies were initiated and between time points.

The NGF (R&D Systems) was biotinylated as previously reported [23]. Briefly, 4 mg of EZ-link biotin–poly(ethylene oxide)–amine (Pierce) and 0.1 mg of carrier-free NGF (R&D Systems) were added to 142 μL of 2-(*N*-morpholino)ethanesulfonic acid (MES)-NaOH buffer (Pierce) at pH 5. Then, 8 μL of 5 M *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) (Pierce) was added to the solution. After 2 h with vigorous stirring, the reaction was quenched with water, and the biotinylated NGF was dialyzed with 500 g mol^{-1} molecular-weight cutoff (MWCO) tubing (Spectrum) to purify the NGF. The mole ratio of biotinylation was determined to be 15.1:1 biotin/NGF through a competitive displacement of 2-(4′-hydroxybenzeneazo)benzoic acid (HABA) dye from avidin (Pierce). The biotinylated NGF was then lyophilized and redissolved in PBS to a concentration of 16 μg mL^{-1}.

Stimulation was applied with a constant-voltage power supply (HP 6614C). For the stimulated samples, 3 V was applied between the PPy sample and a platinum wire mesh at 24 °C. Each cycle lasted for 5 min. The stimulation occurred for the prescribed time (30 or 150 s), after which the samples remained in the solution for the remainder of the cycle. Aliquots were also sampled for a 5 min period after the stimulation cycles were completed. Two stimulation periods were applied for each of the samples. At the end of each period, the supernatant was removed from the sample and stored at –4 °C for ELISA quantification. NGF presence was quantified with the Human β-NGF DuoSet ELISA Development kit (R&D Systems).

PC-12 Cell Studies: PC-12 cells (American Type Tissue Collection) were grown in growth medium (85 % RPMI-1640 medium with 2 mM glutamine, 12.5 % horse serum, and 2.5 % fetal bovine serum (Gibco)) with 5 ng mL^{-1} of unmodified NGF, biotinylated NGF, stimulated NGF, or no NGF. Stimulated NGF was dialyzed (500 MWCO, Spectrum) and lyophilized before addition. Cells were cultured on Vitrogen (Cohesion, Inc.) coated plates at low density (6250 cells cm^{-2}) to allow for neurite measurement. After 4 days of incubation at 37 °C, cells were fixed in 4 % paraformaldehyde (Electron Microscopy Sciences) in PBS for 20 min. The cells were then washed three times for 5 min each in PBS. Neurites of length greater than a cell body were measured using Axiovision software, and the lengths of the measurements on each cell were averaged for comparison.

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