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Electrostatic Assembly of Conjugated Polymer Thin Layers on Electrospun Nanofibrous Membranes for Biosensors

Xianyan Wang,[†] Young-Gi Kim,[†] Christopher Drew,[†] Bon-Cheol Ku,[†] Jayant Kumar,[†] and Lynne A. Samuelson*[,‡]

Center for Advanced Materials, Departments of Chemistry and Physics, University of Massachusetts Lowell, Lowell, Massachusetts 01854, and RDECOM Natick Soldier Center, Natick Massachusetts 01760

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ABSTRACT

We report a new fabrication approach to highly sensitive optical sensors by combining the techniques of electrospinning and electrostatic layer-by-layer adsorption. A fluorescent probe, hydrolyzed poly[2-(3-thienyl) ethanol butoxy carbonyl-methyl urethane] (H-PURET), was electrostatically assembled onto the surface of cellulose acetate (CA) electrospun nanofibrous membranes. The fluorescence of these membranes can be quenched by extremely low concentrations (ppb) of methyl viologen (MV^{2+}) and cytochrome *c* (cyt *c*) in aqueous solutions. This high sensitivity is attributed to the high surface-area-to-volume ratio of the electrospun membranes and efficient interaction between the fluorescent conjugated polymer and the analytes.

New approaches to developing highly sensitive detection techniques for chemical and biochemical agents continues to be a major challenge because of the increasing demands for faster, simpler, and less expensive detection methods. In recent years, although a number of ultrasensitive fluorescent optical sensors for a variety analytes have been demonstrated,[1−3] new strategies are still being developed.

We have previously reported the use of electrospun polymer membranes containing a fluorophore as highly responsive fluorescent optical sensors.[4,5] These sensors had K_{sv} values in excess of 10^5, which were 2 to 3 orders of magnitude higher than those from thin films made from the same sensing material. We attribute this increase in sensitivity to the inherent high surface-area-to-volume ratio found in electrospun membranes. Electrospinning is a relatively simple and versatile method for creating high-surface-area polymeric fibrous membranes. In a typical process, a large static voltage is applied to a polymer solution to generate fine jets of solution that dry into an interconnected membranelike web of small fibers.[6] The fiber diameters are generally in the range of 10−1000 nm. Electrospun nanofibrous membranes can have surface areas approximately 1 to 2 orders of magnitude higher than those of continuous thin films. It is expected that applications such as sensors and catalysts, where large surface areas are desired, can benefit by taking advantage of the large surface area inherent in electrospun membranes to improve performance.

For quenching-based fluorescent optical sensors, the sensitivity of the device can be dramatically affected by the accessibility of the sensing elements to the quencher or analyte. Although electrospun fluorescent membranes have shown significantly improved sensitivities over continuous thin films,[4] there remains room for improvement. Typically, the mean diameter of the electrospun fibers in our experiments was between 100 and 400 nm, thus limiting the diffusion of the quencher to fluorophores located in the interior of the fiber.[1] To address this limitation, a novel approach was employed to localize and immobilize the fluorescent sensing elements onto the surface of the electrospun fibers. The sensors made by this method thus had both the high surface-area-to-volume ratio of the electrospun membranes and optimal exposure of the fluorescent sensing element to the quenchers. This, in turn, led to higher device sensitivities.

The immobilization of inorganic materials and conductive polymers on the surface of electrospun fibers has been previously demonstrated.[7,8] Our group has used electrospun fiber membranes as substrates for the solution deposition of continuous thin coatings of titanium dioxide (TiO_2) and tin dioxide (SnO_2).[8] This technique effectively coated the individual electrospun fibers, thus leaving the inherent high surface area of the electrospun membrane intact. Here, we report a simple and versatile method for immobilizing the

* Corresponding author. E-mail: lynne_samuelson@uml.edu. Phone: (978)-934-3792. Fax: (978)-458-9571.
[†] University of Massachusetts Lowell.
[‡] RDECOM Natick Soldier Center.

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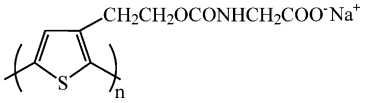
Chart 1. Structure of H-PURET



sensing materials on the surface of electrospun fibers using the electrostatic layer-by-layer (ELBL) assembly technique. ELBL is a powerful approach to creating highly tailored polymer thin-film structures.[9] This process involves the alternate adsorption of anionic and cationic polyelectrolytes on a charged substrate by sequential dipping of the substrate into the appropriate aqueous polyelectrolyte solutions. This yields a thin polymer film on the substrate. The ELBL assembly technique has been successfully applied to sensor fabrication.[10,11] One of the attractive advantages of this technique is the versatility in that a vast range of functional groups can be incorporated within the structure of the film. Furthermore, this technique is not limited to planar substrates and has been demonstrated in the ELBL assembly onto 2- and 3-D architectures.[12] We expected that the combination of electrospinning and the ELBL assembly with different electrospun substrates and sensing layers or functionalities will afford new properties and tremendous flexibility for fabricating sensors.

Conjugated polymers (CPs) have been shown to be promising materials for fluorescent optical sensors for such target analytes as explosives (TNT, DNT),[1] biological materials (peptides, proteins, and nucleic acids),[2] and other electron acceptors (methyl viologen MV^{2+}).[3] A key advantage of conjugated polymer-based sensors over sensors using small fluorescent dye molecules is the potential of the conjugated polymer to exhibit collective properties that are sensitive to very minor perturbations.[13] In particular, the efficient energy migration in CPs results in amplified sensitivity. The disadvantages of CPs are that their synthetic routes are often difficult and laborious and the processibilitiy is often poor. To the best of our knowledge, there has been no report on the electrospinning of pure conjugated polymers. Therefore, the immobilization of CPs on the surface of electrospun fibers is an effective approach for taking advantage of both the high surface-area-to-volume ratio of electrospun membranes and the benefits of localizing small quantities of CP florophores on the fiber surface. All of these factors are expected to provide facile fabrication and improved sensitivity of the sensors.

In this study, a fluorescent conjugated polymer, hydrolyzed poly[2-(3-thienyl)ethanol butoxy carbonyl-methyl urethane] (PURET), was synthesized according to published procedures.[14] The sodium salt of hydrolyzed PURET (H-PURET) was prepared as described previously.[15]The fabrication of the nanofibrous membranes as the substrates for the electrostatic self-assembly of the sensing polymer proceeded as follows. The spin-dope solution consisted of 9%, by weight, cellulose acetate (CA) dissolved in 2:1 acetone/dimethylacetamide (DMAc). A live electrode wire from the dc power source (Gamma High Voltage Research, Inc., model HV ES30P/100) was inserted into the pipet that contained the spin dope. Electrospun fibers were collected on a tin oxide-coated conducting glass slide. The applied electrospinning voltages in this work ranged from 20 to 25 kV. The distance between the pipet tip and the glass slide ranged from 15 to 20 cm. The collection time was about 90 s.

The ELBL self-assembly technique was used for the immobilization of the fluorescent conjugated polymer for sensing. The electrospun cellulose acetate (CA) membrane is insoluble in water but carries a negative charge from the partial hydrolysis of surface ester groups. Commercially available poly(allylamine hydrochloride) (PAH) of average molecular weight (70K) was used as a polycation, and the fluorescent conjugated polymer H-PURET was used as a polyanion. The deposition process was carried out in two steps for each dipping cycle. In the first step, the electrospun CA membrane was immersed in a 10 mM aqueous PAH solution for 5 min at room temperature and subsequently washed with DI water for 3 min. After the deposition and washing step, the substrate was dried with nitrogen gas. In the second step, the substrate with a single layer of PAH was immersed into a 1 mM solution of the polyanion, H-PURET, for 5 min, followed by the same washing and drying procedures. This dipping sequence was repeated five times to build up the fluorescent sensing layers sequentially.

To monitor the deposition process, UV−visible absorption spectra were recorded using a GBC UV/vis 916 spectrophotometer. X-ray photoelectron spectroscopy (XPS) was carried out with VG ESCALAB (VG Scientific Lit.) using Mg Kα radiation ($h\nu = 1253.6$ eV) as the excitation source. The morphology of the membranes was determined using an Amray 1400 scanning electron microscope (accelerating voltage 10 kV). The sensing capabilities of the membranes were determined by measuring the fluorescence quenching in the presence of the analyte with a Perkin-Elmer LS 55 fluorescence spectrofluorometer. The electrospun-membrane-coated glass slides were fixed in a 1-cm quartz cuvette that was filled with the analyte solution. The excitation wavelength that was used was 475 nm, and the emission spectra were measured from 500 to 800 nm.

H-PURET is a water-soluble, fluorescent, conjugated polymer with a negatively charged side chain. The weight-average molecular weight determined by gel permeation chromatography was between 68 500 and 345 000 g/mol after a polymerization time of 1 to 12 h. The maximum UV−vis absorbance and fluorescence emission of the H-PURET solution occur at 410 and 530 nm, respectively. Previous studies demonstrated that up to 200 bilayer-thick multilayer films have been fabricated using the layer-by-layer complexation−fabrication technique, with H-PURET and europium as the polyanion and cation, respectively.[15] Figure 1 shows the UV−vis spectra of assembled layers with sequential dipping cycles. The maximum UV absorbance occurs at 430 nm. An increase in absorbance as a function of the number of dipping cycles was observed. X-ray photoelectron spectroscopy (XPS) was performed to confirm the presence of H-PURET in the membrane. The binding energy of the S_{2p} peak around 165.5 eV shown in Figure 2 is indicative of H-PURET on the surface of the electrospun fiber. The scanning electron microscope (SEM) image of H-PURET

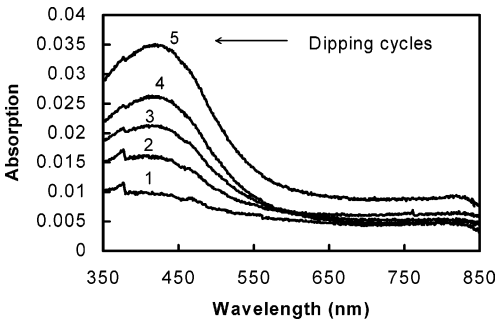


Figure 1. UV−vis absorption spectra of a multilayer film as a function of the number of dipping cycles.

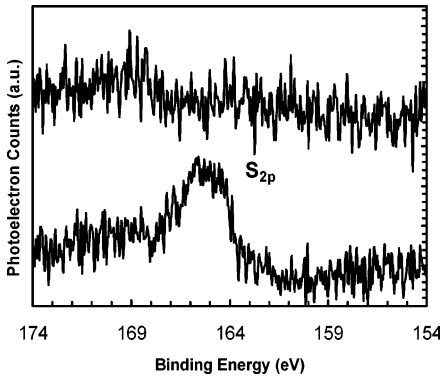


Figure 2. XPS spectra of an electrospun membrane. (Upper) Before H-PURET adsorption. (Lower) After H-PURET adsorption.

assembled on an electrospun film of cellulose acetate is shown in Figure 3. It was observed that the electrospun fibers were randomly oriented as a porous membrane. There was a distribution of fiber diameters ranging from 100 to 400 nm. Some spherical bead structures were also formed during electrospinning and were incorporated into the membrane. This type of porous structure of the electrospun membrane provides a surface-area-to-volume ratio that is roughly 1 to 2 orders of magnitude higher than that known for continuous thin films. Further increases of the surface-area-to-volume ratio may be achieved by changing the conditions of the electrospinning process such as voltage, solvent, concentration, and working distance, which results in either a smaller diameter of fibers, fewer beads, or increased porosity at the fiber surface. The control of film morphology is important to the optimization of sensing capabilities.

The electron-transfer protein cytochrome *c* (cyt *c*) and electron acceptor methyl viologen (MV^{2+}) were selected as the quenchers for the sensitivity studies. Chen et al. recently proposed a novel fluorescent biosensor based on conjugated polymer poly[lithium 5-methoxy-2-(4-sulfobutoxy)-1,4-phenylenevinylene] (MBL-PPV).[2] They demonstrated that the fluorescence of MBL-PPV is readily quenched by cyt *c* and MV^{2+}. Cyt *c* is a heme-containing respiratory protein. The detection of cyt *c* has become standard practice for the confirmation of myocardial infarction (MI) and to monitor patient response to treatment. Methyl viologen can also be tethered to a ligand that is sequestered by binding to a specific, biorelevant target. Their results present opportunities for greatly improved PPV-based biosensors.

In our work, the quenching behaviors of the sensors to cyt *c* and MV^{2+} were studied by the measurement of the fluorescence spectra of the sensing films as a function of different quencher concentrations. Figure 4 shows the fluorescence spectra of the membrane varying with the concentration of cyt *c*. The results showed that the fluorescence intensity decreased with increasing concentration of cyt *c*. Similar behaviors were observed for MV^{2+}. It was found that the fluorescence could be quenched with extremely low analyte concentration. It has been proposed by McQuade et al.[1] that the fluorescence emission of conjugated polymers can be made to respond to very minute analyte quantities because of their efficient energy migration. Thus, a single quencher molecule can potentially quench hundreds of repeat units of the conjugated chain.[13] In this work, the localization of the fluorescent polymer on the surface of an already high-surface-area nanofibrous membrane resulted in highly efficient fluorescence quenching.

The fluorescence quenching sensitivity can be quantified through measurements of the Stern−Volmer constant, K_{sv} described in eq 1.[16,17]

$$\frac{I_0}{I} = 1 + K_{sv}[\text{quencher}] \qquad (1)$$

where I_0 and I are the intensities of fluorescence in the absence and in the presence of the quencher, respectively. When all other variables are held constant, the higher the K_{sv}, the lower the concentration of quencher required to quench the fluorescence and thus the greater the detection sensitivity. As a consequence, the development of high-sensitivity fluorescent optical sensors focuses on the improvement of K_{sv}.

Figure 5 shows the Stern−Volmer plots of the sensors. A linear relationship between quencher concentration and I_0/I is obtained, showing homogeneous quencher-accessible sites

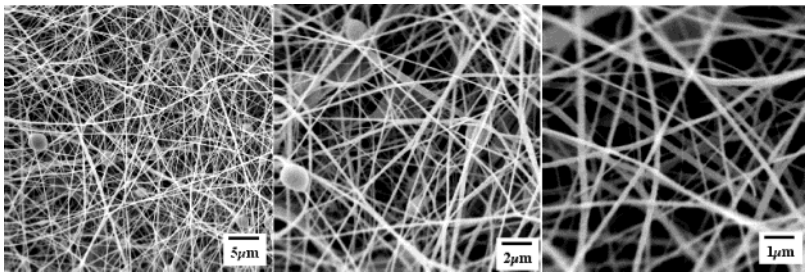


Figure 3. SEM images of an electrospun memebrane (left, 2000×; middle, 5000×; right, 10 000×).

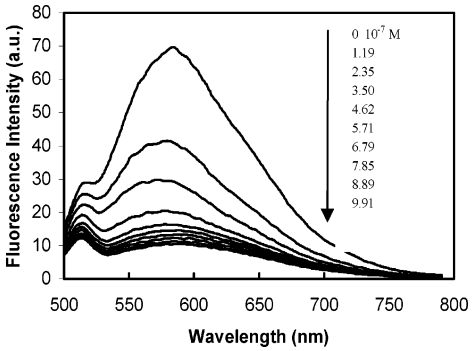


Figure 4. Fluorescence emission spectra of the sensing film with varying cytochrome *c* concentration.

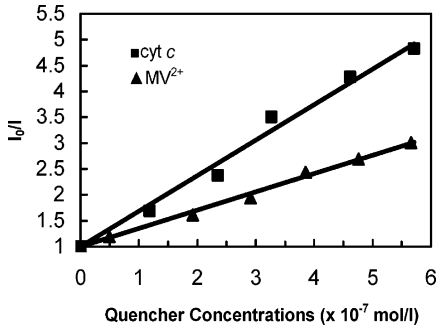


Figure 5. Stern−Volmer plots of the sensing films as a function of quencher concentration.

in the sensing films under the experimental conditions. Stern−Volmer constants of the electrospun films, calculated from slopes of the plots, were found to be 6.9×10^6 and 3.5×10^6 M^{-1} for cyt *c* and MV^{2+}, respectively. These values are still 1 to 2 orders of magnitude lower than the reported values. There are advantages to using solid sensing films, and our results suggest a promising approach to the fabrication of sensors and biosensors. Further improvements in sensitivity are expected by optimizing both the morphology of the electrospun membranes and the deposition process for the conjugated polymer layers.

In summary, we have successfully developed polymer thin-film optical sensors for cytochrome *c* and methyl viologen detection by a unique combination of electrospinning and electrostatic layer-by-layer self-assembly techniques. The remarkably high sensitivities of the solid-state devices are attributed to the high surface-area-to-volume ratio of the films and efficient interaction between the fluorescent polymer and the quenchers. Further efforts will focus on the optimization of the performance of the sensors.

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