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# Biosensing using photolithographically micropatterned electrodes of PEDOT:PSS on ITO substrates

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ABSTRACT

Improving the performance of electrochemical sensors based on indium tin oxide (ITO) can be achieved via intrinsically conducting polymers such as poly (3, 4 ethylenedioxythiophene): polystyrene sulfonate (PEDOT:PSS). However micropatterning of PEDOT:PSS to form microelectrodes on such substrates has been challenging. Here we demonstrate a technique for the precise micropatterning of a conductive ink on ITO using photolithography to realize a covalently-attached ion-transport matrix for stable biosensing. This micropatterning is accomplished using an all water-based and ambient temperature processing. We present electrochemical characterization of the material composite via electrochemical impedance spectroscopy, cyclic voltammetry and differential pulse voltammetry. A sensitive sensor is fabricated for the neurotransmitter dopamine, with a linear range from 1 to 50 μM. The sensor signal is very stable under cyclic increase and decrease in the concentration of dopamine. This sensing can be achieved even in the presence of ascorbic acid at 1000 times higher concentration than dopamine. Further, enzymes can be immobilized in the conductive matrix to form highly selective sensors. Such robust micropatterning strategies can greatly expand the use of conducting polymer substrates in biosensing applications.


## 1. Introduction

Intrinsically conducting polymers (CPs) such as poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) and its derivatives have attracted lot of interest in organic electronics and electrochemical sensing due to their unique electron mobility characteristics [1,2]. PEDOT:PSS is commercially available, has high thermal stability, favorable optical and mechanical properties and has been used for organic thin film transistors, light emitting diodes and photovoltaic devices. High conductivities and transmission over 90% in the visible spectrum have been reported [3]. PEDOT:PSS is a candidate to fabricate MEMS structures, neuronal electrodes and implantable sensors, due to high biocompatibility and stability in biological environment [4]. For sensing and actuation, it is often necessary to have micro/nanoscale nodes and circuits. Most reports using PEDOT:PSS or CP-based biosensors typically demonstrate sensing via macroscale or microscale deposition on an electrode [5,6]. This strategy has two issues – first, the physical deposition does not involve a bond with the electrode, which means that the polymer layer is not durable; second, control of deposition is not very precise [7]. These shortcomings may be addressed using microfabrication techniques – inkjet printing, stamping, laser patterning, screen printing, electrohydrodynamic printing and soft lithography [8–10]. However, limited resolution, throughput, or lack of pattern uniformity over large areas are challenges with such methods [10].

Strategies for robust microfabrication are therefore crucial to utilize this polymer for such applications. Photolithography continues to be a primary choice to fabricate microstructures of high complexity in a fast and reproducible fashion [11]. However, the acidity of PEDOT:PSS adversely affects the crosslinking and decomposition characteristics of conventional acid-sensitive photoresists resulting in damage of patterns. As a result, complex and multi-step lithographic processes have been developed, [10,12] including hydrofluoroethers for direct development of photoresists, [13] orthogonal solvents, [14] or direct crosslinking of PEDOT:PSS using water soluble bis(fluorinated phenyl azide) as a photoinitiator [15]. In general, these processes have not resulted in high resolution or intricate architectures, or are not suitable for biomolecule immobilization [16]. The development of a relatively benign, ambient temperature and easy to handle fabrication process for sensors using PEDOT:PSS is therefore highly desirable.

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PEDOT:PSS is a widely used anode buffer layer for Indium Tin Oxide (ITO) [17]. It provides ohmic contact via a low series resistance for the ITO to transport positive charges efficiently, and block negative charges. In addition it acts as a planarization layer for the rough surface to increase device yield. In an earlier work from our group, we reported on a conductive ink of PEDOT:PSS formed as a composite with the silk protein sericin to form an all-water based photolithography process [18]. The PEDOT:PSS acts as an ion/electron transducer, while the biochemically modified sericin protein provides a soft, photocrosslinkable, water permeable matrix. Here, we discuss the photolithographic micropatterning of PEDOT:PSS to form electrodes via a biofriendly and green process, using only water as a solvent. For the first time, we demonstrate precise, site-specific micropatterning of PEDOT:PSS on electrically conductive substrates for biosensor applications. While hybrid systems such as hydrogel-conductive polymers patterned with inkjet printing and stencil printing were reported, the electrochemical characterization and biosensing experiments were performed by coating the composite material on carbon or platinum electrodes [19]. The micropatterned system itself was not directly used for the electrochemical study. In contrast, here we show that microelectrodes of conductive polymers can be precisely and specifically patterned using a protein carrier to enable photolithography.

Here, we show how microfabricated PEDOT:PSS electrodes on ITO can be employed as highly selective and sensitive electrochemical biosensors for the detection of the neurotransmitter dopamine (DA). DA is an important neurotransmitter in the mammalian central nervous system, and low levels of DA may result in disorders including Parkinson's disease, making it an important target for detection [20]. However, the electrochemical detection of DA in real biological systems is complicated by the coexistence of interfering compounds. In particular, ascorbic acid (AA) is oxidized at the nearly same potential at a bare electrode, resulting in an overlap of voltammetric response [21]. AA itself is an important antioxidant in metabolic processes that has been used to treat colds and even some forms of cancer [22]. This system can be used for electrochemistry and detection of DA and AA, separately, and in the presence of each other in a highly sensitive manner. The work therefore shows the utilization of ITO/glass microelectrodes, and the effect of a soft, biocompatible, conductive, micropatterned coating as a biosensor.

## 2. Experimental section

### 2.1. Fabrication of micropatterned ITO coated glass electrodes

ITO/glass slides (Delta Technologies, Loveland, CO) were first washed with DI water and 100% pure ethanol. The washed slides were further cleaned with RCA cleaning solution (20:4:1of $H_2O$:$H_2O_2$:$NH_4OH$). Sensors were microfabricated as follows: SU-8 2002 (Microchem Corp, Westborough, MA) was spin coated on the glass substrate following standard protocols. Microelectrode patterns were formed by photolithography by using Karl-Suss MA-56 mask aligner with a UV source (5.7 mW/cm$^2$ irradiance) for 20 s. The patterns were developed using SU-8 developer.

### 2.2. Formation of photopatternable conductive ink

The conductive ink is composed of three components – silk sericin protein, the conductive polymer PEDOT:PSS and DMSO. The "sericin protein photoresist" (SPP) was synthesized as described earlier [23]. Briefly, sericin was dissolved in 1 M LiCl/DMSO to form 1% w/v solution. Photoreactive methacrylate moieties were conjugated by reacting with a stoichiometric amount of 2-isocyanatoethyl methacrylate in a $N_2$ purged vessel at 60 °C for 5 h. The conjugate was precipitated in excess cold ethanol, washed 3 times in cold ethanol/acetone (1:1) solution and centrifuged. The product was freeze dried for 48 h to obtain the SPP.

Dry re-dispersible pellets of PEDOT:PSS (Orgacon$^{TM}$, Sigma-Aldrich, St. Louis, MO) were dispersed in water, ultrasonicated for 20 min and filtered with 0.25 μm syringe filter to obtain 0.8% w/v solution. 5% (v/v) DMSO was added to PEDOT:PSS to enhance the conductivity and improve the plasticity of the conductive ink. SPP was added to PEDOT:PSS solution to form an ink with ~11% (w/w) PEDOT:PSS. Darocur 1173 (BASF) 0.1 μl/1 mg of SPP was added as a photoinitiator to the conductive ink before use.

### 2.3. Fabrication of conductive ink patterns on ITO microelectrodes

ITO surfaces were functionalized with 3-(trichlorosilyl) propyl methacrylate (TPM) by chemical vapor deposition to obtain a self-assembled monolayer (SAM) having acrylate moieties as pendant groups. These acrylate groups enable covalent bonding of the conductive ink by UV assisted crosslinking. The conductive ink was drop cast on the patterned ITO/glass substrate and allowed to air dry. Micropatterning was performed by aligning the ITO microelectrodes with a dark field mask (Karl-Suss) and UV exposure for 50 s. The patterns were developed in water to obtain microelectrodes with defined conductive ink patterns.

### 2.4. Electrochemical characterization and sensing

Cyclic voltammetry (CV), Differential pulse voltammetry (DPV) and Chronoamperometric (I-t) techniques were used. The electrolyte used was PBS buffer (0.1 M, 7.4 pH). A standard three cell setup used Ag/AgCl and Pt electrodes as reference and counter electrodes respectively, while the as-prepared samples formed the working electrode. 1 M KCl solution was used as a salt bridge. CV experiments were done on a CHI 401 instrument (CH Instruments, Inc., Austin, TX) at a scan rate of 0.1 V/s over a −1.0 to 1.6 V potential window. DPV and I-t measurements were conducted on a CHI 660 electrochemical workstation. The DPV parameters: step size 2 mV, pulse size 50 mV, sample period 0.02 s, pulse time 0.2 s. DPV response for dopamine and ascorbic acid were recorded. I-t response for dopamine were measured at 0.28 V constant potential. Limit of detection (LOD), limit of quantitation (LOQ) and sensitivity were calculated as per ICH guidelines:$LOD = \frac{3.3\times S_{y/x}}{m}$ μM,$LOQ = \frac{10\times S_{y/x}}{m}$ μM, Sensitivity = $\frac{m}{A}$ μ$A$/μMcm$^2$, where, $S_{y/x}$ = Standard error at y-intercept, $m$ = slope of the calibration curve (A/μM). Both values are obtained from regression analysis of calibration curves. $A$ = area of sensor exposed to electrolyte (cm$^2$) [24].

## 3. Results and discussion

Indium Tin Oxide (ITO) electrodes are widely used in electrochemistry due to stable electrochemical and physical properties, optical transparency, high electrical conductivity, and a wide potential window [25]. Biosensor platforms such as optical, electronic, electrochemical and electro-chemiluminescence biosensors have been shown using ITO [26–28]. While ITO/glass is relatively cheaper than other conventional electrodes such as gold, silver and platinum, recent price increases have encouraged the use of other materials to enhance their properties. PEDOT:PSS is often introduced on ITO substrates to provide ohmic contact and transport charges efficiently. It presents a low series resistance and acts as a planarization layer to avert shorts and increase device yield. Indeed, drop-casting of PEDOT:PSS is a simple method that has been used to form electroactive coatings on various rigid and flexible substrates including ITO, glassy carbon, gold, polypropylene, PDMS and glass [29–33]. Even though this is useful for films in dry media as gas