Micropatterned Films of Conducting Polymers with Entrapped Biomolecules for Neuronal Cell Adhesion

S. Park[1], D.R. Nocera[1], M. R. Abidian[1,2,3], S. Majd[1,4]
Department of Bioengineering[1], Chemical Engineering[2], Materials Science and Engineering[3], Engineering Science and Mechanics[4], The Pennsylvania State University, University Park, PA

Statement of Purpose: Conducting polymers (CPs) are easy to process and have tunable physical and chamical properties including conductivity, volume, color, and hydrophobicity. Therefore, CPs have been employed in a broad spectrum of biomedical applications ranging from implantable electronics, and biosensing to tissue engineering and drug delivery. Among CPs, polypyrrole (PPy) is particularly appealing for biomedical applications due to its biocompatibility and excellent chemical stability. PPy can be electropolymerized into thin films and serve as substrates for *in vitro* cell cultures[1]. Patterned films of conductive polymers, particularly with various surface chemistries, provide an excellent platform to study cellular behavior. Herein we introduce a novel and versatile technique of hydrogel-mediated [2] electrodeposition to directly pattern CP films with spatially-addressable chamistries. This unique technique enables the incorporation of bioactive molecules in the CP network in a very simple one-step process. We aim to apply these micropatterned CP films to study neuronal cell attachment and growth towards potential application in neural tissue engineering.

Methods: We prepared patterned films of PPy on an electrode surface in a single-step and solution-free process by employing an agarose hydrogel stamp as the carrier of the polymer precursor solution (pyrrole (Py) and dopants) for electropolymerization. Upon current application to the three-electrode set-up shown below (**Figure 1**), polymerization of Py occurred only in the contact areas between the stamp and electrode surface. The physical and chemical properties of the patterned CP films were characterized through optical microscopy, scanning electron microscopy, and impedance spectroscopy. We further investigated the effect of various parameters including monomer/dopant concentration, hydrogel agarose content, current density, and electrodeposition time on the resultant films.

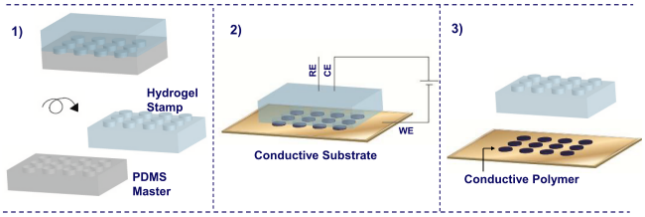

Figure 1. Cartoon illustration of hydrogel-mediated electropolymerization of patterned PPy films.

Furthermore, we examined the ability of this technique to create spatially-addressable patterned films of PPy by selective inking posts on a hydrogel stamp, applied for electrodeposition. Lastly, we tested the capability of the hydrogel stamp to deliver fragile biomolecule to the polymer network, which is essential for cell adhesion and growth. To this end, we included D-biotin in the inking solution of polymer precursor that was loaded onto the hydrogel prior to electropolymerization.

Results: Using this technique, we were able to pattern various geometries (e.g. circles and linear patterns) and sizes (40 µm-1 mm) of PPy films with different dopants. The impedance spectroscopy on these films showed that gold substrates with patterned CP films had lower impedances compared to the bare gold substrate indicating an improved electrical conductivity, as expected. Further, individual inking of selective posts of a Py containing hydrogel with a dopant solution resulted in selective deposition of CP films in the corresponding areas, enabling the generation of any desired pattern. This result demonstrates the capability of this method to fabricate a platform with various surface chemistries by selectively inking posts of a hydrogel stamp with different dopant solutions. Finally, we entrapped bioactive protein, D-biotin, in the PPy network and recognized by its binding partner, fluorescent-labeled streptavidin. As shown in Figure 2, greater fluorescence intensity was observed with the CP film with incorporated D-biotin compared to the control CP film where only monomer and dopant were applied to the hydrogel prior to electrodeposition. We also used attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and confirmed the presence of the carbonyl bond in D-biotin at 1700 cm^{-1}.

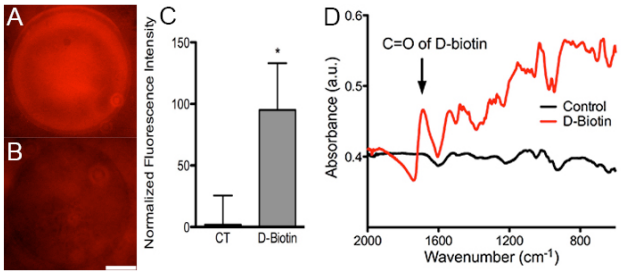

Figure 2. Incorporation of D-biotin in patterned PPy film was confirmed by fluorescence immunohistochemistry (A: Control PPy film; B: PPy film containing D-biotin; scale bar = 200 µm), C) Normalized fluorescence initensity, and D) ATR-FTIR signal.

Conclusions: We present a simple one-step method to micropattern films of conductive polymers by using hydrogel stamp as a carrier of conducting polymer precursor solution during electropolymerization. This multifaceted technique enables the fabrication of a patterned CP film with addressable various surface chemistries in a parallel process. Furthermore, applying hydrogel stamps made it possible to encapsulate bioactive molecules. We are currently applying this method for high-throughput studies of neuronal cell growth and differentiation.

References: 1. Onoda M. et al. Thin Solid Films. 2009; 518: 743-749. 2. Majd S. et al. Angew Chem Int Edit. 2005; 44: 6697-6700.