Conducting Polymer Films with Biomolecular Gradients for Cell Adhesion Control

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

Statement of Purpose: Biocompatible conducting polymers (CPs) such as polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) are popular biomaterials in implantable electronics, biosensing, tissue engineering, and drug delivery. These polymers are easy to process and have tunable physical and chemical properties including conductivity, volume, color, and hydrophobicity. Thus, patterned films of CPs, particularly with various surface chemistries, provide an excellent platform to study cellular behavior. We recently developed a novel and versatile technique of hydrogel-mediated electrodeposition to directly pattern CP films with spatially-addressable chemistries[1]. This unique technique enables the incorporation of biomolecules within the CP network in a very simple one-step process. Herein, we extend this technique to create CP substrates with gradients of biomolecules to study cell attachment and growth towards potential application in neural tissue engineering.

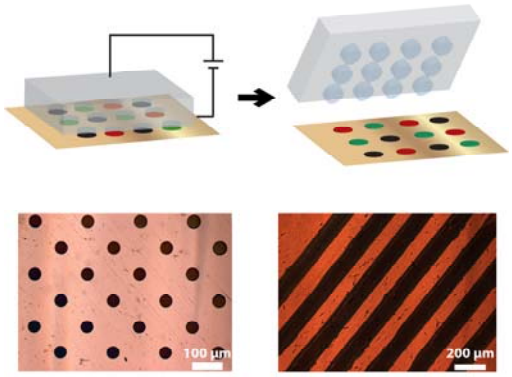

Figure 1. Schematic illustration of hydrogel-mediated electropolymerization of patterned PPy films and optical images of patterned substrates[1].

Methods: We prepared patterned films of PPy on a gold substrate in a single-step and solution-free process by employing a topographically-patterned agarose hydrogel as the carrier of the polymer precursors (pyrrole (Py) and dopants). Upon current application, polymerization of Py occurred only in the contact areas between the hydrogel and gold (**Figure 1**). By selectively inking individual posts on a hydrogel stamp with several different combinations of monomer (Py) and dopants (polystyrene sulfonate (PSS), dodecyl benzene sulfonate (DBS), perchlorate), we fabricated a spatially addressable substrate with various chemistries. The physical encapsulation of fragile biomolecules, such as biotin or laminin, within the polymer network was achieved by adding these biomolecules to the polymer precursor solution during the hydrogel-inking step. These patterned films of CP with various dopants and entrapped biomolecules were analyzed by optical microscopy, immunofluorescence assays, impedance spectroscopy, electron dispersion spectroscopy (EDS), and Fourier-transform infrared (FTIR) spectroscopy. For the preliminary cell viability tests on the patterned PPy substrates, human glioblastoma (U87) cells were cultured for 48 hours, and the live-dead assay was performed and analyzed by optical and fluorescence microscopy.

Results: Using the hydrogel-mediated electrodeposition technique, we patterned various geometries (e.g. circles and linear patterns) and sizes (40 µm-1 mm) of PPy films with variety of dopants. The impedance measurements 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. For the substrate with patterned PPy with multiple distinct dopants, we analyzed individual spots using EDS and FTIR to confirm the presence of desired dopant within these spots. Using EDS we were able to detect chlorine ion from the spots containing perchlorate, and using FTIR we were able to differentiate PSS and DBS spots from each other by the long C-H chain peak (near 2900 cm^{-1}) from DBS. The presence of biomolecules, biotin and laminin, in the PPy network was confirmed by immunoflourescence assays and FTIR. In FTIR measurements, biotin and laminin (in separate samples) was detected from the corresponding C=O bond peak near 1700 cm^{-1}. In the case of PPy films with gradients, FTIR analysis from several regions demonstrated differential presence of biomolecules in these films. U87 cell culture performed on this substrate showed biased attachment and growth of the cells on the laminin-concentrated portions (**Figure 2**), indicating a potential application of this technique to provide an effective substrate to control cellular behavior.

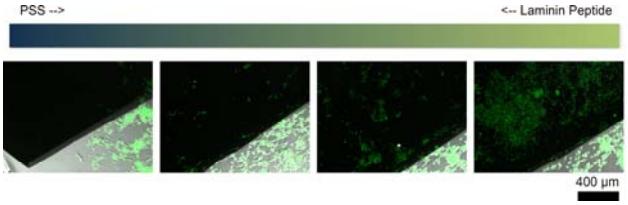

Figure 2. The images of U87 cells (phase + green (live cells)) on PPy films with gradients of the laminin peptide.

Conclusions: We present a simple and versatile method to micropattern films of CPs. This multifaceted technique enables the fabrication of patterned CP films with high fidelity and with spatially addressable surface chemistries. Moreover, this unique approach enables easy encapsulation of biomolecules and generation of films with biomolecular gradients. Future studies will apply these patterned substrates for high-throughput studies of cellular adherence and growth.

References: 1. Park S, Guang Y, Madduri M, Abidian MR, Majd S. Adv Mater. 2014. 26:18, 2782-2787.