FEATURE

BY YUJIE ZHU AND JOSE M. MORAN-MIRABAL

Micro and nanostructured materials

for the study and monitoring of biomolecular interactions

The ability to control, monitor and study biomolecular interactions, such as recognition, binding, catalysis and signal transduction, is critical not only for understanding fundamental cell biology but also for the design of efficient high-throughput biosensing and diagnostic methods.

Micro and nanostructured surfaces have been targeted as powerful tools to investigate biomolecular interactions because they yield high-density arrays of biomaterials at biologically relevant scales. On one hand, they can produce motifs that can be used as simplified surrogates for the complex cellular microenvironment. This has enabled the in vitro study of targeted biomolecular interactions, which would be hard or impossible to study in vivo. On the other hand, the demand for efficient detection of proteomic and genomic biomarkers, as well as the need for rapid detection of pathogenic threats has placed great emphasis on the production of biofunctional micro and nanostructured materials that act as recognition elements in high-throughput biosensing platforms.

Such materials enable the specific binding and sensing of target biomolecules in complex mixtures with high sensitivity and low noise. Because of the potential applications in cell biology and in biosensing and diagnostic tools, much research has focused on the ability to create bioactive micro and nanostructured surfaces. To date, numerous techniques have been developed to accomplish this goal including: self-assembly, micro and nanomachining, quill-pin spotting, dip-pen nanolithography, inkjet printing, microcontact printing, polymer stencil lift-off, electrospinning, and micro-fluidic networks, among others. Our laboratory focuses on the development of techniques to produce surfaces that enable the immobilization of biomaterials in controlled micro to nanoscale patterns, and their application for the study of targeted biomolecular interactions. Below, we detail two specific techniques that we currently employ, and discuss their potential applications in the fields of cellular and molecular biology, and in the development of biosensors and point of care devices.

Polymer stencil lift-off for biomaterial micropatterning

Biomaterial micropatterning refers to the host of techniques used to deposit biomaterials on solid surfaces with controlled feature sizes of micron to nanometer dimensions. Micropatterning is a powerful and rapid means for producing arrays of biomaterials for biosensing assays and for the study of interactions between the patterned materials and target analytes. A number of biocompatible techniques have been developed aimed at placing biological ligands at well-defined locations on substrates, with the most widely used being microcontact printing (μCP). Although strategies for DNA and protein patterning are relatively mature, supported lipid bilayer (SLB) and cellular patterning are more challenging because they require the materials to remain in 'never-dry' condition and cannot be easily achieved by any of the currently available techniques. An additional shortcoming of current techniques lies in the ability to deposit a multiplicity of biomaterials to form intricate patterns. Our research group is working on the development of new approaches to overcome these challenges.

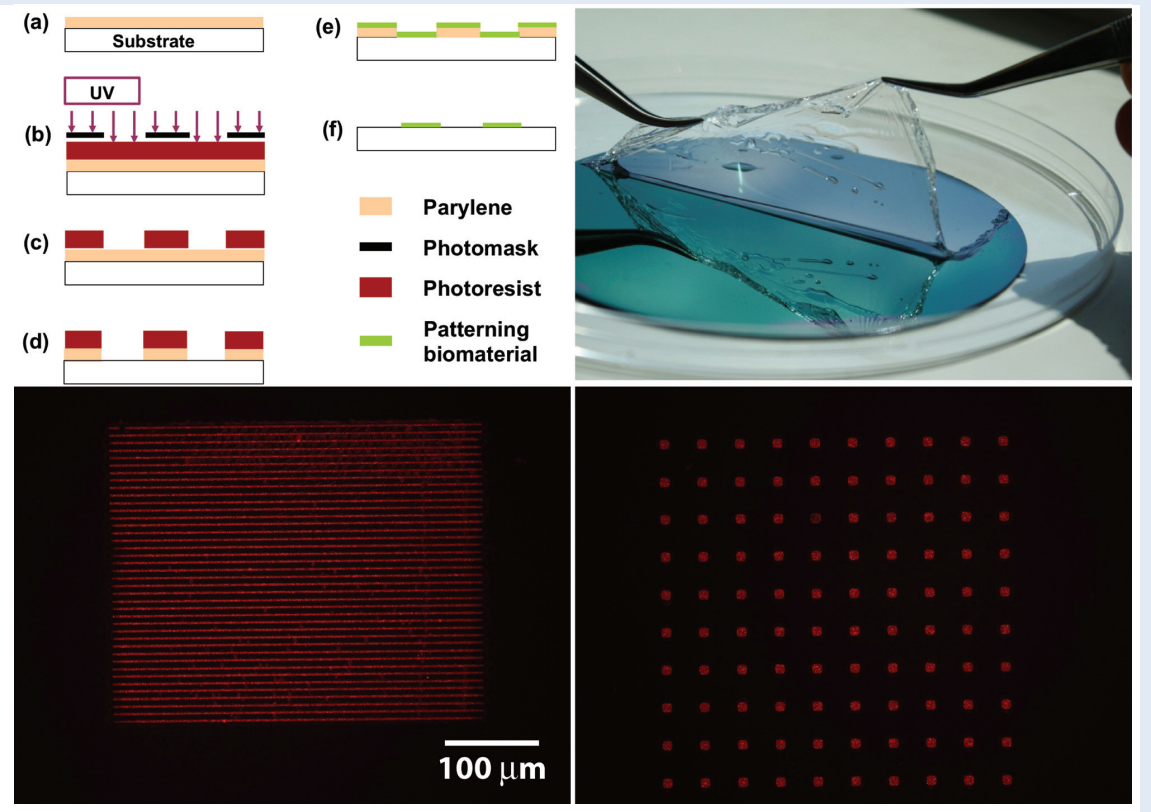
Among the techniques developed for biomaterial micropatterning, polymer stencil lift-off (PSLO) is one of the most robust and is utilized in our lab due to its advantages in terms of biocompatibility, pattern transfer fidelity, and its applicability to patterning under aqueous conditions. PSLO is a relatively new patterning method that enables the formation of arrays of micro to nanometre-sized biomaterial domains over large surface areas. In this approach, Parylene C (dichloro-[2,2]-paracyclophane) is the most commonly used polymer because it produces chemically inert, pinhole-free, thin polymer films. Furthermore, these films do not swell and can be mechanically peeled off from the surface under aqueous conditions, which makes them ideal templates for patterning biomaterials that need to be kept in hydrated environments (e.g. lipid membranes and cells). The principle behind PSLO micropatterning is illustrated in Figure 1. First, a thin conformal polymer film is deposited onto the solid surface through chemical vapour deposition. A photoresist layer is then coated on the polymer and patterned using photolithography. With the patterned photoresist as a mask, the sample is subjected to a controlled oxygen plasma reactive ion etch that removes the polymer and exposes the underlying substrate surface, effectively creating a polymer stencil. The biomaterials of interest can then be applied and bound onto the exposed substrate, while the polymer stencil keeps all the covered areas protected. After the excess biomaterials are washed away, the stencil can be me-

FEATURE

chanically easily peeled off due to its weak adhesion to the substrate, leaving the biomaterial micropattern on the surface.

Currently, research in our lab focuses on supported lipid bilayer (SLB) micropatterning through the PSLO technique. SLBs are interesting model systems because they preserve many of the distinctive properties of cell membranes, such as fluidity and functionality, while at the same time allowing control of the membrane composition and ease of access for their study through quantitative analytical techniques. Thus, SLBs have become increasingly popular and offer opportunities for the study of lipid-lipid, ligand-receptor[1], and cell-membrane[2,3] interactions, as well as for the production of membrane based biosensors.[4–6] SLB micropatterning provides the additional capabilities of controlling the size of the SLB domains and of producing SLB arrays of heterogeneous compositions. Furthermore, the combination of SLB micropatterning with microfluidic devices provides a powerful tool for studying biomolecule interactions with low sample consumption and high throughput. With the PSLO technique, our group is able to produce micron and sub-micron sized patterns of SLBs with homogenous and heterogeneous lipid compositions under aqueous conditions (Figure 1). We use micropatterned SLB arrays as models for the study of the effect of domain size and environmental factors (e.g. temperature, cholesterol concentration, ionic concentration) on the behavior of lipid mixtures. Through these simplified model systems we aim at understanding more complex systems such as the plasma membrane.

Figure 1



The polymer stencil lift-off technique produces well-defined, high quality micron and sub-micron supported lipid bilayer patterns. Top: Schematic description of the PSLO technique and illustration of the lift-off process. Bottom: sample images of line (3 μm wide) and square (10 μm wide) patterns formed using PSLO of fluorescently labeled SLBs with heterogeneous lipid mixture compositions.

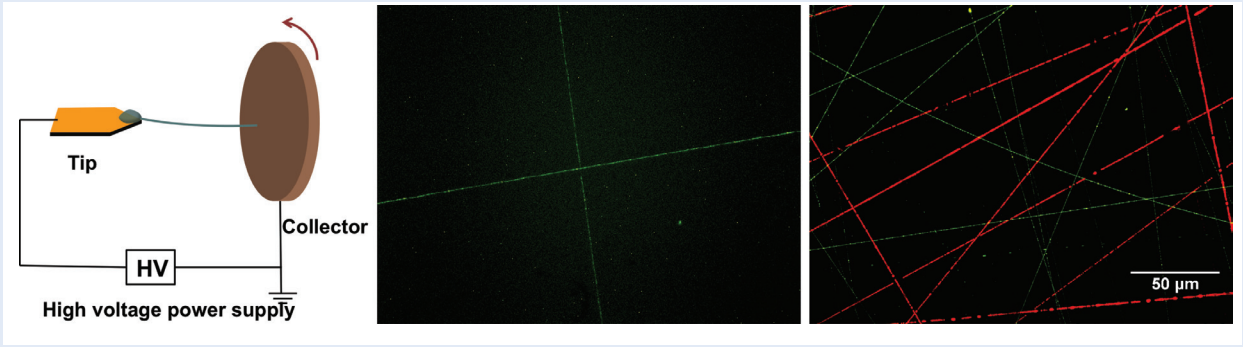
Electrospinning of Functional Nanofibers

Developed over 70 years ago and popularized by Reneker in the 1990's, electrospinning is another powerful technique for generating micro and nanostructured materials. In recent years, there has been increased interest in the study of electrospun nanofibers due to the surge in the demand for nanostructured materials. With electrospinning, not only a variety of polymers, but also biomolecules, nanoparticles, and even cells embedded in a carrier polymer, can be used to produce nanofibers with different physical, chemical and biological properties. In particular, fibers made of functional polymers can possess unique mechanical[7], electrical[8], electrochemical[9], magnetic[10], and optical properties.[11] With easily tailored properties and functionalities, electrospun nanofibers have promising applications in drug delivery, energy storage, tissue engineering, sensing and lab-on-a-chip applications.

One of the most attractive features of the production of functional nanofibers through electrospinning is that can be done on the bench top in a very simple manner. In a typical electrospinning setup, such as the one implemented in our laboratory, a droplet of viscous solution is applied onto a sharp conductive tip and high electrical potential is applied between the tip and a static or rotating grounded collector. As a result of molecular ionization, charge redistribution and the external electric field, the droplet deforms until the electrostatic forces acting on the charged solution overcome surface tension. At this critical point a Taylor cone forms and a continuous jet is extracted from the solution. As the jet travels through the air from the tip to the collector, it is thinned out due to solvent evaporation, stretching and bending forces, which results in the formation of very narrow solid nanofibers that can be deposited on a substrate affixed to the collector. The electrospinning process is mainly affected by the material properties of the polymer solution (molecular weight and polydispersity, viscosity, surface tension, and conductivity of the solution) and process parameters (amount of solution applied, electric voltage, distance between the tip and the collector, and motion of collector). With proper control of the solution viscosity, the voltage applied and the distance between the tip and the collector substrate, fibers with diameters in the sub-micron range can be easily produced via electrospining.

Our current research explores bench top techniques to fabricate mi-

Figure 2



Electrospinnning is a simple and low-cost technique for producing nanostructured materials. Left: Schematic depiction of a standard electrospinning setup. Right: Fluorescence images of photoluminescent nanofibers produced from polymer solutions doped with different fluorogenic molecules.

crostructured conductive tips that can be integrated with continuous flow microfluidic systems for the electrospinning of conductive polymer nanofibers. Our conductive tips are typically fabricated by directly cutting polymer films, which are subsequently coated with a conducting layer. This approach allows us to test different shapes and sizes and tailor the electrospinning tip to the particular polymer blend that we wish to electrospin. Carrier polymers that we use for electrospinning include polystyrene (PS), poly-ethylene oxide (PEO), and poly-vinyl alcohol (PVA) dissolved in different polar and non-polar solvents such as water, ethanol, chloroform, and toluene, among others. The ability to pair the electrospinning tip morphology with a variety of polymer and solvent combinations allows us to routinely produce fibers with diameters smaller than 500 nm. Furthermore, the ability to collect the fibers in static or rotating collector modes allows us to generate disordered non-woven mats or single aligned fibers (Figure 2). Fluorescence microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM) are the main techniques our group utilizes in the characterization of the electrospun nanofibers. Figure 2 shows a fluorescence micrograph of a collection of 100-300 diameter photo-luminescent polyethylene oxide nanofibers doped with two different fluorophores. Our research aims at employing electrospinning as a simple and low-cost technique for the fabrication of functional polymeric nanostructures to be used as highly sensitive biosensors for lab-on-a-chip applications.

Conclusion and outlook

With the increasing demand for understanding more complex biological phenomena as well as the need for highly sensitive diagnostic techniques, micro and nanostructured materials will play a preponderant role in the study of biomolecular interactions and the development of novel sensing devices. On one hand, the development of high throughput and low-cost techniques to create more uniform and precisely controlled biomaterial micropatterns under aqueous conditions should expand the range of biological systems that can be studied and monitored in vitro. This will not only enhance our ability to study membrane and cell behavior under controlled environmental conditions, but should also expand the range of platforms available for diagnostics based on membrane-binding analytes and for the screening of drugs that target membrane associated proteins. On the other hand, the use of simple techniques for the fabrication of nanostructured bioactive surfaces should contribute to the development of architectures that exploit the high surface area and tailored functionality provided by electrospun nanofibers, such as tissue engineering scaffolds, separation matrices, or highly sensitive electrodes.

References

1. Moran-Mirabal, J. M.; Edel, J. B.; Meyer, G. D.; Throckmorton, D.; Singh, A. K.; Craighead, H. G. Biophysical journal 2005, 89, 296–305.
2. Wu, M.; Holowka, D.; Craighead, H. G.; Baird, B. Proceedings of the National Academy of Sciences of the United States of America 2004, 101, 13798.
3. Mossman, K.; Groves, J. Chemical Society Reviews 2007, 36, 46.
4. Fang, Y.; Frutos, A. G.; Lahiri, J. Journal of the American Chemical Society 2002, 124, 2394–2395.
5. Zhou, X.; Moran-Mirabal, J. M.; Craighead, H. G.; McEuen, P. L. Nature Nanotech 2007, 2, 185–190.
6. Bally, M.; Bailey, K.; Sugihara, K.; Grieshaber, D.; Vörös, J.; Städler, B. Small 2010, 6, 2481–2497.
7. Verbridge, S. S.; Parpia, J. M.; Reichenbach, R. B.; Bellan, L. M.; Craighead, H. G. Journal of Applied Physics 2006, 99, 124304.
8. Liu, H.; Reccius, C. H.; Craighead, H. G. Applied Physics Letters 2005, 87, 253106.
9. Liu, H.; Kameoka, J.; Czaplewski, D. A.; Craighead, H. G. Nano Letters 2004, 4, 671–675.
10. Li, D.; Herricks, T.; Xia, Y. Applied Physics Letters 2003, 83, 4586.
11. Moran-Mirabal, J. M.; Slinker, J. D.; DeFranco, J. A.; Verbridge, S. S.; Ilic, R.; Flores-Torres, S.; Abruña, H.; Malliaras, G. G.; Craighead, H. G. Nano Lett 2007, 7, 458–463.

Yujie Zhu is a graduate student and Dr. Jose M. Moran-Mirabal is an Assistant Professor at the Department of Chemistry and Chemical Biology at McMaster University.

NEW PRODUCTS

Electrofusion

The Hybrimune® is the newest addition to the BTX line of electrofusion systems. The Hybrimune® is an advanced pulse generator capable of delivering variable AC pulse frequencies; utilizing patented technology to enhance the alignment of cells in large volumes up to nine mls in one run. Researchers performing monoclonal antibody production can achieve the highest efficiencies in scalable throughputs (up to 180 million cells fused in milliseconds). Published data demonstrates that Hybrimune® technology can yield 10 to 20 times greater antigen plus specific clones compared to PEG.

Web: www.btxonline.com

Pumps

Supercritical Fluid Technologies, Inc. introduces the completely self-contained SFT-10 Liquid Carbon Dioxide Pump. The SFT-10 pump can pressurize carbon dioxide up to 10,000 psi (69 MPa) at flow rates from 0.01 to 24.0 mL/min. These characteristics make the SFT-10 an ideal pump for use in supercritical fluid extraction as well as a variety of other high pressure applications, including supercritical fluid reaction chemistry and chromatography. The SFT-10 utilizes reliable, dual sapphire syringe pump technology to achieve high pressures rapidly while its Peltier chiller has superior cooling capability. It maintains the temperature at the pump heads low enough to ensure the carbon dioxide remains liquid. The SFT-10 may be acquired as a stand alone CO2 pump or used as part of Supercritical Fluid Technologies' SFT-100 / SFT-100XW Supercritical Fluid Extractors.

Web: www.supercriticalfluids.com.

Titrator

JM Science's AQUACOUNTER® Karl Fischer Volumetric Titrator (AQV-2200S) is rugged, reliable, long lasting and eco-friendly with small volume titration cells requiring only 20mL of titration solvent for accurate measurements. Less reagent volume reduces waste and makes it easy to replace fresh solvent for the next series of measurements. This unit comes with accessories kit and is ideal for users who have many kinds of samples to be analyzed or exchange KF reagents frequently or after each measurement. A two-channel option means easy plug-and-play. End-users can add various peripherals, such as a second channel, and the system recognizes the new channels and begins working with them immediately. Intuitive control panel display shows current status, data and function keys displayed on a large, colourful screen. Result data with curves can be viewed on your PC internet browsing program without optional software. Save and load parameters and results via USB flash memory. A built-in thermal printer is easy to load and has high-resolution printouts.

Web: www.jmscience.com