Fabrication of Biomolecular and Polymeric Nanostructures by Proximal Probes

NSF NIRT Grant 0210590

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The overarching goal of the proposed research is to address both scientific and engineering aspects of nanopatterning of surfaces and in-situ nanofabrication that will directly impact biotechnology, biologically inspired engineering, transducers, and electronics at the nanoscale. This requires the development of suitable SPL instrumentation in conjunction with a suite of prototypical patterning techniques. The proposed interdisciplinary research program is focused on the fabrication of biomolecular and polymeric nanostructures by proximal probe techniques - these techniques include Dip-Pen Nanolithography (DPN), Reactive DPN, Electrochemical DPN, and Scanning Near Field Optical Lithography (SNOL). To facilitate these fabrication methods, a scanning probe lithography (SPL) instrument is currently being developed that provides high spatial resolution and positioning repeatability to perform the necessary nanofabrication steps. Initially we have concentrated on surface functionalization chemistries; however, the nanopatterning methods proposed are not confined to the particular chemistries chosen.

The research objectives are to

- develop SPL instrumentation that features multi-dimensional nanopositioners and uses engineered SPM tips specifically designed for nanolithography,
- develop a "virtual nanopattern generator" that seamlessly works with the hardware control of the SPL instrument,
- develop new fabrication methods for structures on the nanometer length scale with biomolecules and stimulus-responsive macromolecules using DPN and SNOL techniques.

The greatest impact of this proposal will be the development of easy-to-use nanofabrication research tools for the scientific community. The impact on research includes

- development of robust and multipurpose SPL instrumentation and design software,
- nanofabrication of biopolymer surface structures by active patterning,
- first demonstration of surface initiated nanopolymerization (SINP),
- first demonstration of direct nanografting of elastin like polypeptides (ELPs) and biotin,
- first demonstration of patterning biomolecules with light activation at the nanoscale (PLAN),
- first demonstration of fabricating nanoscale polymeric electronic structures using direct nanopolymerization (DNP) of organic materials.

The impact on education includes

- new curriculum for graduate students, combining elements of engineering, materials science, surface chemistry and physics,
- ample training opportunities for undergraduate students in nanotechnology through participation in research projects,

- considerable industrial collaboration providing technology transfer and training opportunities for faculty, research associates and students.

Since faculty/student interaction with collaborating companies is necessary for the success of this research as well as benefiting education and training, we intentionally have adopted NSF's GOALI approach in this proposal. The proposed research incorporates faculty from the Pratt School of Engineering's Mechanical Engineering and Materials Science (MEMS) and Biomedical Engineering (BME) Departments and Trinity College of Arts & Sciences Departments of Chemistry (CHEM) and Physics (PHY) as well as significant industrial collaboration from Piezomax (Madison, WI), Veeco Digital Instruments (Santa Barbara, CA), and GlaxoSmithKline (RTP, NC). Strong industrial collaboration for the instrumentation and fabrication efforts will provide us with an efficient conduit to the industrial community, facilitating intellectual exchange between engineers and scientists working towards a common goal and providing a method for knowledge and technology transfer.

In the past year the Duke NIRT team has made significant progress in the development of new nanomanufacturing processes that are derived from DPN. These include the following achievements:

(1) Development of a generic, modular approach to biomolecular lithography based on **nanoscale, covalent patterning of biotin** onto alkanethiol self-assembled monolayers (SAMs) on gold [1]. This approach enable the facile patterning of any biomolecule that can be synthesized as a biotin conjugate by a simple incubate and rinse procedure.

(2) The **first demonstration of the in situ fabrication of stimuli responsive polypeptide nanostructures** [2-5]. We have demonstrated that a recombinant stimuli responsive Elastin-like polypeptide (ELP) can be attached to COOH-terminated SAMs that are nanopatterned by DPN and that these biopolymer nanostructures exhibit a hydrophilic-hydrophobic phase transition at the surface. ELP nanopatterns with feature sizes ranging from 200 nm to 2.5 μm were fabricated by this method on gold surfaces against a background of a nonfouling, oligoethylene-glycol terminated, alkanethiol SAM. We also demonstrated that these smart nanostructures could reversibly bind an ELP fusion protein from solution by simultaneously triggering the phase transition of the ELP. Nanopatterning with stimulus-responsive biopolymers holds significant promise for the fabrication of devices for biotechnology applications that require the capture of a target protein directly from a complex mixture and for devices where the transport, separation and detection of many biomacromolecules must be performed in aqueous solutions, with applications in biosensors and proteomic chips, and nanofluidic devices.

(3) The **first demonstration of surface initiated nanopolymerization (nanoSIP)** using an atom-transfer radical initiator that is covalently nanopatterned on a gold surface by DPN [6]. Two different polymers have been successfully grown, in situ from gold using this method; a protein and cell-resistant poly(oligoethylene glycol methacrylate) and a stimuli-responsive poly(N-isopropylacrylamide). The combination of DPN with SIP is an important enabling nanotechnology for nanomanufacturing, because it provides a new capability in the in situ, bottom-up fabrication of polymeric nanostructures, which are likely to be a critical component of many nanoscale devices.

References

[1] J Hyun, SJ Ahn, W Lee, **A Chilkoti**, and S Zauscher. Molecular recognition mediated fabrication of protein nanostructures by dip-pen lithography, *Nanoletters,* **2:** 1203-1207 (2002).

[2] J Hyun, W.K Lee, N. Nath, **A Chilkoti** and S Zauscher. Stimulus-responsive elastin-like polypeptide nanopatterns, for submission to *Nanoletters*.

[3] J. Hyun, WK Lee, SJ Ahn, **A Chilkoti**, and S Zauscher, Fabrication of "smart" protein nanostructures using molecular recognition and dip-pen nano-lithography." Polymer Preprints (PMSE), 226th ACS National Meeting, New York, NY.

[4] N Nath, **A Chilkoti**, J. Hyun, S Zauscher, and WK Lee, Non-covalent, environmentally modulated molecular recognition between ELP biopolymers: A convenient route to reversible protein arrays. Polymer Preprints (POLY), 226th ACS National Meeting, New York, NY.

[5] S Zauscher, **A Chilkoti**, SJ Ahn, J Hyun, and WK Lee (2003) Fabrication of surface confined, stimulus-responsive polymer nanostructures using dip-pen nanolithography. Polymer Preprints 44(1), 457-458.

[6] H Ma, J Hyun and **A Chilkoti.** Surface initiated atom transfer radical polymerization of an oligoethylene glycol functionalized comb polymer: A new route to nonfouling biomaterials, *Adv. Mater*., in press.