Deposition of Biotemplates using Dip-Pen Nanolithography

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Abstract

Dip-Pen Nanolithography (DPN) is a powerful and versatile technique for nano- to micro-scale patterning.[1] DPN entails loading the probe of an atomic force microscope (AFM) with a desired molecule which is then deposited in a specific pattern onto the substrate of interest. Since the introduction of DPN in 1999 a variety of molecules have been deposited, including proteins,[2,3] alkylthiols (self-assembled monolayers)[1] and metal salts.[4] Deposition may be driven by covalent, electrostatic, or electro-chemical forces. Here we describe the use of DPN for nano-scale patterning of biotemplates which can subsequently be used as biomineralization precursors or molecular building blocks for nanoscale structures.

Overview of Dip-Pen Nanolithography

An effective method available for patterning molecules on a micro- or nano-scale is dip-pen nanolithography (DPN) which is performed using an atomic force microscope (AFM).[1] The AFM probe is coated with the desired molecule and patterned onto the substrate of interest in a fashion similar to an old-style quill pen (figure 1). Line widths in the range of 10 - 20 nm resolution are obtainable. The molecular deposition may be driven by covalent, electrostatic or electrochemical forces and a dynamic range of molecules have been deposited, including alkylthiols (self-assembled monolayers),[1] proteins,[2,3] metal salts,[4] and many others.[5] DPN is typically performed in a serial fashion, but parallel deposition, active probes, and many other advances are currently being developed.[6,7]

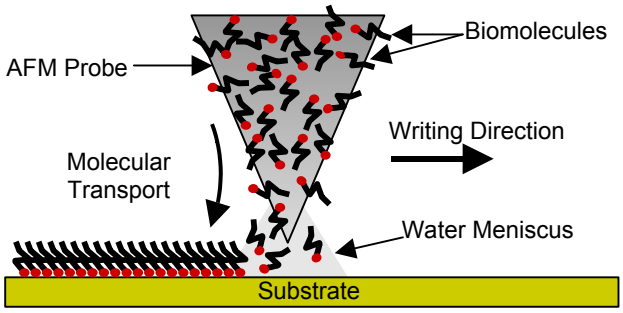

Figure 1.Cartoon of dip-pen nanolithography process utilizing an AFM probe loaded with molecular ink.

Biomolecule Deposition via DPN

Biological organisms exhibit incredibly diverse mechanisms for a variety of chemical reactions under mild ambient conditions. These reactions often involve specialized proteins that are responsible for precipitating or catalysis of organic and inorganic molecules. Our research group seeks to exploit these reactions available in nature by extracting or mimicking the reaction components and incorporating them to improve existing technologies.

We have deposited a variety of biological molecules using DPN. Deposition was originally performed on a Digital Instruments Multimode Scanning Probe Microscope with a Nanoscope IIIa controller. We have recently acquired an NSCRIPTOR (NanoInk, Chicago, IL) which is an AFM specifically tailored to perform DPN. Patterns are created with InkCad software which is provided with the NSCRIPTOR system.

Electrostatic Peptide Deposition

Our group has patterned gold, silicon, and mica surfaces with charged peptides by way of electrostatic interactions.[8] The substrate surface and peptide must have opposite net charges to enable deposition. Figure 2 shows the deposition of a slightly negatively charged 19 amino acid peptide onto a positively charged gold surface. The AFM probe was held in the center of the spot for two minutes as the peptide diffused in a circular pattern.

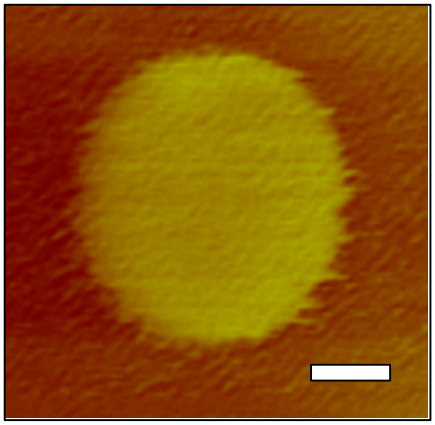

Figure 2. AFM height image of peptide deposited electrostatically by DPN. Scale bar is 1 micron.

The nanoscale environment is unique in that many products will be larger than their original DPN-patterned design. This allows one to seed crystals and monitor their growth over time. Liu et. al have accomplished this with poly-DL-lysine hydrobromide crystal growth by imaging a freshly cleaved mica substrate with a lysine coated probe.[9]

Electrochemical Deposition of Biomolecules

Histidine tags, 6 histidine residues, are routinely used in biology for the purification of proteins. The His-tag is generally inserted at

the N- or C- terminal of the protein of interest. Using electrochemical DPN, we have been able to immobilize His-tagged peptides and proteins onto a nickel surface.[3] In this method, the AFM probe was loaded with the tagged biomolecules. A bias was applied to the probe during the DPN process which chelated the biomolecules onto ionized regions of the nickel surface.

Patterning Biomineralization Reactants via DPN

Biomineralization occurs in many biological organisms in order to create specialized structures composed of inorganic materials.[10] The transformation of inorganic molecules into nano- and microstructured components is controlled, in most cases, *in vivo* by proteins. Titanium (IV) bis(ammonium lactato) dihydroxide solution (TiBALDH) is a water soluble precursor for titania particles which can be precipitated by charge localization.[11,12] Titania particles have many applications, including uses as photocatalysts, solar cell components, white pigments, and could benefit from the development of ambient mineralization and patterning methods.[13]

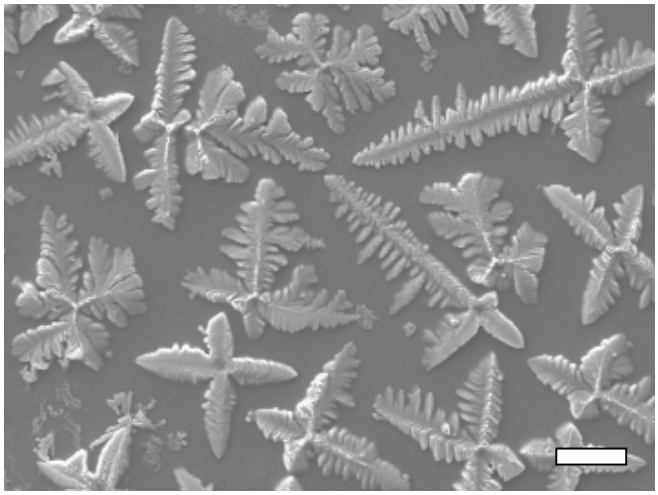

Figure 3. Scanning electron micrograph of dendritic structures formed by PLL and TiBALDH reaction. Scale bar is 10 microns.

When TiBALDH is exposed to a surface coated with poly-L-lysine (PLL) under ambient conditions, titania particles are formed. Depending on the ratio of precursor to peptide, the products can completely cover the surface with titania particles or with patches of dendritic structures having fractal dimensions around 1.6 (figure 3).We are currently optimizing the patterning of PLL and TiBALDH using DPN to offer controlled, patterned growth of these dendritic structures. For example, the water soluble titania precursor can be patterned onto a substrate via DPN. Similarly, poly-L-lysine can also be deposited (figure 4).

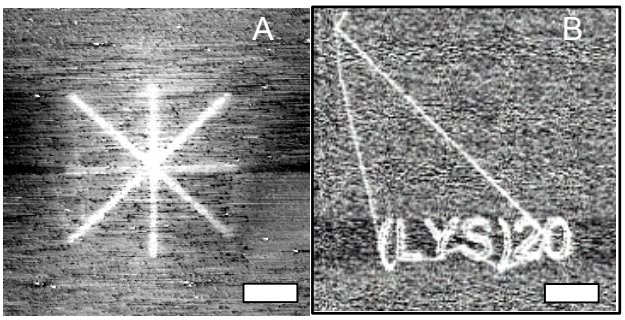

Figure 4. NSCRIPTOR lateral force mode images of a) TiBALDH and b) poly-L-lysine deposited by DPN. Scale bars are 1 micron.

Discussion

In order to exploit the advantages of biomimetic approaches to materials chemistry then techniques that facilitate the spatial deposition of biomolecules that can perform specific chemistries on surfaces is critical. DPN is an attractive technique for patterning molecules onto substrates with high resolution. DPN also allows reactions to be studied by localizing them and permitting systematic changes made to reaction volumes, concentrations, etc.

Acknowledgements

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Author Biography

Laura Sowards has worked in the Air Force Research Labs for 10 years. She began as a part-time undergraduate student. She spent three years studying the structure-property relationships of conjugated dye systems and earned a Masters of Science in physical chemistry at Wright State University. Upon graduation, she joined a newly formed biotechnology group and studied thermal detection in snakes and beetles. Her focus then shifted to soft lithography and molecular patterning techniques.