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Nanopatterning Proteins with a Stamp Tip for Dip-Pen Nanolithography

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Abstract

The fabrication of a nano-porous polyoxazoline stamp tip for scanning probe nanolithography has been reported, and the patterning of nanometer scale high-molecular weight proteins at ultra-fast speed demonstrated using the stamp tip. A nano-porous polymer-coated silicon tip was constructed using ring opening polymerization of 2-methyl-2-oxazoline on the surface of the tip employing a simple solution polymerization method. Because polyoxazoline is a hydrophilic and biocompatible polymer, these tips can easily absorb and release water-based biomolecules via their pores. Using dip-pen nanolithography, the patterning of Human IgG and PSA proteins using these stamp tips has been demonstrated. The results showed that protein nano-patterns can be generated at least five times faster with this method than with a conventional silicon tip.

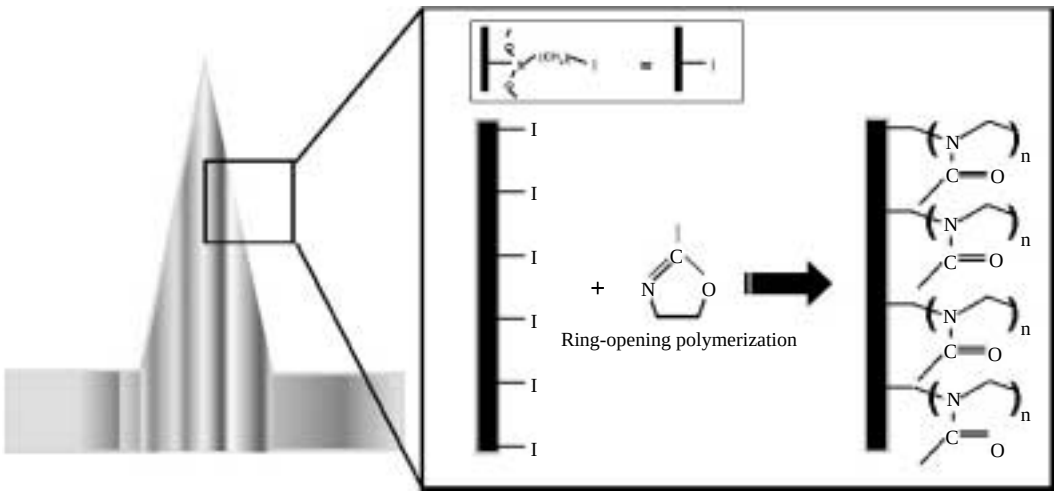
Keywords: Atomic force microscopy (AFM), Polyoxazoline, Protein nano-patterns, Dip-pen nanolithography (DPN), Self-assembled monolayers (SAMs)

Introduction

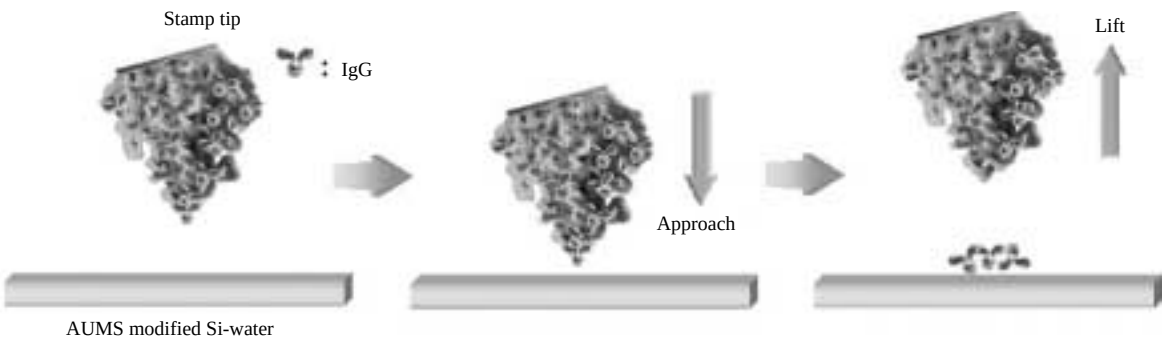
Since the invention of dip-pen nanolithography (DPN) in 1999[1], it has been used widely in various research areas, such as basic chemistry and physics, proteomics, diagnostics, molecular electronics and materials science[2]. DPN is a scanning probe microscopy (SPM)-based direct-write nanofabrication tool, which uses an atomic force microscopy (AFM) tip to deliver molecules to a surface via the water meniscus formed between the tip and the surface. This technique, in principle, allows the direct transport of any of a number of materials to a variety of substrate surfaces, but with the resolution of AFM. In a typical DPN method, a bare silicon cantilever can be dip-coated with ink molecules, which are then transported to a substrate by bringing the tip into contact with the surface. Thus far, much of the work has been performed with a variety of ink molecules, such as small organic molecules[1], DNA[3], proteins[4,5], conducting polymers[6], colloidal particles[7], collagens[8], metal ions and sols[9]. J. Liu and co-workers developed another direct-write patterning method; electrochemical DPN (E-DPN), where the water meniscus formed at the tip-substrate interface was used as a transport medium, and also as a nanoscale electrochemical cell[2]. With this system, they showed that metal nano-features could be deposited via electrochemical reduction onto a silicon surface.

The application of the DPN system to the biochip industry, however, has a few restrictions: 1) slow patterning of high-molecular-weight biomolecules due to the low diffusion rate, 2) difficulty retaining the biological activity of biomolecules on the surface of the tip as a result of drying, and 3) short operating time due to the limited ink volume on the surface of the tip.

In this paper, a new and simple method for the fabrication of a nano-porous polyoxazoline-coated tip is reported. This sponge-like tip can easily absorb water-based biomolecules into its porous matrix, which can be easily released when the tip contacts the solid surface. Also, because polyoxazoline is a hydrophilic and biocompatible nonionic polymer[10], the absorbed biomolecules can be hydrated by the presence of water molecules in the polymer matrix; therefore, will retain their biological activities. Herein, the use of the stamp tip, in combination with the DPN technique, for the creation of protein nanopatterns on the surface of the silicon substrate has been demonstrated.



Scheme 1. Schematic diagram for the polymerization of polyoxazoline on the tip surface.



Scheme 2. Schematic illustration of the stamping system.

Results and Discussion

A stepwise polymerization process for the fabrication of a nano-porous polyoxazoline-coated AFM cantilever is shown in Scheme 1. In order to create active polymerization sites on the surface of the tip, the bare surface was functionalized with 11-iodoundecyltrichlorosilane, as an initiator, by self-assembly. Thereafter, polymerization of 2-methyl-2-oxazoline monomers on the I-functionalized surface of the tip was performed using a simple solution polymerization method. In our previous work, gas phase polymerization was required to create the nano-porous polyoxazoline structures on the surface. However, the same polyoxazoline nanostructures could be obtained on the surface of the tip via solution polymerization by just changing the initiating agent. This was presumed to be due to the inhomogeneous growth of the polymer on the surface.

The process for generating protein nanostructure arrays is shown in Scheme 2. After dip-coating protein inks onto the surface of the stamp tip, the coated tip can be brought into contact with the surface of the substrate to transport ink molecules from the tip to the surface. It should be noted that the physical contact between the tip and the surfaces was used as a driving force to transport the ink molecules, and the DPN process was governed by the diffusion of materials between the tip and the surface of the substrate via the meniscus of the water. Therefore, a protein nanostructure can easily be generated by touching the tip onto the surface, and this method would be expected to remain unaffected by humidity, temperature or contact time.

As proof-of-concept experiments, human IgG and prostate specific antigen (PSA) proteins were used to generate nanoarrays with our DPN stamping system.

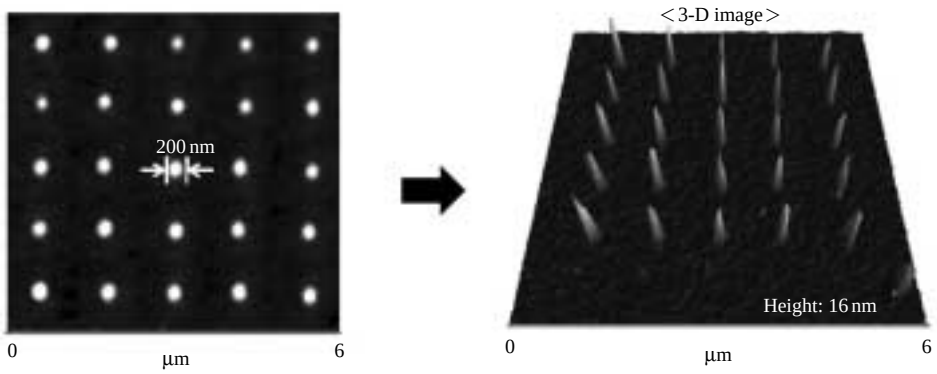


Figure 1. AFM topography images of human-IgG protein nanostructures DPN-generated with a stamp tip onto AUMS modified silicon surfaces.

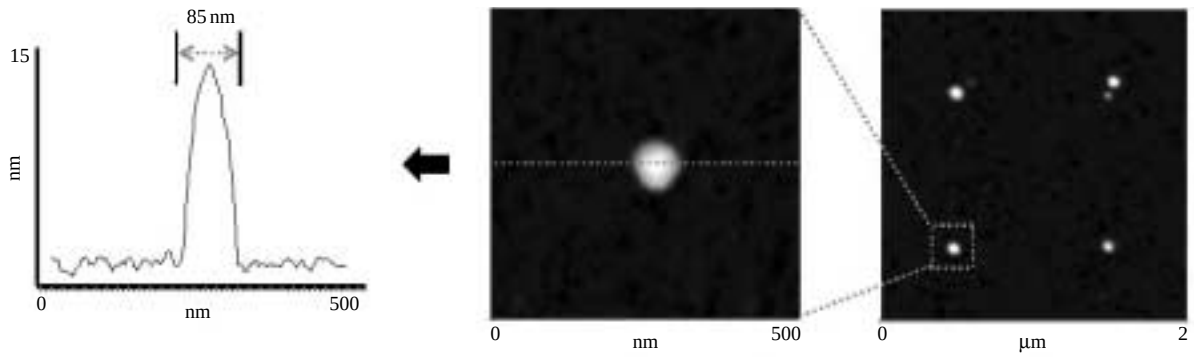


Figure 2. AFM topographical images of PSA nanostructures on AUMS modified silicon surfaces.

Figure 1 shows human-IgG nanoarrays on N-2-aminoethyl-11-aminoundecyltrimethoxysilane (AUMS) modified silicon surfaces using the conventional DPN method employing a nano-porous stamp tip. An array consisting of 25 dots, with a diameter of 250 nm and a spacing of one micron, was constructed. Note: the array was able to be constructed in less than 25 seconds, with a contact time of one second for each dot, which was five times faster than the results from previous DPN for the same protein using just a silicon tip[4,5]. Therefore, this system does not use the diffusion of materials as the driving force for depositing the molecules, but rather physical contact. Moreover, the size of the dot does not change, even when the contact time is increased to 10 min. This is also evidence that our system works by physical stamping. The average measured height; 16 nm, for the IgG dots was consistent with a single monolayer of protein adsorbed onto the surface.

Figure 2 shows an AFM topographical image of the PSA nanostructures fabricated using DPN with the same stamp tip. The height of the PSA nanoarray profile shows that each PSA protein feature is about 15 nm high and 85 nm wide, which is also consistent with a single monolayer. It is also of interest that the size of the protein nano-features depends only on the tip used, and a different sized dot can not be made with the same tip, even of the operating conditions, such as humidity, temperature and contact time, are changed. This means that in future work the size of the protein features can probably be changed by changing the size of the tip-end.

Conclusions

The development of a nano-porous polyoxazoline stamp tip for DPN for the generation of high-molecular weight proteins, at an ultra-fast deposition speed, has been reported. A nano-porous polyoxazoline coated tip was successfully fabricated by the polymerization of 2-methyl-2-oxazoline monomer using a simple solution polymerization method. The stamp tip, in combination with DPN, has also been demonstrated to pattern IgG and PSA proteins on a nanometer scale. Finally, it has been shown that the method employing the stamp tip can generate protein nanopatterns at least five times faster than with a conventional silicon tip.

Materials and Methods

Materials

The monomer for the polymerization of polyoxazoline, 2-methyl-2-oxazoline, was purchased from Aldrich Chemical Co. (USA). 11-iodoundecyltrichlorosilane (IUCS) was purchased from JSI silicon Co., and N-2-aminoethyl-11-aminoundecyltrimethoxysilane (AUMS) from Gelest, Inc. (USA). Human IgG protein and prostate specific antigen (PSA) were purchased from Chemicon (Temecula, CA, USA) and Biodesign (Saco, USA), respectively. All other chemicals (methanol, ethanol, toluene, acetonitrile, sulfuric acid and hydrogen peroxide) were of ACS grade, from Aldrich Chemical Co., and used without further purification. Silicon wafers were purchased from Silicon Quest International, Inc. (CA, USA).

Preparation of Substrates

The silicon wafers were cleaned by immersion in a "piranha solution" (H_2SO_4/30% H_2O_2=7 : 3 (v/v)) (CAUTION: Piranha solutions are extremely dangerous and should be used with extreme caution) at 80˚C for five min, subsequently washed three times with pure water and then dried with nitrogen gas. The cleaned substrate was then placed for 30 min in a 10 mM AUMS solution in toluene, then washed thoroughly with toluene and ethanol, dried under nitrogen gas and baked at 100˚C for 10 min. These amine-terminated substrates were used for the stamp-on type nanolithography of proteins.

Fabrication of Nanoporous Tip

Silicon probes (M2N, Inc., Korea, spring constant= 40 N/m, model=STP4) were cleaned with a "piranha solution" and then immersed for one hour in a 10 mM IUCS solution in toluene to form an I-terminated self-assembled monolayer on the surface of the tip. Washing and drying were then performed using the same procedure mentioned above. The I-functionalized silicon tips were immersed in a 1% (v/v) acetonitrile solution of 2-methyl-2-oxazoline monomer, and then heated at 70˚C for one hr to allow sufficient time for polymerization. These polyoxazoline-coated tips were washed several times with acetonitrile, and then dried with N_2 gas.

Nanopatterning and Imaging

All "stamp-on" nanolithography patterning was conducted using a XE-100 AFM system (Park systems, Inc., Korea), with a nano-porous polyoxazoline -coated tip, under ambient conditions (20-50% RH and room temperature). The stamp-on tip was immersed in protein solutions (100 μg/mL phosphate buffered saline (PBS), pH=7.0) for five seconds to load the protein inks onto the surface of the tip. All topographic images of the generated protein nanopatterns were obtained using the same XE-100 AFM system, with conventional silicon cantilevers (spring constant=40 N/m, model=STP (30)) in the non-contact mode.

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