Fabrication of a Nano-Porous Polyoxazoline-Coated Tip for Scanning Probe Nanolithography

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Summary: A nano-porous polyoxazoline-coated silicon tip was developed by ring opening polymerization of 2-methyl-2-oxazoline. The tip surface modified with the nano-porous polyoxazoline has pore sizes from 30 nm to 100 nm, which can easily absorb large-molecular-weight biomolecules in their pores. In combination with dip-pen nanolithograpy (DPN) technique, this can generate protein nanostructures 60 times faster than what one can obtain with the conventional silicon tip. We discuss the fabrication of the nano-porous polymers on the surface and how to use this novel tip for scanning probe lithography-based nanopatterning of biomolecules.

Keywords: atomic force microscopy (AFM); dip-pen nanolithography (DPN); polyoxazoline; self-assembly; surfaces

Introduction

The dip-pen nanolithography (DPN) technique[1] is a promising atomic force microscopy (AFM)-based nanofabrication tool. It allows one to pattern ‘‘inks’’ such as small organic and inorganic molecules, charged polymers, DNA, and proteins on a variety of surfaces, as ‘‘papers’’, with a coated AFM tip. In principle, any materials can be deposited by the DPN method, controlling for environmental conditions such as humidity, temperature, tip coating procedures, and substrate-ink interactions. In addition, the direct-write capabilities of DPN technique offer many advantages over indirect methods for making arrays, such as facile processing, the elimination of the need for resists, and the generation of multi-component arrays with extraordinary complexity.[2] Although the method offers potential advantages, the DPN system has three major restrictions for applying biochip industry: 1) slow patterning speed of a high-molecular-weight molecules due to a low diffusion rate; 2) short operating time due to a limited ink volume on the tip surface; 3) difficulty of retaining biological activity on the tip surface against drying. Recently, in order to improve these restrictions, a tip made of poly(dimethylsiloxane) (PDMS) and the PDMS-coated Si_3N_4 stamp tip were fabricated.[3,4] They showed that the radius of the PDMS-coated tip was a little larger than that of the normal tip due to the coating layer, and the surface of the tip was very smooth in the nanometer length scale. They have used these modified stamp tips to deposit small thiol-inks and PAMAM dendrimer-G6-OH.[3,4] However, the results revealed that the transport of materials was still dependent upon the diffusion model of DPN ($t^{1/2}$ dependence), and no nanopatterns were shown with high-molecular-weight biomolecules at ambient conditions (room temperature and relative humidity (RH) lower than 30%). This means that the PDMS-coated tip still works not by a physical stamping mechanism, but by the diffusion mechanism via water meniscus formed between the tip and the surface.

In this study, we discuss a new type of nano-porous polyoxazoline-coated tip fabricated by ring opening polymerization of

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2-methyl-2-oxazoline on the silicon tip surface. Polyoxazoline is a well known hydrophillic and biocompatible nonionic polymer.[5] When biomolecules are absorbed into the hydrophilic polyoxazoline nanoporous matrix, these biomolecules can be easily hydrated by water molecules so as to retain their biological activities. This sponge-like tip can generate a nanoarray of large-molecular biomolecules by a physical stamping mechanism when the tip makes contact with the substrate surface. We will show how one can use this novel stamp-tip to pattern large-molecular-weight biomolecules with ultra-fast speed at ambient conditions.

Experimental Part

Materials

2-methyl-2-oxazoline, methyl-p-toluenesulfonate, diethyl ether, acetonitrile, and all solvents (Aldrich Chemical Co., USA) were dried and distilled under nitrogen. 1- bromoprophyltrichlorosilane, 16-mercaptohexadecanoic acid, sulfuric acid, hydrogen peroxide, and all of the other chemicals were purchased from the Aldrich Chemical Co. (USA) and used without further purification. IgG proteins (rabbit IgG) were purchased from Chemicon (CA, Temecula, USA) and used as source materials for patterning protein nanoarrays. Protein solution was prepared at a concentration of 500 μg/ml in a PBS buffer.

Substrate Preparation

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 10 minutes. They were subsequently washed several times with deionized water and dried with N_2. For modification of the surface with Br-terminated initiators by the self-assembly method, the cleaned silicon wafers were immersed in a 1 mM toluene solution of 1-bromoprophyltrichlorosilane for 10 minutes and were subsequently washed several times with toluene and dried with N_2.

Gold substrates were prepared by placing the cleaned silicon wafers into a thermal evaporator chamber and coating them with a 5 nm Ti adhesion layer, then subsequently coating then with 50 nm gold under vaccum conditions. For DPN of IgG molecules, the surface of gold substrates was modified with a 16-mercaptohexadecanoic acid (MHA) by soaking the substrates in 10 mM ethanol solutions for 2 hours. The MHA-modified gold substrate was rinsed three times with the ethanol, and dried under N_2. All modified substrates were used within 3 hours of preparation.

Fabrication of Nanoporous Tip

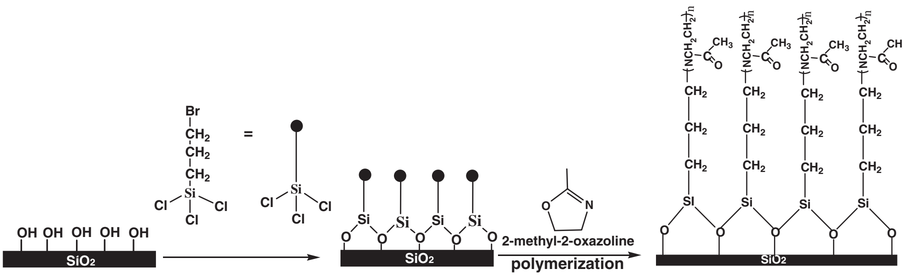
Silicon probes (M2N, Inc., Korea, spring constant = 40N/m, model = STP4(30)) were cleaned and modified using the same procedure for silicon wafer substrates as mentioned above. Nano-porous polyoxazoline was fabricated by ring opening polymerization of 2-methyl-2-oxazoline on the Br-functionalized silicon tip surface. The polyoxazoline-coated stamp tip was then rinsed several times with deionized water and dried with N_2.

Nanopatterns Using the Polyoxazoline-Coated Tip

A nano-porous polyoxazoline-coated AFM tip was soaked in a 500 μg/ml protein solution for 5 seconds and blown dry with nitrogen gas prior to use. All patterning experiments described herein were conducted with the XE-100 AFM system (PSIA, Inc., Suwon, Korea) under ambient conditions at 10–30% RH and room temperature.

Characterization

All topographic images of nano-porous polyoxazoline on the silicon oxide surface and IgG nanopatterns were obtained with the XE-100 AFM system (PSIA, Inc., Suwon, Korea) with conventional Si cantilevers (M2N, Inc., Korea, spring constant = 40N/m, model = STP4(30)) in the tapping mode. The morphological structures of the



Scheme 1.
Process of surface polymerization of polyoxazoline.

nano-porous polyoxazoline on the solid substrate and the polymer-coated tip were obtained by scanning electron microscopy (SEM, JSM-6700, 15-30 kV).

Results and Discussion

As control experiments, the polymerization of 2-methyl-2-oxazoline monomers was conducted on the silicon wafer surface (Scheme 1). Self-assembled monolayers of 1-bromoprophyltrichlorosilane as a surface initiating agent could be formed on the piranha-treated silicon surface via silanization reaction. A Br-functionalized silicon wafer was then placed on the top of the reactor facing down, and the monomer was vaporized slowly at 100 °C. This gas phase polymerization is a very important process in constructing nano-porous polyoxazoline structures on the surface. We could not obtain rod-like nanostructures of polyoxazoline with a solution polymerization method. Figure 1 shows scanning electron microscopy (SEM) images of the resulting polyoxazoline polymers with different reaction times. Polyoxazoline nanostructures were gradually grown on the surface with the increasing of the reaction time

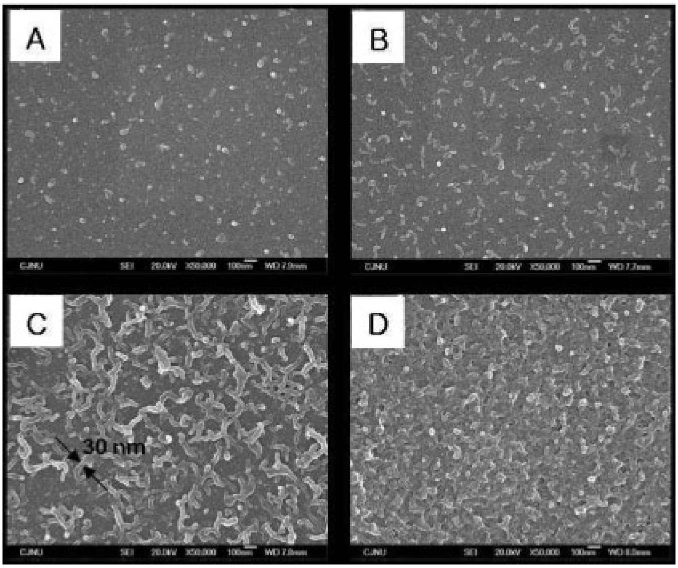


Figure 1.
SEM images of polyoxazoline on the silicon surface. Reaction time: A, 30 sec; B, 1 min; C, 10 min; D, 3 hrs.

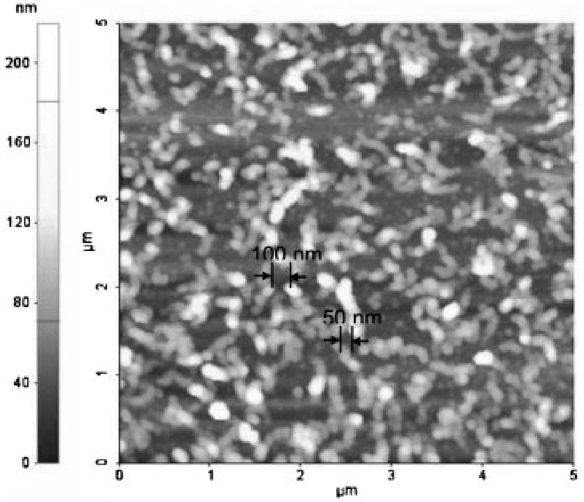


Figure 2.
AFM image of rod-type polyoxazoline nanostructures on the silicon surface. Reaction time: 10 min.

(Figure a, b, c), and it covered the whole surface after 3 hours (Figure D). The growth of rod-type polymer nanostructures is presumably due to the inhomogeneous feeding of monomers into the active sites in the gas phase. Another reason seems to be steric hindrance. Once polymers start to grow at a site, the growing rod-type nanostructures could sterically inhibit the initiation reaction of polymerization around itself. The AFM topographic image was also measured for the sample with a reaction time of 10 minutes. As shown in the SEM and AFM images, the diameter of the polymer rod is approximately 30 nm, and there are a lot of nano-pores sizing from 50 nm to 100 nm on the silicon surface. Note that this is enough size to absorb large-molecular-weight biomolecules (for example, IgG = 10 nm)[6] in their pores. In addition, hydrophilic polyoxazoline can hydrate biomolecules so as to retain their biological activities. In order to fabricate a nano-porous polyoxazoline-coated stamp tip, the same experimental procedure mentioned above was carried out on the tip surface.

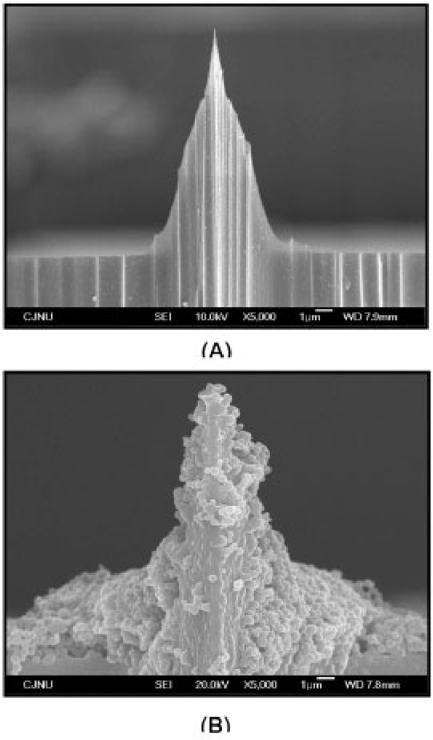


Figure 3.
Typical SEM images of a stamp tip: A) Bare silicon tip, B) Nano-porous polyoxazoline-coated stamp tip.

A typical SEM image of the fabricated nano-porous polyoxazoline-coated stamp tip is shown in Figure 3(b). The results

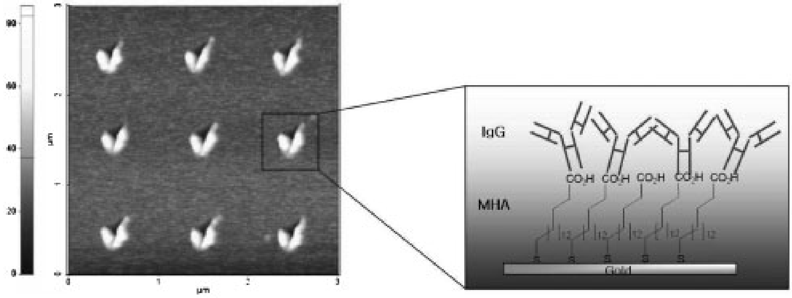


Figure 4.
AFM topography image of DPN-generated rabbit IgG protein nanostructures on MHA modified gold surfaces. The contact time was 0.5 seconds for each dot.

indicate that the tip radius is a little larger than that of the normal silicon tip (Figure 3(a)) due to the polyoxazoline coating layer. In addition, the end of the tip can be fabricated with different shapes and geometries, with the result that one can even generate different shapes of nanopatterns.

Proof-of-concept is demonstrated with the rabbit IgG proteins. Figure 2 shows an AFM topographic image of IgG nanostructures on 16-mercaptohexadecanoic acid (MHA)-modified gold surfaces generated by a conventional DPN method with a nano-porous stamp tip. An array consisting of 9 dots with a diameter of 250 nm and a spacing of 1 μm was constructed from IgG proteins. It is very important that the array was fabricated in less than 3 seconds with a holding time of 0.5 seconds for each dot. This is 60 times faster than the conventional DPN method with a silicon tip.[7] Since the stamp tip was modified with hydrophilic nano-porous polyoxazoline, the sponge-like stamp tip can easily absorb water-based biomolecules in their porous matrix. Therefore, it can easily generate nanopatterns of IgG molecules when the tip makes contact with the solid surface. Interestingly, all the dots exhibit uniform V-shaped features, which is presumably transcribed from the end of the polymer-coated tip. Furthermore, the size of dot is independant of the contact time. These results indicate that the transport mechanism is dominated by physical stamping rather than by diffusion (the driving force of DPN).

Conclusions

We give an account of the fabrication of a nano-porous polyoxazoline-coated tip for stamping-type DPN applications. As proof-of-concept, we demonstrated that an IgG nanoarray can be constructed on the MHA-modified gold surface. Significantly, this approach can generate protein nanostructures 60 times faster than what one can obtain with the conventional silicon tip.

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