Fabrication of Silicon Dioxide Nano Array for Bio-mimicking of Molecular Interactions

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ABSTRACT

We employed atomic force microscopy (AFM) with bias control to fabricate oxided nanopatterns on silicon surface with feature size down to 50nm. The relationship of silicon dioxide nanopatterns against humidity was studied and then the optimal parameter was used to make oxide nanoarry for interaction of biotin and streptavidin. The scanning function of AFM was utilized to verify the different height of biomolecules. According to our experimental results, using nano biochip of silicon dioxide can decrease the monitoring scale to nanometer and can be the nano-platform for monitoring the behavior of biomolecular interaction. We anticipate mimicking the correlation of single molecular behavior and an array of biomolecular behavior to understand the coincidence of them.

Keywords: electrochemical atomic force microscopy, nano biochip, silicon dioxide nanopatterns

1. INTRODUCTION

Biomolecular interactions play important role in the physiological and pathological phenomena at molecular level. It is rather important to develop new tools for biological studies to gain further understandings based on both qualitative and quantitative data [1-2]. This has resulted in an active research topic, biomimetic, for imitating nature processes at different levels of substances, devices, or even systems [3]. The studies of conformation changes of myosin-actin interactions and the gating of ionic channel across cellular membrane have resulted in the creation of various forms of molecular motors or molecular switches for potential applications in electronics and medicine [4-5]. However, the nature of molecular interaction requires measurement tools with spatial and temporal resolution across micro and macro scale to fully elucidate its mechanism. For example, patch clamp method has been successfully used to measure the electrical signals caused by the state transition of single ionic channel on the cellular membrane for understanding the stochastic behavior of biomolecular interaction [6]. In principle, such a method is restricted in spatial resolution due to the dimension of mechanical fabrication of micropipette and number of electrodes for statistical treatment. It would be rather interesting to develop a new method which is capable of large area measurement for group behavior with statistical meaning and yet has high temporal and spatial resolution for the addressing of each individual molecule.

Recent advancements in nano-optics and nanotechnologies have enabled novel approaches for possible realizations of above mentioned biomimicking devices. For examples, total internal reflection fluorescence (TIRF) can be utilized to get signals ranging within several hundred nanometers of specimen, and the specific labeling fluorophores that are excited by evanescent wave can enhance the interacting those signals. Fluorescence resonance energy transfer (FRET) and fluorescence quenching can apply to analyze the change of molecular conformation at the scale of below 100nm, so they can become high sensitive biomolecular probing tools [7-8]. Scanning near-field optical microscopy (SNOM) is also one of noticeable field to subcellular studying due to its outstanding spatial resolution without the limit of light diffraction. It is possible to detect the configuration of subcellular features in situ and in vivo [9-10]. Depended on these highly developed techniques, studying biomolecular behaviors, especially for single molecular detection, have been not

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any more difficult to be proceeded.

Accompanied with the development of nano biotechnology, many newly probing materials, such as quantum dots and metal nanoparticle, have been proposed to explain the model of single molecular interaction, for instance, protein and protein interaction. Many kinds of nanofabrication techniques have been applied not only to produce nanosensors to investigate the biomolecular interaction but also to fabricate biosystem to mimic their behaviors [11-13]. Atomic force microscopy (AFM) is a very critical tool to image and to fabricate the nanofeatures. Excepting the measuring resolution of atomic level, AFM has excellent advantages for bio-specimen because it can operate in the air and liquid environment and can break through the limitation of transparent electron microscopy (TEM), where the specimen must be dry in vacuum. The ion channels on the plasma membrane have been imaged by Muller [14], and the conformational changes of gap junction stimulated by Ca^{2+} ion have been identified by AFM scanning. Many groups also have devoted to use the AFM as a tool to assess the interacting force between biomolecules by attaching them on one side of AFM tip and the other side fixed on the substrate for the purpose of understanding the nanomechanics of interaction force of single molecule [15].

Scanning probe lithography (SPL) includes two operating types, scanning tunneling microscopy (STM) and atomic force microscopy (AFM), and it is also one of high-resolution nanopatterning methods, which is used a sharp tip in the end of cantilever to pattern the feature at nanometer scale directly [16]. Many techniques of SPL have been explored for patterning purpose, such electric-field enhanced oxidation, resist exposure using field emitted electrons and dip pen nanolithography. The electric-field enhanced oxidation can produce a thin oxide layer on the surface of metal and silicon by SPL, and the oxide layer can be part of electrical nanodevice, for example, nano size field effect transistor [17]. In the field to biological application, the silicon dioxide (SiO_2) based biochips have been applied to many fields related to biochemistry and immunochemistry. R. Danczyk in 2003 described the detail procedure of immobilization on the glass surface of silicon dioxide [18]. Therefore, the oxide nanopatterns of silicon surface using SPL can be utilized directly for the application of nano biochip. Moreover, the roughness of silicon wafer is ultra-flat due to nature crystallization, so it can help reduce the background interference to evaluate the performance of biochip.

In this studying, we demonstrated the fabrication of the nano biochip assembled by nanodot array with diameter of 50 nm which was used electrochemical atomic force microscopy (EC-AFM) to form a thin layer on the silicon surface. Then the biomolecule, biotin, was immobilized on the surface of oxide layer to be as the platform for monitoring biomolecular interaction. In this way, the observing scale can be down below 100 nm and interaction can be happened without any disturbance of artificial design.

However, more and more Micro-Electro-Mechanical Systems (MEMS) and Nano-Electro-Mechanical System (NEMS) techniques have been implanted to apply to this field to explore those phenomena happen at the scale of nanometer, which has never been studied before. Hence, the single molecule detection has been possible, and the dynamics of single molecular interaction also can be expected depended on the bio-mimicking system made by MEMS/ NEMS. Therefore, some of theoretical models based on the experimental records by these tools, especially for those records which are the interacting signals of group molecules, are essential to be identified.

2. MATERIALS AND METHODS

2.1 Preparation of substrate of nano biochip

The N-type silicon wafer (Swiftek Corp., Taiwan) was cut into small pieces with area of $1cm^2$ and then proceeded by the sonicated cleaner (Branson, U.S.A.) for 10 minutes soaked in ethanol solution (above 95%). The buffered oxide etchant (BOE) was used to remove the oxide layer of silicon surface. After washing by DI water and drying by nitrogen gas, these small chips were sealed by containers individually to block air before nanofabrication. When the silicon surface is exposed in the air, the performance of oxide pattern will be bad with the time goes by due to the generation of oxide layer.

2.2 Factor of humidity to affect nanopatterns

For the purpose to investigate the effect of humidity to nanopattern size, a set of default linear nanopattern with the width of 100nm, 200nm, 300nm, and 400nm was designed. Under constant bias, 30V, and constant temperature, 25°C, the related humidity was controlled by environmental Chamber (NanoInk, Il, U.S.A.) and was increased with the range from 30% to 60%. In the constant value of humidity, nanopatterns were made with the invariable speed to explore the relationship of humidity against the dimension of nanopatterns.

2.3 Preparation of nano biochip

All the following chemicals were purchased from Sigma Chemical Co. 3-Mercaptopropyltrimethoxysilan (MPS) (1% in ethanol) and a heterobifunctions crosslinker, GMBS (0.25mM in ethanol), were used to amine-modified surface of silicon dioxide on nano biochip. The bond of carboxylic group (COOH) was active by 1-Ethyl-3- (3-Dimethylaminopropyl)carbodiimide (EDC) (0.1M in water). The concentration of biotin (10mM, Pierce, Il, U.S.A) and streptavidin (0.14mM, Invitrogen Co, Cal, U.S.A) were diluted by phosphate buffered saline (PBS) buffer solution.

2.4 Nanopatterning using electrochemical atomic force microscopy (EC-AFM)

The AFM probe (Nanosensor®, U.S.A) is also N-doped for electrochemical experiment and be operated in contact mode with a spring constant of 0.19N/m. After the nanolithography was finished, AFM scanning images were obtained using Nscriptor™ (NanoInk, U.S.A). The design of oxide nanopatterns were realized by the software NSCRIPTOR™ (NanoInk, U.S.A), and all AFM images of the nanopattern were obtained by lateral force microscope (LFM) with scanning rate of 2Hz. The AFM scanning of nano biochip was carried out by the tapping mode probe, whose spring constant is 40 N/m and resonance frequency is 300kHz, to avoid causing damage of biomolecules on the chip. The analysis software, NanoRule (Pacific Nanotechnology Inc., U.S.A), was used to estimate the pattern size in the AFM images. The diameter of dot pattern is the full width at half maximum (FWHM) of line profile of AFM image, and the line profile is presented the image intensity caused by the patterns.

3. RESULTS AND DISCUSSIONS

The oxidation function of EC-AFM is depended on the electrochemical reaction occurred within the nanocell of water meniscus, which is forming by the humidity in the air while the AFM probe contact with substrate that is metal or silicon (Fig. 1(a)). By adding a bias, which is larger than the binding energy of outer electron of atom in the substrate, between probe and substrate, the electron will be out of atomic orbit to create a thin oxide layer of substrate. The oxide pattern will be produced via moving the probe. Fig. 1(b) shows the AFM images resulted by reverse and forward biases respectively. Only the forward bias, which would cause the reduction on the side of AFM probe and oxidation on the side of substrate, can have nanopatterns on the substrate surface. According to general the electrochemical theory, the AFM probe is anode and the substrate is cathode. When the bias is correct direction, the oxide pattern can be produced obviously (shown in the Fig. 1 (b)). According to this result, forming oxide pattern was proven by the principle of electrochemical reaction.

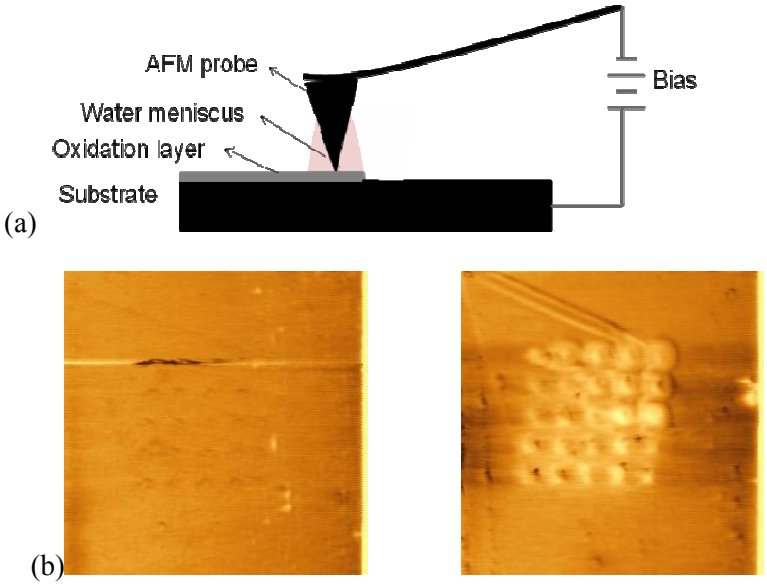
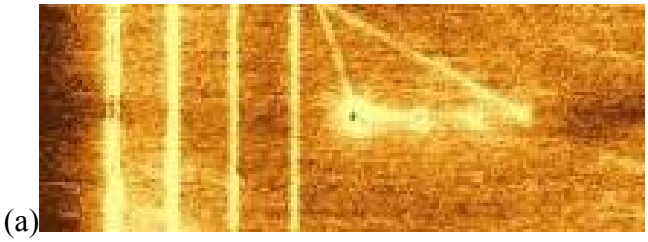
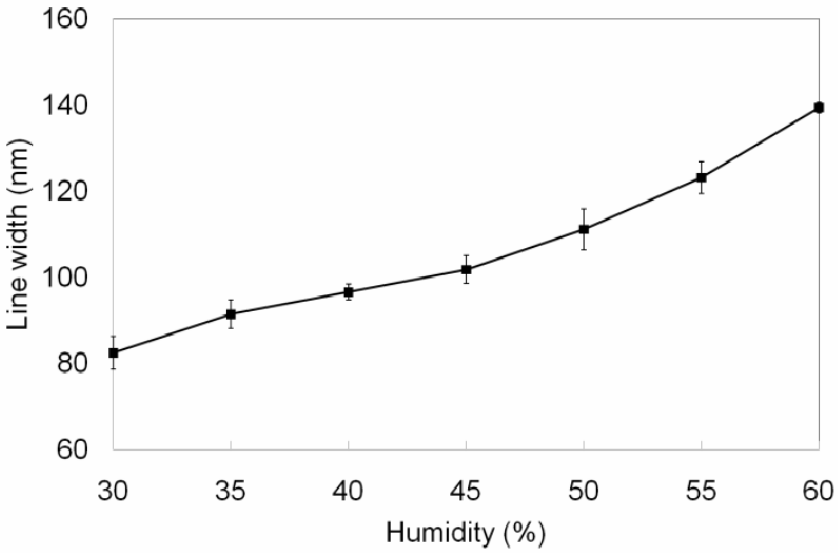


Fig. 1. (a) Schematic of EC-AFM. Applying a bias between the AFM probe and substrate causes a thin layer of oxidation of substrate. The substrate can be metal or doped silicon wafer. The water meniscus is the nanocell for electrochemical reaction. The arrow indicates the moving direction; (b) the patterns in the left image is caused by positive bias for AFM probe as cathode and substrate as anode, whereas the right side image there is no obvious patterns showing while the bias is opposite.

The water meniscus is the zone where the reaction of oxidation- reduction takes place and the dimension of this water meniscus controlled by the amount of humidity, which is critical to affect the size of oxide patterns. It is essential to evaluate the effect of humidity for deciding the parameter of nanolithography. A set of linear nanopatterns with the widths of 100, 200, 300, and 400nm was designed to evaluate the effect of humidity. As shown in the Fig. 2(a) the oxide nanopatterns were recorded by the later force microscopy (LFM) image. The bias was sustained at 30V, and the designed pattern was repeated three times under the humidity of 30%, 35%, 40%, 45%, 50%, 55%, and 60%. The Fig. 2 (b) demonstrated the positive tendency of the relationship of nanopatterning width versus humidity by evaluating the linear nanopatterns of 100nm. According to this result, the lower humidity leads to make better spatial resolution of nanopatterns. Moreover, at the humidity ranging from 35% to 40%, the actual line width of nanopattern is approached to the defaulted size. As a result, in the following nanopatterning fabrication, the environmental humidity was maintained at 35%.



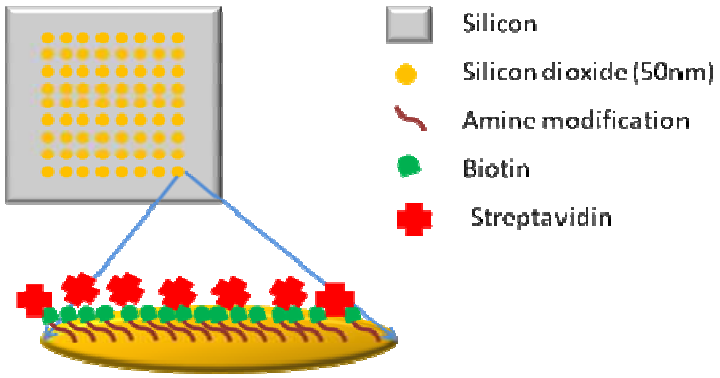
(a)



(b)

Fig. 2. (a) A set of nanopatterns was designed for evaluating the effect of humidity, and this AFM image was done with humidity of 50% ; (b) width of linear pattern as a function of humidity under the applied bias, 30V. Humidity increases with an interval humidity of 5% from 30% to 60%.

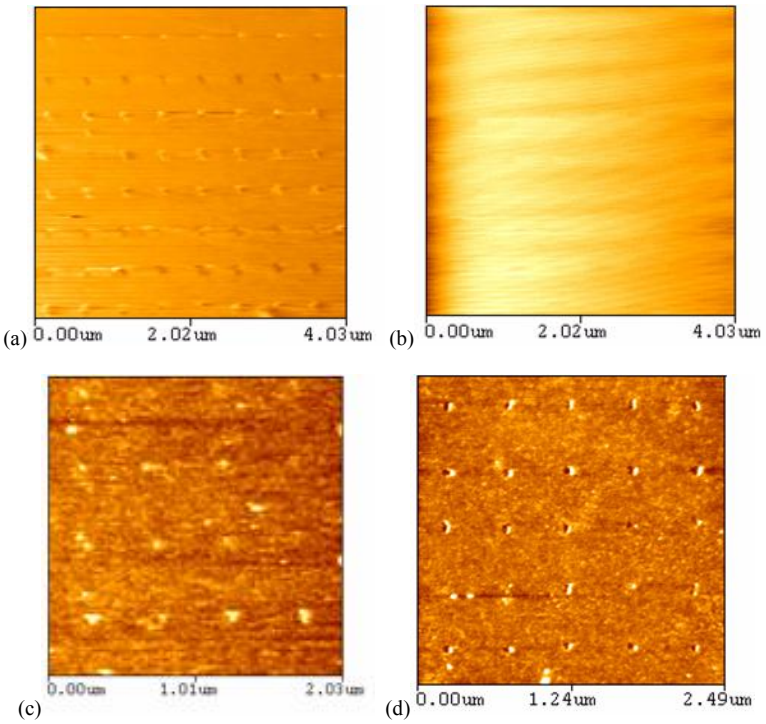
The nanodot array of 70 by 70μm^2 with pitch of 0.5μm was designed for nanopatterns of biochip, and it was served as the acting site for biomolecular interaction. The dwell time which is the contacting time of probe to substrate was 0.1s. As shown in Scheme 1, the nanopatterns, which can be as the zone for inaction with streptavidin, were created with diameter of 50 nm, and the surface was preprocessed by amine-modification in order to immobilize with biotin. The height difference of nanopattern, which was recorded by the scanning function of AFM (in Fig. 3), can be used to represent the results with and without biomolecular immobilization. The condition of biomolecular inaction, which is limited in the scale of nanometer, can be observed by optical microscopy by labeling dyes or quantum dot in the advanced applications.

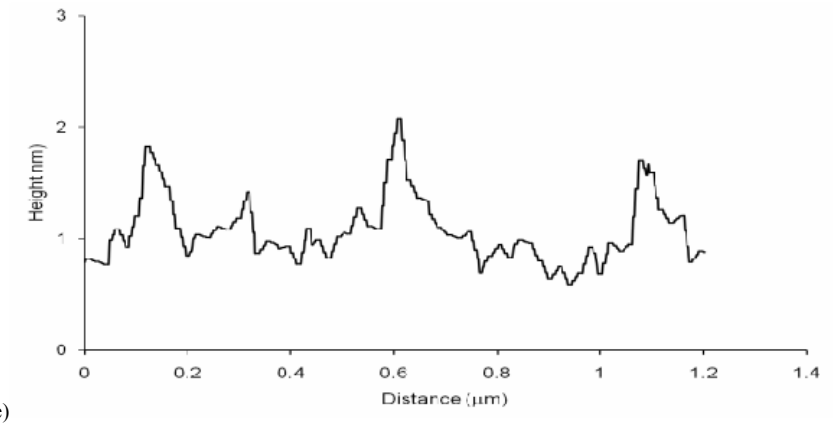


Scheme. 1. Scheme of nano biochip.

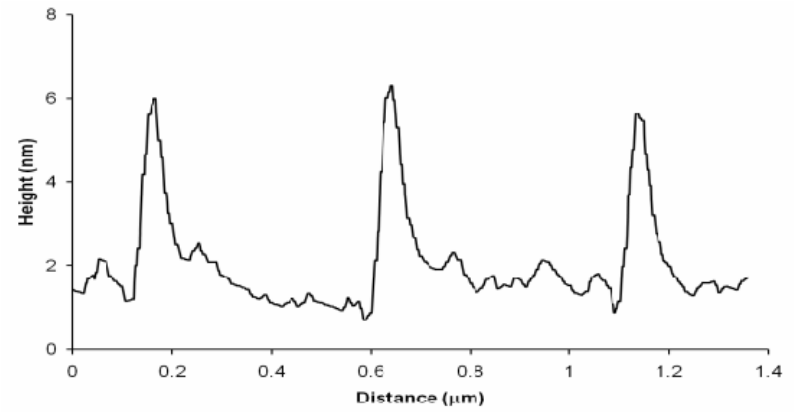
After nanolithography of EC-AFM, the bias was turned off after nanofabrication, and the pattern was scanned by original probe. Fig. 3 (a) and (b) are the LFM image and topography respectively, and the diameter of dot is about 50 to 60 nm. Because the silicon dioxide layer is very thin, the oxide nanopatterns can't be presented in the topography. However, LFM image is caused by the twisting deformation of probe while doing scanning and is more sensitive to those samples with less change of height difference but with various assembled materials. The nanodot array in this studying is silicon dioxide and the substrate is silicon. Therefore, oxide pattern can be identified by LFM image but cannot be shown in topography because they are very thin.

After interaction of biotin and streptavidin, the surface of nano biochip was scanning by tapping mode AFM in order not to do damage for biomolecule. Fig. 3(c) is the topography of only binding biotin and Fig. 3(e) is the corresponding line profile by choosing arbitrary three points of Fig 3(c). The height of nano biochip against background is 1nm. Fig. 3(d) is the topography of binding biotin and streptavidin, and Fig. 3(f) is the corresponding line profile by choosing arbitrary three points in Fig. 3(d). The height of nano biochip against background is 4 to 5nm, and the theoretic dimension of streptavidin is about 4.5nm. Therefore, the height change in Fig. 3(e) and (f) can be assumed as the result of molecular binding.

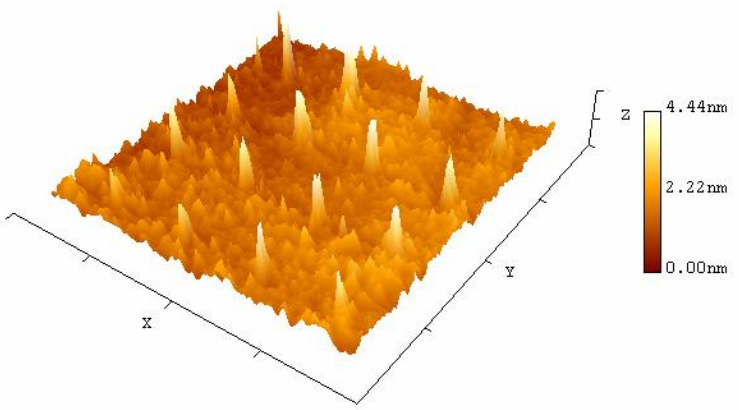




(e)



(f)



(g)

Fig. 3. (a) LFM image of oxide nanodot array; (b) topography of oxide nano-dot array; (c) topography of nano biochip with immobilization of biotin; (d) topography of nano biochip with streptavidin and biotin; (e) the corresponding line profile of (c); (f) the corresponding of line profile of (d); (g) three dimension of AFM image of streptavidin and biotin.

According to the above experimental results, the operating condition of nanopattern by EC-AFM has been optimum and the biomolecules also have been labeled successfully on the nano biochip, which can be used to identify the height difference of molecules without labeling any fluorophores on biomolecule. Based on this fundament, it can be

anticipated that this nano biochip can provide more exact interacting signals at the scale of nanometer for analyzing the stochastic behavior of biomolecular interaction in the following experiment.

4. CONCLUSION

We employed atomic force microscopy (AFM) with bias control to fabricate oxided nanopatterns on silicon surface with feature size down to 50nm. The relationship of silicon dioxide nanopatterns against humidity was studied and then the optimal parameter was used to make oxide nanoarry for interaction of biotin and streptavidin. The scanning function of AFM was utilized to verify the different height of biomolecules. According to our experimental results, using nano biochip of silicon dioxide can decrease the monitoring scale to nanometer and can be the nano-platform for monitoring the behavior of biomolecular interaction. We anticipate mimicking the correlation of single molecular behavior and an array of biomolecular behavior to understand the coincidence of them.

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