Electrochemical DNA Biosensor with Nanometer Scale Using Nano-Patterning Lithography Machine

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Major challenges in the field of electrochemical DNA hybridization biosensors are the immobilization of DNA and the detection of hybridization signals. The method of DNA immobilization using the nano-patterning machine and detection for DNA hybridization signals has been proposed. Here, two gold electrodes were deposited on SiO_2 layer and the gap between the electrodes was fabricated by electron beam lithography. 3-aminopropyltriethoxysilane (APTES) solution was selectively treated to immobilize the amino-modified oligonucleotides onto the SiO_2 layer between the electrodes. The recognition of DNA hybridization was accomplished by metallic aggregation of nano-particles. The results showed that DNA is immobilized with nanometer scales and the method for detecting hybridization signals is useful. The experimental results were verified by I-V curves. The conductance between two electrodes changed with the density of the Au-nanoparticles immobilized onto the oxide layer. These results can be applied to the DNA chip and the multi-functional sensors which will be researched in the further study.

Key Words : Electrochemical, DNA Biosensor, Nano-patterning, Nanometer Scale

1. Introduction

Since the junction transistor was invented in 1947, the field of microelectronics has developed substantially. The integrated circuits and devices have been invented subsequently and their growth rates have exponentially increased due to micro-electromechanical systems (MEMS) technology and nanotechnology. The minimum feature size has decreased from 2 um in 1980 and 0.18 um in 1999 to 0.1 um in 2003 in volume production according to the roadmap projected by the SIA (Semiconductor Industry Association). It is, however, becoming increasingly difficult to keep downscaling due to physical limitations such as wavelengths of radiation used for lithography, interconnect schemes, etc. Especially, in the field of the semiconductor industry, the dominant technology over the last 30 years in high resolution lithography has been optical lithography. Many engineers and scientists have been recently trying to fabricate sub 0.1um patterns and coming up with several alternatives to optical lithography (Lawes, 2000). There are some next generation lithographic techniques including electron beam lithography (Driskill-Smith et al., 2004), x-ray lithography (Henry et al., 2000), ion beam lithography (Hirscher et al., 2002), extreme ultraviolet (EUV) lithography (Fay, 2002), immersion lithography (Wei et al., 2004), nanoimprint lithography (Stephen et al., 1996 ; Tan et al., 1998), etc.

Electron beam lithography is a general method to create mask patterns and offers high resolution due to the small wavelength of electrons (<0.1 nm for 10~50 keV electrons). The resolution of

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an electron lithographic system is not limited by diffraction, but rather by electron scattering in the resist and by the various aberrations of the electron optics. Although the biggest disadvantage of electron beam lithography is the low throughput and it is not suitable for mass production, it offers high resolution of sub 0.1 um.

Meanwhile, another topic of interest concerning nanotechnology is DNA. While researchers have been aspiring to fabricate or manipulate structures at the micrometer and nanometer scale, nature has been performing these tasks and assembling structures accurately and efficiently using biological molecules such as DNA and proteins. Although the electronic properties of DNA remain controversial (Endres et al., 2004; Jung et al., 2004), DNA has the potential as an important tool in nanotechnology and nanometer scale electronics. A major reason for that is DNA's use, both as mediator for fabricating nanostructures and as a template for assembling nanometer scale circuits in nanoelectronic devices (Bashir, 2001; Braun et al., 1998).

On the other hand, these DNA-mediated fabrication technologies would have conversely a great impact on DNA chips and DNA biosensors. Park et al. have reported an electrochemical method for detecting DNA hybridization with Au-nanoparticle tagged DNA probes (Park et al., 2002). A basic electrochemical DNA biosensor is designed by the immobilization of a single-stranded oligonucleotide (probe) on a transducer surface to recognize its complementary DNA sequence via hybridization. Major challenges in the field of electrochemical DNA hybridization biosensors are the immobilization of DNA and the detection of hybridization signals. There have been a number of methods to immobilize DNA probes onto glass. Recent studies show several methods to immobilize DNA probes by covalent bond onto silicon dioxide (SiO_2), which needs for thorough pretreatment (Lobert et al., 2003; Zhang et al., 2004). As a method of detecting hybridization signals, the labels such as antiancer agents, organic dyes, metal complexes, enzymes or metal nanoparticles can be electrochemically used (Kerman et al., 2004). Electrochemical methods have a great advantage compared to the other existing measurement systems due to rapid, simple and low-cost on-site detection.

In this work, we fabricate an electrochemical DNA biosensor with two Au-electrodes using electron beam lithography and immobilize DNA probes onto the gap between the electrodes through covalent bond formation. After hybridizing with Au-nanoparticle tagged complementary DNA targets, the conductance between the electrodes is discussed according to the density of the Au-nanoparticles.

2. Materials and Methods

All the oligonucleotides with functional groups were purchased from GenoTech Corp. (Daejeon, Korea). Four types of oligonucleotides were used in this experiment:

H_2N-5′-CCACGGACTACTTCAAAACTA-3′-SH
(probe for immobilization test)
H_2N-5′-CCACGGACTACTTCAAAACTA-3′-SH
(complementary probe)
H_2N-5′-ATCGATCGATCGATCGATCGA-3′-SH
(non-complementary probe)
SH-5′-TAGTTTTGAAGTAGTCCGTGG-3′
(target)

Thiol groups of oligonucleotides bind covalently with Au-nanoparticles (13 nm in diameter) and amino groups of oligonucleotides with the aldehyde-modified surface of the wafer, respectively. The oligonucleotides with amino groups at 5′ and thiol groups at 3′ were used for immobilizing the oligonucleotides onto the aldehyde surface of the wafer after attaching the oligonucleotides to Au-nanoparticles. APTES (3-Aminopropyltriethoxysilane) and glutaraldehyde solution were used for modifying the silicon oxide into the aldehyde surface. The complementary and non-complementary amino-modified at 5′ to be immobilized onto the surface were used for hybridizing with the oligonucleotides thiol-modified at 5′.

APTES and glutaraldehyde solution were purchased from Sigma-Aldrich. Zep series (Zeon

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Corporation, Japan) were used for electron beam lithography and Korea Research Institute of Standards and Science provided Au-nanoparticles. P-type Si (100) wafers were used.

2.1 Formation of gold electrodes

The P-type Si (100) wafers were grown in an oxidation furnace at 1000℃ for about 2 h with dried O_2. The thickness of SiO_2 layer measured by Nanocal was 100 nm. The first process was to make a marker for electron beam lithography. The substrate grown silicon oxide layer, was coated at 500 rpm for 3 s and 2500 rpm for 25 s by using a spin coater after 2 ml of PR (DNR-L300) was dropped. The wafer was soft baked at 100℃ for 60 s and exposed for 9 s at 50 um gap by soft contact. This wafer was post baked at 110℃ for 90 s and developed in the solution MIF-300 for 11 s. The rinsed wafer was hard baked at 110℃ for 2 min. The 5 nm Cr layer was deposited on the wafer, Au 100 nm by DC sputtering and PR was removed in acetone. The second process was to fabricate the line patterns whose line width is 50 nm and 100 nm respectively. The patterns fabricated by Leica EBPG-4HR electron beam lithography system. After the patterning the wafer was developed in ZEP-N50 for 100 s. The third process was to etch the metal layer (Cr/Au) by MICP etcher in the solution of gases. The gas solution is Ar 40 sccm, Cl2 20 sccm, CF4 20 sccm, pressed in 5 mTorr. The power was applied RF 500W, bias 60W and then the resist was removed in acetone. The figures show these fabrication processes.

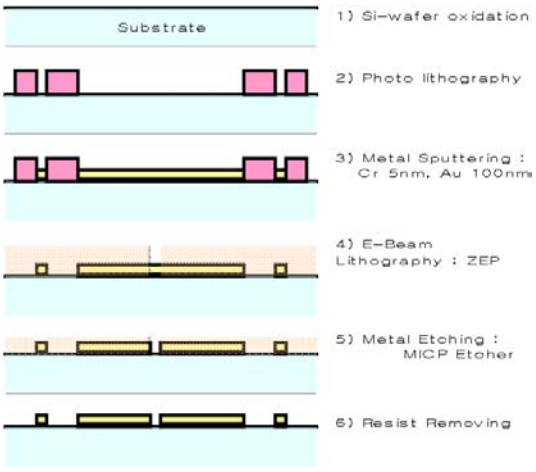

Fig. 1 Fabrication process of gold electrodes

2.2 Immobilization of Au-nanoparticles tagged DNA onto oxide layer

After washing with acetone by ultrasonic for 1 min, the patterned specimens were activated by the O_2 Plasma treatment for 30 s. After washing with deionized (DI) water, the specimens were immersed in the solution of 2% APTES in acetone for 1 h to generate amino group. After washing with acetone by ultrasonic for 1 min twice, the specimens were treated with the solution of 1.25% glutaraldehyde in DI water for 1 h. Then, the specimens were washed with DI water and dried. The solution 2 μl of Au-nanoparticles tagged DNA was dropped onto the patterned specimens. The specimens were washed and dried after 1 h.

3. Results and Discussion

All images were obtained by Field Emission Scanning Electron Microscopy (FE-SEM). As shown in Fig. 2, there is a silicon oxide layer between the two Au electrodes. The electrodes were electrically insulated by the oxide layer, but the adhesive force between the Cr layer and the Au layer was not strong enough. It was shown that some areas of Au electrodes were apart from

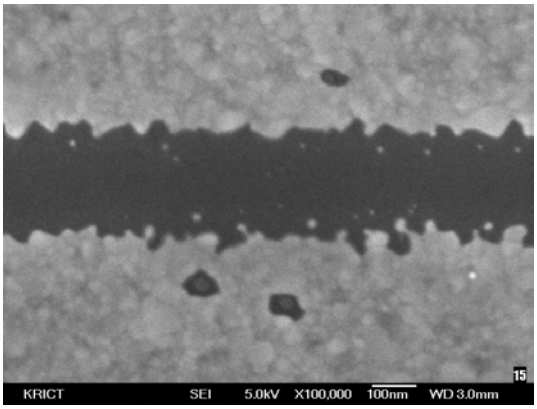

Fig. 2 Au-electrodes

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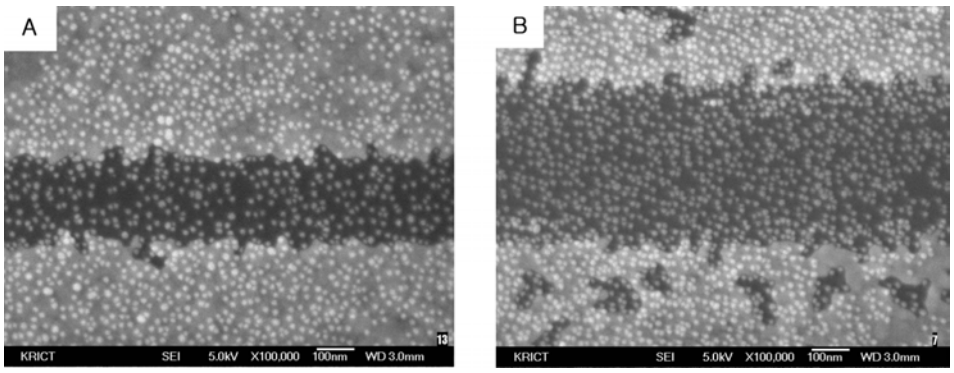

Fig. 3 Immobilization of Au-nanoparticles tagged DNA onto oxide layer. (A) The concentration of Au-DNA assembly solution is 20 μM. (B) The concentration of Au-DNA assembly solution is 50 μM.

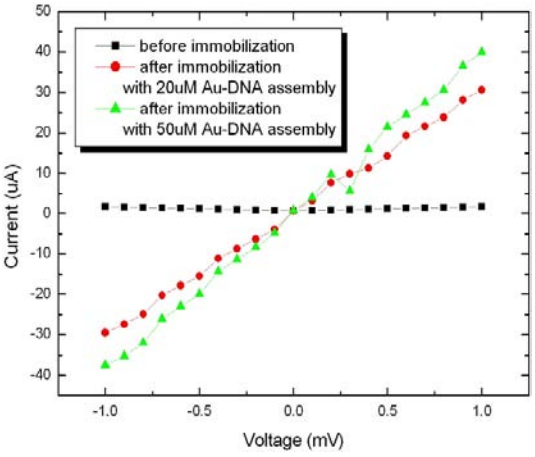

Fig. 4 The conductive values of the electrodes

Cr layer and the edges of the electrodes were eroded. However, these defects had no effect on the experiments.

The conductance between the electrodes changed with the density of Au-nanoparticles immobilized onto the oxide layer. Two types of electrodes were fabricated. One is 50 nm gap distance between the electrodes (A), and the other is 100 nm (B). After pretreatment of APTES and glutaraldehyde solution, a little droplet of 20 μM Au-DNA assembly solution was pipetted onto the electrode A and a little droplet of 50 μM Au-DNA assembly solution onto the electrode B. The results were shown in Fig. 3.

The density of Au-nanoparticles immoblilized onto the oxide layer affects the conductance between the electrodes. There are many elements to enhance the density including oxidation methods, the concentration of the immobilizing solution and the density of Au-DNA assembly solution, etc. Our works were discussed in terms of the density of Au-DNA assembly solution. The conductance between the electrodes was measured by HP4156A. The measured values were shown in Fig. 4. The conductive value of Fig. 3(A) was $3.0\times10^{-2}[\Omega^{-1}]$ and that of Fig. 3(B) was $4.0\times10^{-2}[\Omega^{-1}]$.

4. Conclusion

The fabrication of micrometer and nanometer structures was processed by electron beam lithography for making Au electrodes of electrochemical DNA biosensors. Nanometer scale electrodes have the advantages of the reduction of non-specific reaction, the miniaturization of sensors, and the enhancement of the sensitivity. APTES and glutaraldehyde solution was treated to immobilize Au-DNA assembly onto the surface. These two solutions make Au-DNA assembly to bind covalently onto the surface. The conductance between the electrodes changed with the density of the Au-nanoparticles immobilized onto the oxide layer. The results were meaningful to figure out the electrical characteristic of electrochemical DNA biosensors using Au-DNA assembly. Approaches to increase the density of immobilized Au-nanoparticles and decrease the gap distance between the electrodes will be done in the future study.

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