Bio-Molecules Immobilization on Nanowire Electronic Devices

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ABSTRACT

Nano-scale Field Effect Transistor based devices have shown great promise in bio-molecular detection. However, immobilization of bio-molecules on nano-scaled devices is a challenge. We investigated methods for immobilizing oligonucleotide and peptide nucleic acid on nanowire devices. Oligonucleotide and peptide nucleic acid were covalently linked on the nanowire surface which is covered by native silicon dioxide. Standard silanization chemistry combined with a bi-functional linker approach was used in the immobilization process. Using this approach, negatively charged target nucleic acids were closely attached to the nanowire. Streptavidin-Alkaline phosphatease conjugate was used as indicator to confirm immobilized oligonucleotide on the surface. Biotin labeled oligonucleotides with complimentary sequences was used to report the presence of PNA on the surface.

Keywords: nanowire transistor, surface cleaning, oligonucleotide, peptide nucleic acid, immobilization.

1 INTRODUCTION

Nanowire field effect transistors (nano-FET) have been developed and investigated in bio-detection for the last decade [1, 2, 3, 4 and 5]. It has shown promises for improving biosensor characteristics such as sensitivity, simplicity and economic viability. Compared with traditional bio-detection techniques such as optical spectroscopy, mass spectroscopy or immune precipitation, advantages of electronic nano-FET bio-detection are: direct detection without labeling, ultra-sensitivity without amplification and simple device implementation without the requirement of expensive equipment.

It has been reported that nano-electronic bio-detection achieved much higher detection sensitivity, at least on the order of 10^3, fM verses pM and nM [6,7,8] Our previous work demonstrated electronic detection of methylated DNA, bacterial 16S rRNA and toxins on nano-FET biosensors [9, 10, 11 and 12]. However, there are challenges in the development of nano-FET biosensors. One of these challenges is immobilization of bio-molecules on nano-scaled sensing surface and the distance between captured bio-molecules and nanowire, which is critical in electronic detection. Since the detection mechanism is based on molecular charge on the sensing surface, which affects electronic properties of semiconductor nanowire and generates detectable signal. These issues must be addressed in the development and application of nano-FET biosensors. Investigations and studies of silicon nanowire surface modification have been reported and showed improvement [13, 14, and 15].

Herein, we report the investigation of immobilization oligonucleotide and peptide nucleic acid (PNA) on silicon chips and nanowire device surface. Oligonucleotide and PNA were covalently linked on the nanowire surface which is covered by 1-2 nm native silicon dioxide. To reduce the distance between oligonucleotide and nanowire, a low concentration acetic acid solution was used to wash the silanized surface. Chemiluminescence detection method was used to confirm immobilized oligonucleotide and PNA.

2 METHODS

2.1 Cleaning and Activating Silicon Chips

Slicon chips with a 25nm thin layer of silicon dioxide were used to investigate cleaning and immobilization methods. A set of six nanowire devices was used to demonstrate device surface modification. Two methods were investigated. In method-1, silicon chips were rinsed with ethanol and sonicated in 2% Hellmanex solution for 5 minutes.

After rinsing with ddH_2O chips were sonicated in ddH_2O for another 3 minutes. In method-2, silicon chips were cleaned using Plasma. In both methods, further cleaning was performed in a acid solution of HCl:Meth (1:1) for 30 minutes, rinsed with ddH_2O, and dried in Argon gas.

To activate the silicon dioxide surface, chips were immersed in 5% APTES ethanol solution and silanization at 50°C for 3 hrs, or applied 300 ul APTES on a set of nano-device surfaces in an Argon gas chamber for 1 hr. After rinsing with 1 mM or 1% AcOH and then water, chips or nanowire devices were dried in argon gas and store in desiccators.

2.2 Immobilization of Oligonucleotide and Peptide Nucleic Acid

Oligonucleotide and PNA were immobilized on an activated surface through a bi-functional linker uccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC). This oligonucleotide contains a thio-group at 5'end for covalently linking to the surface, and biotin at 3'end for chemiluminescence detection. To immobilize PNA, a cystein residue was designed at the N-terminal of PNA, which provides thio-group for covalent linkage.

4 mg/ml sulfo-SMCC was prepared in 100mM $NaHCO_2$ buffer, pH 9.0. The chips were soaked in above solution for 1 hr at room temperature (RT), then, washed with ddH_2O and dried in Argon gas. In the modification of nanowire devices, 100ul of sulfo-SMCC solution was applied onto a set of nano-device surface, incubated in a sealed moisture chamber for one hr.

A free SH group is required in both oligonucleotide and PNA for immobilization. A reduction reaction was carried out in the presence of 50mM DTT in 50ul 1uM oligonucleotide for 15 minutes in RT. After reduction, the sample was purified through a Sephadex G-25 spin column to remove DTT molecules. A drop of purified sample was then applied onto a sulfo-SMCC modified surface, and incubated in a sealed moisture chamber overnight. Unbound molecules were washed away with TE buffer and ddH_2O. After drying in Argon gas, these modified chips can be store in dry at 4°C for several months.

Immobilization of oligonucleotide and PNA on a nanowire device was performed using the same method described above.

2.3 Characterization of Immobilized Bio-molecules

The chemiluminescence detection method was used for characterization of immobilized oligo-nucleotide and PNA on the chip surface. To prevent non-specific binding, chips and nanowire devices were blocked using a blocking buffer containing 1mM PEG and 3% BAS for 3 hrs. Strepavidin and Alkaline phosphatease conjugate (SA-AP) was diluted 1:1,000 in a TBS buffer. Chips and devices were incubated in SA-AP solution for 30 minutes at RT. After washing with a TBS buffer (3 x 3minutes), chemiluminescence detection was performed in the presence of substrate AutoGlow 450. Images were taken using a CCD camera.

To determine the presence of PNA on the chip surface, biotin labeled oligonucleotides with complimentary sequences were used as the reporter. Hybridization was performed at 33°C for 3 hrs. Unbound oligonucleotides were washed away. To investigate the specificity of hybridization, three PNAs and three oligonucleotides were used in the tests. Chemiluminescence signal was detected and recorded using CCD camera.

3 RESULTS

3.1 Surface cleaning and activation

To investigate the modification methods, Oligonucleotide and PNA immobilization were first studied on silicon chips with a thin layer of silicon dioxide. Two methods were used and compared for surface cleaning of silicon chips and nanowire devices. In method-1, acid and sonication treatment were used to clean the surface. In method-2, acid treatment combined with plasma cleaning was used. Both methods worked well for surface cleaning of chips. In the device surface cleaning, however, nanowires were damaged in the sonication process using method-1, particularly in the nanowire contact with the gold pads (data not shown). Therefore, plasma cleaning method was used.

The thickness of the salinization layer on the surface is a critical issue in the bio-molecular immobilization on nano-FET biosensors, because the distance between nanowire and molecular charge directly affects detection sensitivity. It is important that molecular charge should be close to sensing surface within 10nm. Therefore, reducing the thickness of the salinization layer on the sensing surface must be addressed. It was reported that using 1mM AcOH to rinse APTES activated surface

reduced the thickness of salinization layer to less than 4nm [15]. The length of a 20 mer oligonucleotide is about 6nm. We applied an additional AcOH washing steps after sialinization reaction which brought the total distance between nanowire and oligonucleotide to 10nm. We found that results were reproducible.

3.2 Bio-molecular immobilization

Immobilization of oligonucleotide on silanized silicon chips was tested using a modified Oligo-dT_{20}. This oligonucleotide contains a thio-group at 5'end for immobilization, and a biotin modified residue at 3'end for chemiluminescence detection. The sequence of the oligonucleotide is 5'-SH-ttttttttttttttttttt- biotin-3'.

Chemiluminescence detection of the presence of biotin labeled oligo-dT_{20} on chip surface is shown in Figure 1.

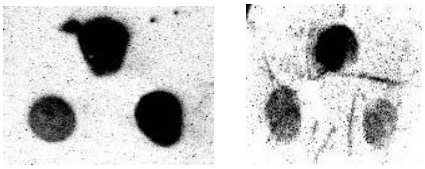

Figure 1 Images of chemiluminescence detection of biotin labeled oligo-dT_{20} on silicon chips.

Alkaline phosphatease activity was detected in the areas of immobilized oligo-dT_{20} on chips. Light signal generated by enzymatic reaction was captured by CCD camera with exposrue time of one minute. Longer exposure time increased the signal. However, significant diffusion was observed in the images.

3.3 Immobilization of multiplex PNA

For the investigation of immobilizing multiplex PNA, three PNA molecules and three biotin labeled oligonucleotides with complimentary sequences were used in the study. The following is the sequences of PNAs and oligonucleotides.

PNA-1 Ac-Cys--TTA-TCT-TCC-TCT-CONH2
PNA-2 Ac-Cys-TTC-ACT-TGT-TAT-CONH2
PNA-3 Ac-Cys-CAC-CAC-TAT-TAT-CONH2

Oligo-1 5'-biotinAAA AGA GGA AGA TAA -3'
Oligo-2 5'- biotinAAA ATA ACA AGT GAA –3'
Oligo-3 5'- biotinAAA ATA ATA GTG GTG -3'

Three PNAs were immobilized on each of four chips. Hybridization was performed as: a) buffer only (top left); b) buffer contains one oligonucleotide (top right); c) buffer contains two oligonucleotides (bottom left); and d) buffer contains three oligonucleotides. Results showed that the presence of all three PNAs on chips, and demonstrated specific hybridization and detection.

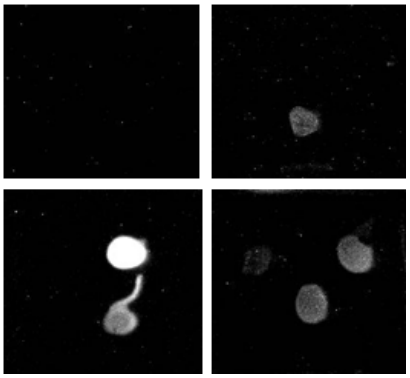

Figure 2 Chemiluminescence detection of PNAs on silicon chips

3.4 Nanowire surface modification

To investigate immobilization of PNA on nanowire devices, a set of six devices (Figure 3 A) was used in the study. The "gap" area of each device is the location of nanowire PNA immobilization was confirmed through hybridization of biotin labeled oligonucleotide which was detected using CCD imaging (Figure 3 B).

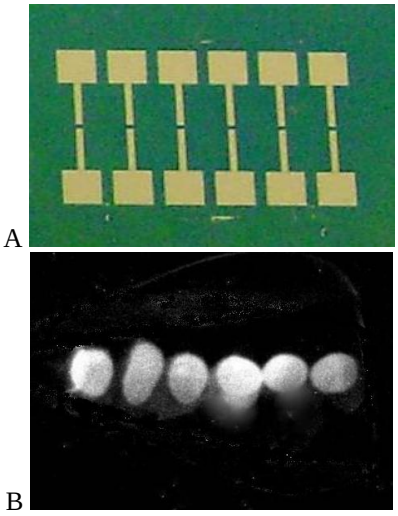

Figure 3 Chemiluminescence detection of PNA immobilized on nano-devices

Alkaline phosphatease activity was detected on six devices as shown in Figure 3B. The image clearly showed six dots. However, the dots are larger than

the actual sensing area. This is because of the exposure time for imaging was set for 1 minute which resulted in light diffusion. Short exposure times, 15, 30 and 45 seconds, were tested. No clear signal was detected due to the time required for enzymatic reaction. The peak of AP reaction is 12 - 15 minutes after applying the substrate.

4 CONCLUSION

Immobilization of oligonucleotide and PNA was investigated in the presented work. Additional AcOH washing step reduces the thickness of salinization layer and brought bio-molecules close to the sensing surface. Chemiluminescence characteriazation of immobilized molecules has shown the presence of oligonucleotide and PNA on silicon chips and nanowire devices. Multiple PNA immobilization and specific hybridization were also demonstrated.

5 ACKNOWLEDGEMENTS

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REFERENCES

1. Stern, E. et al. Label-free immunodetection with CMOS-compatible semiconducting nanowires *Nature* **455**, 519-522 (2007).
2. Anderson, E. P. et al. A system for multiplexed direct electrical detection of DNA synthesis *Sensors and Actuators B: Chemical* **SNB-10185** (2007).
3. Patolsky, F., Zheng, G. and Lieber, C. M. Fabrication of silicon nanowire devices for ultra-sensitive, label free, real time detection of biological and chemical species *Nature Protocol* **1**, 1711-1724 (2006).
4. Hahm, J. and Lieber, C. M. Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors. *Nano Letter* **4,** 51-54 (2004).
5. Uslu, F. Label free fully electronic nucleic acid detection system based on a field-effect transistor device *Biosensors and Bioelectronics* **19**, 1723-1731 (2004).
6. Wang, Z. and Jin G. Silicon surface modification with a mixed silanes layer to immobilize proteins for biosensor with imaging ellipsometry *Colloids and Surfaces B: Biointerfaces* **34**, 173-177 (2004).
7. Barbaro, M. et al. Fully electronic DNA hybridization detection by a standard CMOS biochip *Sensors and Actuators B: Chemical* **118**, 41-46 (2006).
8. Kerman, K. et al. Peptide nucleic acid modified carbon nanotube field effect transistor for ultra-sensitive real-time detection of DNA hybridization *NanoBiotechnology* **1**, 65-70 (2005).
9. N.N Mishra, P. Winterrowd, R. Nelson, S. Rastogi, W.C. Maki and G.K.Maki, " Fabrication and Characterization of Nano-Field Effect Transistor for Bio-safety" (Nano Science and Technology Institute, Nanotech 2007, May 20-24, Santa Clara, CA; ISBN 1420061836 **2,** 465-468 (2007).
10. N. N. Mishra, W. C. Maki, R. Nelson, P. Winterrowd, S. K. Rastogi, E. Cameron, B. Filanoski and G.K. Maki, "Polysilicon Nano-wires Based Nano-device on Silicon Chip: Fabrication and its Application", Nano Science and Technology Institute , NSTI-Nanotech 2008, **2**, 607-610 (2008)
11. Mishra, N., Maki, W., Nelson,R., Winterrowd, P., Rastogi, S., et al. "Ultra-Sensitive Detection of Bacterial Toxin with Silicon Nanowire Transistor" *Lab-on Chip* **8**, 868-871 (2008)
12. B., Rastogi, S. and Maki, G. "Nanowire-transistor based ultra-sensitive DNA methylation detection", *Biosensor & Bioelectronics*, **23**, 780-787 (2008)
13. Man-Fai Ng, Liping Zhou, Shuo-Wang Yang,Li Yun Sim, Vincent B. C. Tan, and Ping Wu Theoretical investigation of silicon nanowires: Methodology, geometry, surface modification, and electrical conductivity using a multiscale approach. *Physical. Review.* 76, 155435 (2007)
14. Diao, J. Ren, D. Engstrom, J. R. and Lee, K. A. Surface modification strategy on silicon nitride for developing biosensors *Analytical Biochemistry* **343**, 322-328 (2005).
15. Y. Han, D. Mayer, A. Offenhäusser and S. Ingebrandt. Surface activation of thin silicon oxides by wet cleaning and silanization *Thin Solid Films*, 510: 175-180 (2006).

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