Journal of the Korean Physical Society, Vol. 55, No. 1, July 2009, pp. 232∼235

P-type Si-nanowire-based Field-effect Transistors for Electric Detection of a Biomarker: Matrix Metalloproteinase-9

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(Received 26 August 2008)

We studied the electric detection of a biomarker by using p-type Si-nanowire-based field-effect transistors (FETs) for biological applications. A combination of electron-beam lithography and a lift-off process was utilized to fabricate individual 50-nm-thick Si nanowire FETs. The gate-dependent $I - V_{SD}$ curves revealed that the conductance of a Si-nanowire FET increased with increasing negative V_G. The conductance of the Si nanowire FET depended upon the existence of negatively charged streptavidin binding to a biotin with a peptide and Matrix metalloproteinase-9 (MMP-9), cutting the peptide. Our results suggest that Si-nanowire FETs can be used to detect MMP-9 activity.

PACS numbers: 11
Keywords: Matrix metalloproteinase-9 (MMP-9), Gate effect, Field effect transistor (FET), Nanowire, Streptavidin-biotin binding

I. INTRODUCTION

Technological convergence of nanotechnology (NT), biotechnology (BT), and information technology (IT) has become a new paradigm in the 21st century. Semiconducting nanowires for biological applications are representative examples in NT-BT convergence technology because since they can provide the opportunity of investigating novel ways to detect biomolecules. Recent studies [1–8] demonstrated that field-effect transistors based on semiconducting nanowires were a useful platforms for real-time, label-free, highly-sensitive, direct electric detection of biological and chemical species in solution, excelling over conventional optical methods. Many research groups have been developing nanowire-based biological sensors to detect viruses [2], DNA [3], proteins [4], and so on for the diagnosis of various diseases.

In this work, we present field-effect transistors (FETs) based on p-type Si nanowires grown by using chemical vapor deposition for electric detection of a biomarker, Matrix metalloproteinase-9 (MMP-9). Matrix metalloproteinase-9 (MMP-9) is a kind of proteolytic enzyme capable of digesting extracellular matrix components [9]. MMP-9 has recently received particular attention because it is involved in a number of diseases, such as cancer, angiogenesis, alopecia, and metastasis. MMP-9 appears to have potential as a biomarker or a therapeutic target for these diseases [10,11].

The underlying principle of the electric detection of MMP-9 using a Si nanowire-based FET is shown in Fig. 1. The peptide substrate (biotin-GGPAG↓CHAK-NH_2) of MMP-9 was chemically synthesized and immobilized on the surface of the Si nanowire. Biotin was attached to the end of peptide substrate in order to accommodate streptavidin. When streptavidin binds to a biotin linked to the peptide substrate on a Si-nanowire FET, the conductance of the FET changes from the baseline value due to surface charge of streptavidin (pI $\sim$ 5 to 6), which is negatively charged [12]. The conductance of the FET drops when MMP-9 cleaves the peptide. For a p-type nanowire FET, the conductance increases when the surface charge of the streptavidin is negative. Our results demonstrate that Si-nanowire FETs are powerful

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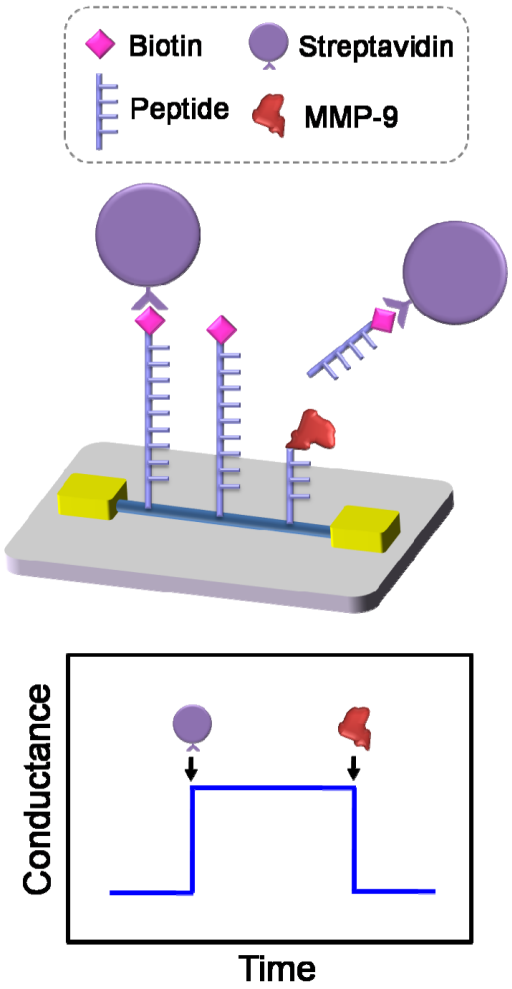

Fig. 1. Si nanowire principle of the electric detection of MMP-9 by using a Si nanowire-based FET configured as a sensor with peptide, where the cutting of MMP-9 gives rise to a decrease in the conductance of the FET.

platforms for detecting MMP-9 activity for direct and rapid diagnosis of various diseases.

II. EXPERIMENT

Si nanowires were synthesized by using chemical vapor deposition (CVD) with 20-nm gold nano-clusters as catalysts, silane as a reactant, and boron as a *p*-type dopant with a B/Si of 1 : 1,000. Si nanowires with average diameter of 50 nm [13] were dispersed on a thermally oxidized Si (100) substrate with an underlying conducting Si used as a back gate. A combination of electron-beam lithography and a lift-off process was utilized to fabricate an individual 50-nm-diameter nanowire device as shown in Fig. 2. Rapid thermal annealing (RTA) was performed under vacuum conditions ($<10^{-6}$ Torr) at 300 – 600 °C for 3 min in order to make contacts between the Ti/Au electrodes [14–17] and to passivate the

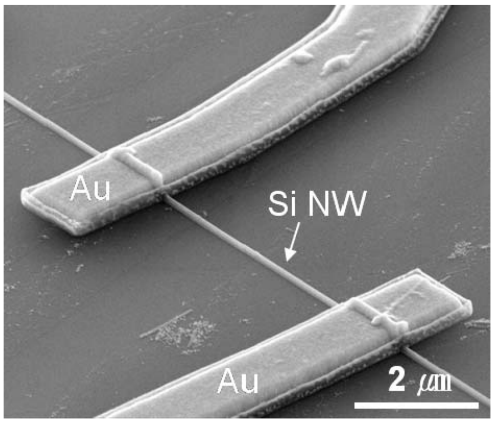

Fig. 2. A SEM image of a 50-nm-thick Si nanowire device with Ti/Au contacts for electrical transport measurements.

Si-SiO_x interface traps [18]. The $I - V$ curve became more linear and symmetric, and the conductance was increased ten times after RTA. The enhanced two-terminal conductance and stability are attributable to better contacts between Ti/Au electrodes and the Si nanowire and to passivation of defects at Si-SiO_x interface.

The Ti/Au contacts to a Si nanowire were isolated by spin coating a 300-nm-thick electron-beam resist. The active sensing area, spacing between source-drain electrodes, was 3 μm in all devices. A droplet containing MMP-9 was applied to the active sensing area of the nanowire device. In order to immobilize peptides on the surfaces of Si nanowires, we employed an oxygen plasma at 0.3 Torr and 25 W for 60 sec to remove contaminants on the surfaces of the Si nanowires. Then, the nanowires were immersed in a 2% ethanol solution of 3-(trimethoxysilyl)propyl aldehyde containing 4% water and 0.1% acetic acid for 1 hr. Peptides were linked to the Si nanowires by dropping a concentration of 5-μg/ml peptides with 4-mM sodium cyanoborohydride The biotin-peptide used in this experiment has an amine functional group that can immobilize biotin on the surface of a aldehyde-modified nanowire via hydration. All electrical measurements were performed at room temperature under an ambient air environment.

III. RESULTS AND DISCUSSION

Figure 3 presents (a) gate-dependent current *vs* source-drain voltage ($I - V_{SD}$) curves at various gate voltages (V_G) and (b) $I - V_G$ curves recorded for various V_{SD} (0.1 – 1.0 V) for an individual 50-nm-thick Si nanowire All $I - V_{SD}$ curves were found to be linear, demonstrating that the Ti/Au electrodes make good ohmic contacts to the Si nanowire, as seen in Fig. 3(a). The Si nanowire was also found to be *p*-type from the gate-dependent $I - V_{SD}$ curves, showing that the conductance of the nanowire increases with increasing neg-

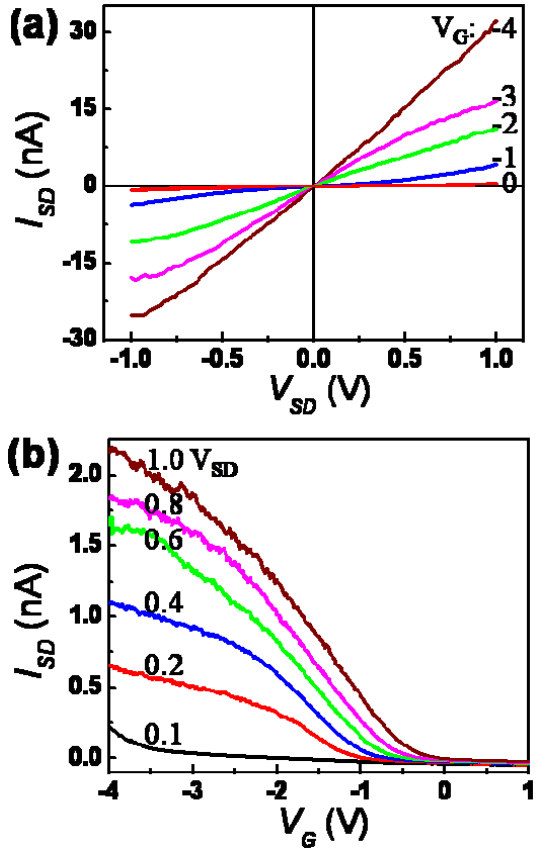

Fig. 3. (a) Gate-dependent $I - V_{SD}$ data at various gate voltages (V_G) and (b) $I - V_G$ curves recorded for various V_{SD} (0.1 – 1.0 V) for a 50-nm-thick Si nanowire.

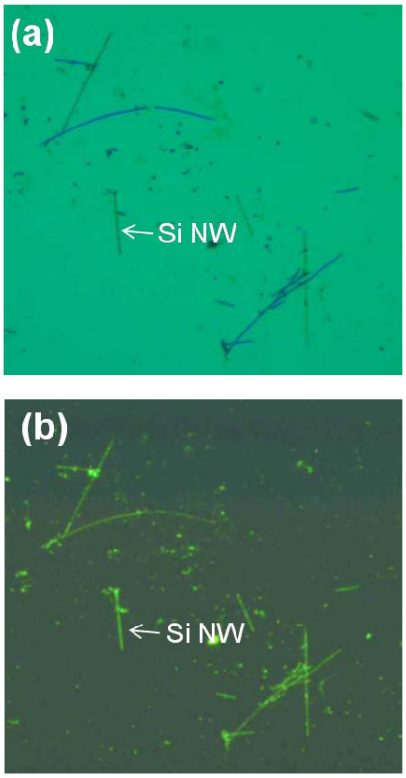

Fig. 4. (a) Optical images of immobilized biotin on surfaces of Si nanowires and (b) a fluorescence image after biotin-streptavidin binding.

ative V_G. The $I - V_G$ curves for the Si-nanowire FET obtained at different source-drain voltages are indicative of a p-channel metal-oxide-semiconductor FET [19–22] [see Fig. 3(b)]. The conductance modulation of the Si nanowire was found to exceed two orders of magnitude by changing the gate voltage from –4 to 0 V. This large switching voltage is believed to be due to the 300-nm-thick oxide dielectric layer used in the Si-nanowire FET. The carrier concentration and the carrier mobility in the p-type Si nanowire FET were determined to be 2.17×10^{17} cm^{-3} and 13.6 cm^2/V·s, respectively from the results of $I - V_G$ curve measurements. We infer from the results of the transport properties that Si-nanowire FETs are sensitive to the surface charge of negatively-charged streptavidin, binding to a biotin with a peptide.

Prior to the electrical detection of MMP-9 by using a Si-nanowire-based FET, fluorescence microscopy was employed in order to investigate the modification of the nanowire's surface after functionalization with the biotin-peptide and subsequent exposure to streptavidin labeled with FITC (3-9 mol of FITC/ 1 mol of streptavidin, Sigma-aldrich Co., USA). Fig. 4. exhibits (a) an optical image of immobilized biotin on the surfaces of Si nanowires and (b) a fluorescence image after biotin-streptavidin binding. One unit of this labeling molecule binds 1 μg of biotin. Control experiments were performed in order to confirm that the green fluorescence resulted from the specific binding of streptavidin to the biotin-peptide on the nanowire's surface. When the bare nanowires (without biotin) were exposed to a 300-nM of streptavidin solution, green fluorescence was not observed (data not shown). However, the nanowires with biotin-peptide modification showed bright green fluorescence [see Fig. 4(b)]. Our results strongly suggest that the fluorescence in the nanowires originates from the biotin-streptavidin interaction and the peptide substrates are successfully immobilized on the surfaces of the nanowires.

Figure 5 shows the real-time conductance response for a Si-nanowire FET functionalized with peptide-biotin during the flow of the buffer solution, 300 nM of streptavidin, and 300 nM of MMP-9. Addition of solution without streptavidin was found to have no appreciable effect on the conductance of the Si-nanowire FET (see Step 1 in Fig. 5). However, for addition of a 300-nM streptavidin sample solution, as indicated by the first arrow in Fig. 5. an abrupt (<5 sec) increase in the conductance was found to occur and then to remain steady (see Step 2 in Fig. 5). The increase in the conductance of the Si-nanowire FET is attributable to the negative surface charge density [3–5] associated with binding of negatively-charged

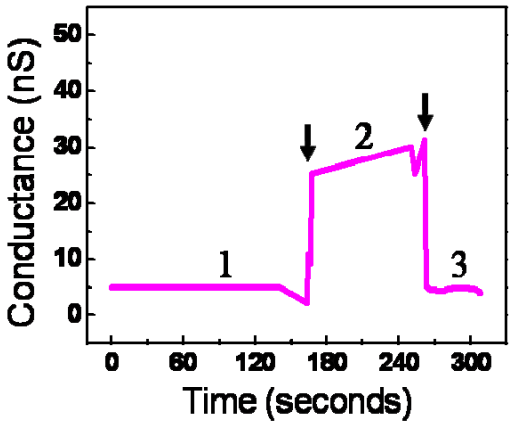

Fig. 5. Real-time conductance response for a Si nanowire FET functionalized with peptide-biotin during the flow of the buffer solution, 300-nM streptavidin and 300-nM MMP-9.

streptavidin at the surface of the Si nanowire, as previously addressed. When 300 nM of MMP-9 was applied to the Si-nanowire FET, the conductance was found to drastically decrease, as indicated by the second arrow in Fig. 5. This is due to the proteolytic activity of MMP-9, which is cutting the peptide and subsequently giving rise to the removal of negatively-charged streptavidin from the surface of the Si-nanowire FET. Our results successfully demonstrate that Si-nanowire FETs can be used for monitoring the enzymatic activity of MMP-9, which is regarded as a biomarker of serious diseases, such as cancer metastasis.

IV. CONCLUSION

We have investigated field-effect transistors (FETs) based on individual Si nanowires grown by chemical vapor deposition for biological applications. An individual 50-nm-thick Si nanowire was found to be *p*-type from the gate-dependent $I - V_{SD}$ curves. The conductance of the nanowire was found to increase with increasing negative V_G, suggesting that Si-nanowire FETs are sensitive to the surface charge of negatively-charged streptavidin binding to a biotin with a peptide. Fluorescence microscopy studies illustrate that the fluorescence in Si nanowires results from the biotin-streptavidin interaction and that the peptide substrates are successfully immobilized on the surfaces of the nanowires. Real-time conductance for a Si-nanowire FET functionalized with peptide-biotin changes due to the binding of negatively-charged streptavidin and to the proteolytic activity of Matrix metalloproteinase-9 (MMP-9). Our results demonstrate the possibility of applying Si-nanowire-based FETs for monitoring the enzymatic activity of MMP-9 as a representative biomarker of various diseases.

ACKNOWLEDGMENTS

This work was supported by the Agency for the Defense Development through Defense Nano Technology Application Center and the Korea Science and Engineering Foundation (KOSEF) through the National Core Research Center for Nanomedical Technology (R15-2004-024-00000-0), Seoul Research and Business Development Program (10816), the Korea Research Foundation (Ministry of MOEHRD) (KRF-2005-042- D00203).

REFERENCES

[1] Y. Cui, Q. Wei, H. Park and C. M. Lieber, Science **293**, 1289 (2001).
[2] F. Patolsky, G. Zheng, O. Hayden, M. Lakadamyali, X. Zhuang and C. M. Lieber, Electrical detection of single viruses, Proc. Natl. Acad. Sci. USA **101**, 14017 (2004).
[3] J. Hahm and C. M. Lieber, Nano Lett. **4**, 51 (2004).
[4] W. U. Wang, C. Chen, K. Lin, Y. Fang and C. M. Lieber, Proc. Natl. Acad. Sci. USA **102**, 3208 (2005).
[5] G. Zheng, F. Patolsky, Y. Cui, W. U. Wang and C. M. Lieber, Nat. Biotechnol. **23**, 1294 (2005).
[6] Y. L. Bunimovich, Y. S. Shin, W.-S. Yeo, M. Amori, G. Kwong and J. R. Heath, J. Am. Chem. Soc. **128**, 16323 (2006).
[7] Y. Lee, J. Kim and J. Park, J. Korean Phys. Soc. **54**, 1769 (2009).
[8] P. Estrela, P. Li, D. Keighley and P. Migliorato, J. Korean Phys. Soc. **54**, 498 (2009).
[9] R. Visse and H. Nagase, Circ. Res. **92**, 827 (2003).
[10] I. Stamenkovic, Semin. Cancer Biol. **10**, 415 (2000).
[11] R. A. Cairns, R. Khokha and R. P. Hill, Curr. Mol. Med. **3**, 659 (2003).
[12] M. Wilchek and E. A. Bayer, Methods Enzymol. **184**, 49 (1990).
[13] Y. S. Woo, K. Kang, M.-H. Jo, J.-M. Jeon and M. Kim, Appl. Phys. Lett. **91**, 223107 (2007).
[14] S.-W. Chung, J.-Y. Yu and J. R. Heath, Appl. Phys. Lett. **76**, 2068 (2000).
[15] Y. Cui, X. Duan, J. Hu and C. M. Lieber, J. Phys. Chem. B **104**, 5213 (2000).
[16] M. C. McAlpine, R. S. Friedman, S. Jin, K. Lin, W. U. Wang and C. M. Lieber, Nano Lett. **3**, 1531 (2003).
[17] R. Martel, T. Schmidt, H. R. Shea and T. Hertel, Appl. Phys. Lett. **73**, 2447 (1998).
[18] W. I. Park, J. S. Kim and G.-C. Yi, Appl. Phys. Lett. **85**, 5052 (2004).
[19] Y. Huang, X. Duan, Y. Cui and C. M. Lieber, Nano Lett. **2**, 101 (2002).
[20] S. Wolf and R. N. Tauber, *Silicon Processing for the VLSI Era* (Lattice Press Sunset Beach, CA, 1986) Vols. 1-2.
[21] S. M. Sze, *Physics of Semiconductor Device* (John Wiley & Sons New York, 1981).
[22] J. P. Gambino and E. G. Colgan, Mater Chem. Phys. **52**, 99 (1998).