Article

Journal of Biomedical Nanotechnology
Vol. 9, 732–735, 2013
www.aspbs.com/jbn

MMP-2 Detective Silicon Nanowire Biosensor Using Enzymatic Cleavage Reaction

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Matrix metalloproteinases are proteolytic enzymes that play a significant role in tissue remodeling related with various pathological and physiological processes such as tissue repair, angiogenesis, cirrhosis, morphogenesis, arthritis, and metastasis. Especially, MMP-2 has been shown to be related with benign prostatic hyperplasia and prostate cancer. Therefore, there is a need to make sensors with high sensitivity that can measure MMP-2 concentrations precisely. Silicon nanowires have been used in the development of high sensitive chemical sensors and biosensors. The high sensitivity of silicon nanowire based sensor originates in its high surface to volume ratio and ability to field-effect induced local charge transfers. In this study, 100 nm silicon nanowire based field-effect transistors (FET) device was fabricated by electron-beam lithography and MMP-2 was successfully measured by conductance versus time characteristics within 1 pM to 100 nM.

KEYWORDS: *Silicon Nanowires, Biosensor, Electron-Beam Lithography, Matrix Metalloproteinase-2, Silicon-on-Insulator.*

INTRODUCTION

In particular, matrix metalloproteinase-2 (MMP-2) has been coming to inform biomarker about several cancers.[1] In the concrete, the MMP-2 level in normal healthy people is lower (420 ng/ml–520 ng/ml) than in patients with prostatic hyperplasia (734 ng/ml–894 ng/ml) and with prostate cancer (1175 ng/ml–1285 ng/ml).[2] Common strategies employed for general protein detection including MMP is enzyme-linked immunosorbent assay (ELISA) that relative rapid and simple assay using antigen-antibody interaction.[3] However, there are some disadvantage to this method including sustained stability, exposure of active sites for antigen binding, and the antibody, which a kind of protein can easily denature with temperature changes.

Especially, a sandwich structure of ELISA system has to use two kinds of antibodies that is labeled enzyme or other detectable probe and deposited on the ELISA plate. It is not only complicated process but also required antibodies in a large amount both to labeling probe on antibody and depositing antibody on plate. Therefore, it is necessary to develop a new protein detection method both not to use antibody and label other probes.

Fortunately, MMPs are proteolytic enzymes, proteases that play a key role in extracellular matrix degradation implicated in tumor cell invasion, metastases and angiogenesis.[4] This actual phenomena leads that MMP-2 is able to degrade some specific peptide sequences included degraded proteins. Therefore, MMP-2 assay with high selectivity is great of significance for the diagnosis of protease-relevant disease and development of therapeutic agents. This method is superior to immune reaction based biosensors from a practical standpoint, since it does not require a label or antibody and is a one-step reaction.

In this study, silicon nanowire (SiNW) patterns were fabricated on a silicon-on-insulator (SOI) wafer[5] using electron-beam (E-beam) lithography in order to measure MMP-2 through enzymatic cleavage reaction on specific

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Received: 7 November 2011
Accepted: 2 March 2012

 1550-7033/2013/9/732/004 doi:10.1166/jbn.2013.1541

peptide sequence (IPVSLRSG).[6] MMP-2 was successfully measured by conductance versus time characteristics within 1 pM to 100 nM using silicon nanowire based field-effect transistor (FET) device.

EXPERIMENTAL DETAILS

Materials

P-type Silicon on insulator (SOI) wafers (insulating layer: 145 nm; silicon layer: 55 nm) was purchased from Soitec Co. (France). 3-(triethoxysilyl)butylaldehyde was purchased from Gelest, Inc. (USA). Distilled and deionized Milipore water (Milli-Q) and N_2 gas were used for cleaning and drying. Peptide (KKGGGGGG-IPVSLRSG-EEEEEE) was synthesized from Peptron Inc. (Korea).

Fabrication of Silicon Nanowire Based FET Device

SiNW on SOI wafer was designed by Auto Cad 2010 in the form which zigzags in order to widen surface area. SiNW patterns were fabricated on a SOI wafer by using E-beam lithography, and the width of nanochannel was 100 nm. Composing FET-based device, chromium (Cr) and gold (Au) were deposited in series to a thickness of 150 nm and 30 nm, respectively. They were overlapped over patterned nanowires with 6 μm width. The number of nanowires was 25 with zigzag pattern, and its width and height were 100 nm and 100 nm, respectively.

Characterization of the SOI Device

All electrical properties of the silicon nanowires on the SOI substrate were obtained by a B1500A semiconductor parameter analyzer system (Keithley Co., USA). The nanowire structures on the SOI substrate were characterized by scanning electron microscopy (SEM, JSM-6700, 15–30 kV).

Fabrication of the Peptide-Functionalized SiNW

The fabricated silicon nanowires were cleaned by O_2 plasma at 50 W and 50 sccm O_2 for 60 sec. After cleaning with O_2 plasma, the silicon nanowires patterned device was immersed in ethanol solution containing 3-(triethoxysilyl)butyl aldehyde for 30 min at room temperature to form self-assembled monolayers (SAMs) and washed with absolute ethanol several times. These functionalized silicon nanowires were incubated in 0.15 M PBS buffer solution (pH 7.4) containing peptide (KKGGGGGG-IPVSLRSG-EEEEEE) which was designed to measure MMP-2 through enzymatic cleavage reaction for 2 hrs at room temperature. After 2 hrs, the silicon nanowires were rinsed with deionized water and PBS buffer and then dried with N_2 gas. Afterwards, the silicon nanowires were passivated with 1 mg/ml of polyethylene glycol functionalized thiol group (SH-PEG) to minimize the non-specific binding events of MMP-2. Finally, MMP-2 in the concentration range of 1 pM and 100 nM was applied to the system.

RESULTS AND DISCUSSION

Characterization of Silicon Nanowire Based FET Device

Uniform sized silicon nanowires were fabricated on the SOI wafer via the electron-beam method for biosensor applications. Using the standard procedures of photolithography, reactive ion etching (RIE), ion implantation, electron-beam lithography, and thermal evaporation, the SiNWs and the connecting electrodes were created and assembled into the SiNW-FET devices, where the width of the SiNWs were about 100 nm. As shown in Figure 1, SiNWs were successfully and uniformly fabricated on the SOI substrate in a zigzag structure. The electrical property of the fabricated *p*-type silicon nanowire devices were assessed using the B1500A semiconductor device analyzer and the SiNW-FET device was operated well as expected (data not shown).

Optimization of Peptide Concentration on SiNW Substrate

In order to detect MMP-2 sensitively, it is essential to determine the concentration of peptides on SiNWs surface.

After cleaning the silicon nanowires with O_2 gas plasma, 3-(triethoxysilyl) butylaldehyde monolayer was formed on the SiNWs by self assembly method. As shown in Figure 2, designed peptide fragments were covalently attached on SiNW surface. Herein, different concentration of designed peptide fragments (5~640 μg/ml) was attached to aldehyde-functionalized SiNW surface, and the conductance variation of SiNW was measured after applying 100 nM of MMP-2 to SiNW surface. The conductance variation of SiNWs is originated from peptide cleavage effect by MMP-2. As shown in Figure 3, it could be concluded that 160 μg/ml of peptide was optimal concentration in order to detect MMP-2 with high sensitivity using the SiNW based FET biosensor. Because the biggest

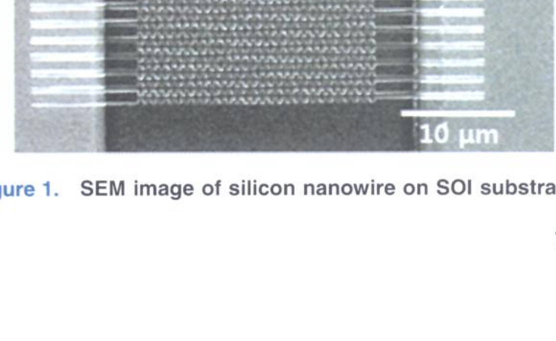

Figure 1. SEM image of silicon nanowire on SOI substrate.

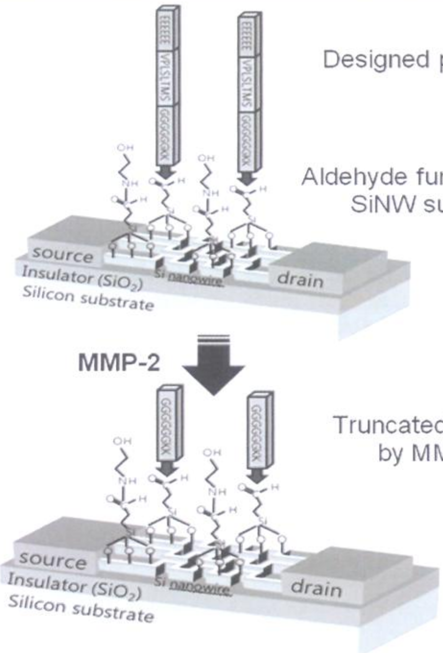

Figure 2. Schematic illustration of the SiNW based FET biosensor for detection of MMP-2 using enzymatic cleavage reaction.

change of conductance was shown at 160 μg/ml of peptide concentration for MMP-2, compared to that at another peptide concentrations.

Detection of MMP-2 Using SiNW Based FET Biosensor

Sensitivity of fabricated SiNW based FET biosensor for MMP-2 is shown in Figure 4. The decrease of negative charges by cleavage reaction of MMP-2 for target peptide sequence (IPVSLRSG) can greatly affect the conductivity of SiNW FET. In case of *p*-type SiNW FET, a decrease of the conductance will be expected when negatively charged peptide fragments (6-glu group) were removed on its sensing surface. MMP-2 was applied to the device at various concentrations ranging from 1 pM to 100 nM (Figs. 4(A and B)). Concentration-dependent

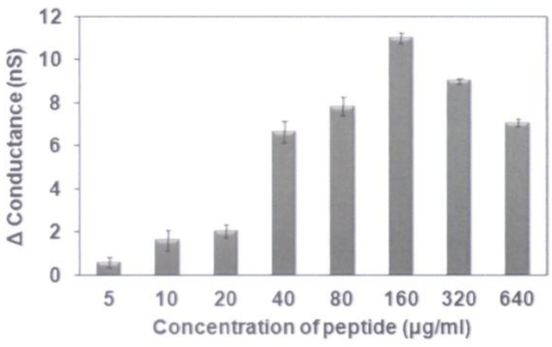

Figure 3. Conductance variation of SiNW based FET biosensor as a function of peptide concentration by MMP-2.

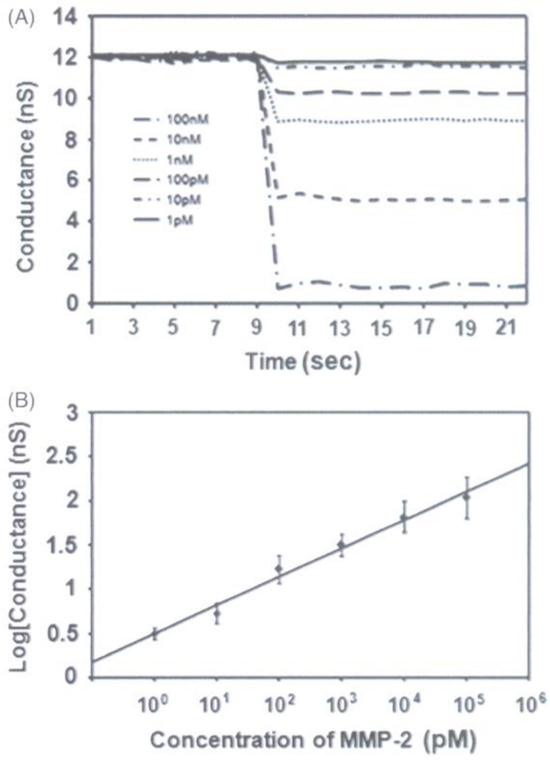

Figure 4. Conductance measurement as a function of target MMP-2 concentration. (A) Conductance response of SiNW based FET biosensor for various concentration of MMP-2. (B) Conductance variation as a function of MMP-2 concentration.

electronic responses were observed for MMP-2 at concentrations ranging from 1 pM to 100 nM. Moreover, the time required for conductance change was approximately 20 sec. This result strongly suggests that a rapid and sensitive sensing device for the detection of MMP-2 was achieved.

CONCLUSION

In this study, we successfully developed a biosensor for detection of MMP-2 using a SiNW FET device that was fabricated by the E-beam lithography. Using the fabricated SiNW FET device, different concentrations of MMP-2 were sequentially measured by conductance versus time at MMP-2 concentrations ranging from 1 pM to 100 nM. The developed SiNW FET sensor possesses several advantages, such as good analytical performance, clear conductance response, high sensitivity and low detection limit with an appropriable linear range.

Acknowledgments: This research was supported by the Original Technology Research Program for Brain Science through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093907), and by the Human Resources Development of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Knowledge Economy (No. 20114010203090) and by Sogang University Research Grant of 2011 (201114007).

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