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Using silicon nanowire devices to detect adenosine triphosphate liberated from electrically stimulated HeLa cells

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ABSTRACT

In this study, we used a biosensor chip featuring Abl tyrosine kinase-modified silicon nanowire field-effect transistors (SiNW-FETs) to detect adenosine triphosphate (ATP) liberated from HeLa cells that had been electrically stimulated. Cells that are cultured in high-ionic-strength media or buffer environments usually undermine the sensitivity and selectively of SiNW-FET-based sensors. Therefore, we first examined the performance of the biosensor chip incorporating the SiNW-FETs in both low- and high-ionic-strength buffer solutions. Next, we stimulated, using a sinusoidal wave (1.0 V, 50 Hz, 10 min), HeLa cells that had been cultured on a cell-culture chip featuring interdigitated electrodes. The extracellular ATP concentration increased by ca. 18.4-fold after electrical stimulation. Finally, we detected the presence of extracellular ATP after removing a small amount of buffer solution from the cell-cultured chip and introducing it into the biosensor chip.

1. Introduction

Thousands of complicated biological and chemical reactions occur in living cells, with most of them requiring an amount of energy for activation. In living cells, the universal unit of energy is provided by adenosine triphosphate (ATP). Extensive research has focused on the role of intracellular ATP as an energy source and its complex biochemical interactions with living cells (Hafner, 2000; Schneider et al., 1999). Several approaches have been developed previously for the detection of ATP. Firefly luciferase (Billard and DuBow, 1998) requires the presence of ATP to produce bioluminescence, allowing ATP detection at the picomolar level when using an enzyme-linked immunosorbent assay (ELISA). Although this approach features an appropriate detection range and limit, its drawbacks are that it is time-consuming and difficult to apply to real-time detection. An enzyme-modified electrode has also been demonstrated for the detection of ATP, with a detection limit on the nanomolar level. This technique was first fulfilled by co-immobilization of the enzymes onto the electrode (Kueng et al., 2004). The enzymatic reaction resulted in a current response that was proportional to the ATP concentration. The selective catalytic activity of an enzyme/ISFET (ion-sensitive field-effect transistor) system has also been adopted for the detection of ATP (Migita et al., 2007). The major drawbacks of enzyme/ISFET and enzyme/electrode sensors are their low sensitivities and slow responses. The use of field-effect transistors (FETs) based on nanowires (Cui et al., 2001; Wang et al., 2005b) or nanotubes (Star et al., 2006; Wang et al., 2007) as biosensors has also been studied extensively in biological research because of their sensitivity and their label-free and real-time detection capabilities. Wang et al. (2005b) reported the highly sensitive and label-free detection of ATP using Abl tyrosine kinase-modified silicon nanowire field-effect transistors (SiNW-FETs), with ATP binding to Abl distinguishable above the background noise at concentrations at least as low as 100 pM.

To date, most SiNW-FETs (Park et al., 2007) have been developed for the detection of pre-concentrated and purified target molecules in low-ionic-strength buffer environments; there has been little study of the use of SiNW-FET for in vitro or in vivo detection of molecules released from living cells (Wang et al., 2007). Lieber's group recently employed an array of SiNW-FETs to monitor the electrical signals from single neurons (Patolsky et al., 2006). They observed highly sensitive detection, stimulation, and inhibition of neuronal signal propagation between nanowires and axon junctions. Although a single-walled carbon nanotube transistor has been developed for the in situ detection of chromograni A (Wang et al., 2007), which is released from neurons, there are very few reports of the use of SiNW-FET or CNT-FET biosensors for the detection of chemical species released from cultured living cells.

In this study, we prepared a system comprising a cell-culture chip and a sensor chip for the detection of ATP released from living cells. The cell-culture chip was developed for both the cell culturing and electrical stimulation of living cells. The HeLa cells were cultured in the medium on the cell culture chip. After cell

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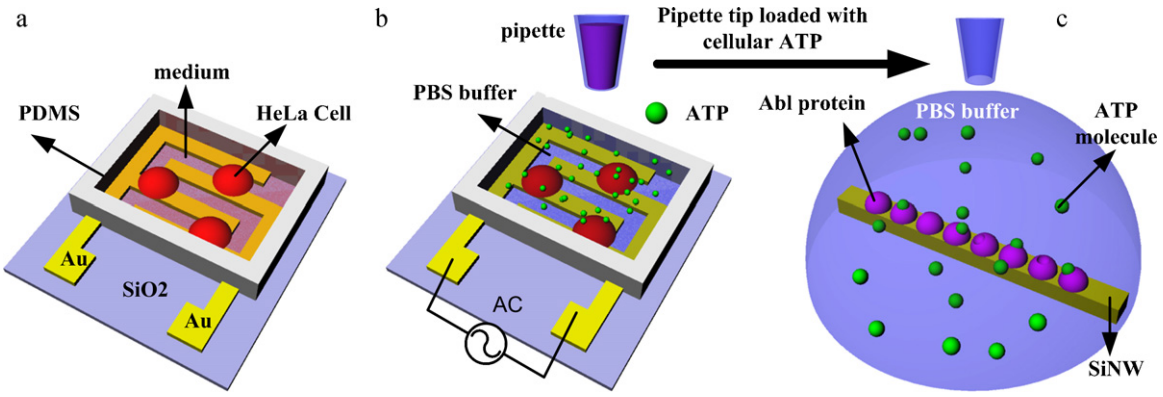

Fig. 1. Schematic representation of (a) cultured HeLa cells on a cell-culture chip, (b) electrical stimulation activating HeLa cells to release ATP, and (c) cellular ATP detected by an Abl-modified SiNW.

attachment (Fig. 1a), the cell culture environment was replaced by phosphate-buffered saline (PBS). Electrical stress caused the HeLa cells to liberate ATP into the PBS solution (Fig. 1b). We then used a pipette tip to remove a portion of the PBS solution, now incorporating the extracellular ATP, from the cell-culture chip and loaded it onto the biosensor chip featuring SiNW-FETs as sensors (Fig. 1c). Taking advantage of the high sensitively and selectivity of the Abl-modified SiNW-FET biosensor, we could detect the extracellular ATP released from the HeLa cells in the cell-culture chip, without the need for pre-concentration or and purification.

2. Experimental

2.1. Chemicals

Deionized water (>18 MΩ cm) was obtained from a purification system. All reagents employed in this study were of reagent grade and used as received. Sylgard 184 base (A) and curing agent (B) were purchased from Dow Corning (Mialand, MI). Abl tyrosine kinase was purchased from New England BioLabs (Beverly, MA). SU-8 photoresists (SU-8 2005 and 2050) were purchased from Microchem (Newton, MA). Dulbecco's modified Eagle's medium (low glucose, DMEM), fetal bovine serum (FBS), 0.05% trypsin/ethylenediaminetetraacetic acid (EDTA) solution, and PBS were purchased from GibcoTM Invitrogen Co. (Carlsbad, CA).

2.2. Fabrication of SiNW-FET biosensors

The SiNW-FET biosensors were fabricated from an SOI (silicon on insulator) wafer having a buried oxide thickness (BOX) of 150 nm and a 50-nm-thick top-silicon film in the ⟨1 0 0⟩ orientation. The thickness of the top silicon layer was further reduced to 30 nm using dry oxidation and wet etching technology. The SiNW-FET biosensor was fabricated using e-beam lithography and subsequent dry etching. The samples were capped with a 10-nm-thick screening oxide layer before being doped through boron ion implantation at an energy of 10 keV; the dopants were then activated at 950 °C for 30 min in nitrogen ambient to ensure their uniform diffusion over the entire SiNW. Contact holes were patterned and Au/Ti metallization was performed and then the devices were sintered at 300 °C in nitrogen ambient for 60 min. To avoid short circuiting or leakage during measurement in the solution environment, SU8-2005 photoresist was patterned to protect the source and drain electrodes.

2.3. Surface modification of SiNW-FET biosensors

Abl tyrosine kinase was covalently coupled to the SiNW surface using a modification of Lieber's method (Wang et al., 2005b); Fig. S1a presents an idealized representation. To clean the surface of the biosensor chip, UV/ozone exposure was performed for 10 min using a commercial ozone cleaning system (UV-1, All Real). The cleaned biosensor chip was then immersed for 30 min in a solution of 1% (v/v) 3-(trimethoxysilyl)propyl aldehyde in EtOH/H_2O (95:5, v/v). The biosensor chip was washed with EtOH, dried, and then heated at 120 °C for 30 min. The aldehyde-presenting chip was placed in a solution of Abl tyrosine kinase (5 μg/mL) containing 4 mM sodium cyanoborohydride; unreacted aldehyde groups were quenched with 15 mM Tris buffer (pH 7.5) for 10 min.

2.4. Atomic force microscopy characterization

The immobilization process (surface morphology) was characterized at its various stages using atomic force microscopy (AFM). A commercial AFM instrument (JSPM-5200, JEOL) was adopted for this morphological analysis using a silicon probe (NSG 11, NT-MDT) having a nominal spring constant of 2.5 N m^{-1}. The atomic force microscope was operated in the tapping mode to minimize mechanical damage of the chemically modified surfaces.

2.5. Characteristics of SiNW-FET biosensors

The surface morphologies and dimensions of the SiNWs were examined using a field-emission scanning electron microscope (JSM-6700, JEOL). The electrical characteristics of the SiNW-FET biosensors were measured at room temperature using a semiconductor parameter analyzer (HP4156C, Agilent), performed on a probe station (VFTTP4, Lakeshore). SiNW-FET biosensors were also characterized in terms of their performance in low- and high-ionic-strength buffer environments.

2.6. Fabrication of cell-culture chip

The cell-culture chip fabricated in this study featured gold finger-like electrode structures and a cell culture well prepared from polydimethylsiloxane (PDMS). The chip substrate was a silicon wafer having a thermal oxide thickness of 3000 Å. The gold finger-like electrodes were fabricated using a standard photolithography and lift-off process; the gap between electrodes was ca. 100 μm. A Sylgard 184 A/curing agent mixture (10:1) was poured over a stainless-steel mold and cured until solidified; the PDMS well was then removed from a mold. After cleaning, the PDMS well (well size: 5 mm × 5 mm × 2 mm) was bonded to the surface of the cell-culture chip.