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Development of electrochemical calcium sensors by using silicon nanowires modified with phosphotyrosine

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Abstract

This paper reports the electrical detection of calcium ions by using silicon nanowires (SiNWs) as channels in a chemically gated field-effect-transistor (FET) configuration. To obtain a selective and sensitive layer for calcium sensing, the SiNWs are modified with a biologically relevant amino acid phosphotyrosine (p-Tyr), which is able to complex calcium ions with high affinity. It is found that when the p-Tyr modified SiNWs are exposed to aqueous solutions containing calcium ions, their conductances increase with the increasing of calcium concentration up to 10 μM. In contrast, when the SiNWs are exposed to sodium or potassium, or when they are modified with tyrosine (Tyr), no significant increase in the conductance is observed. This finding suggests that the calcium ions complexed with the phosphate group of p-Tyr can act as a positive gate voltage on the FET device comprising of n-type SiNWs, and leads to an increase in their conductances. The FET device is also sensitive to magnesium ions. However, the response is 10 times lower than that of calcium at the same concentration. The study reported here may pave the way for designing an intracellular calcium sensor which permits the monitoring of calcium concentration in real time.

Keywords: Electrochemical; Silicon nanowire; Phosphotyrosine; Calcium sensors; Field-effect-transistor

1. Introduction

Calcium ions regulate many biological processes through their interactions with calcium-receptor/binding proteins (Berridge et al., 1998). Multiple calcium-binding proteins with different calcium-binding affinities have been identified in various cellular environments in all eukaryotic cells (Glusker, 1991). Extracellularly, the concentration of calcium ion is maintained at a level of 1 mM, which regulates many biological functions such as signal transduction, calcium-dependent cell adhesion, and proteolytic stability (Shapiro and Colman, 1998; Brown and MacLeod, 2001). Intracellularly, the concentration of calcium ion is only 100 nM, which is 10,000 times lower than the extracellular calcium concentration. This huge concentration gradient is the driving force for several critical cellular functions such as cell proliferation, gene expression, and cell apoptosis. When calcium channels on a cell membrane are open, extracellular calcium ions will flow into the cell and increase the intracellular calcium concentration to the micromolar range, which will then trigger a cascade of cellular events. Therefore, it would be interesting to design a calcium sensor which can be used to monitor the increase of intracellular calcium concentration in real time. To meet this requirement, the first step is to design a sensor that has similar dimensions of a single cell and has a high sensitivity within a biologically relevant range from 100 nM to 10 μM.

Recently, novel electrical and optical properties (Ma et al., 2003; Morales and Lieber, 1998; Holmes et al., 2000; Zhang et al., 2001; Wu et al., 2004; Schmidt et al., 2005; Ge et al., 2005) of silicon nanowires (SiNWs) attract a lot of attention because they have many applications in nanometer-scale bioelectronics and biosensors (Cui et al., 2001; Nagle et al., 2003; Alivisatos, 2004; Patolsky et al., 2004; Zheng et al., 2005; Wang et al., 2005; Risveden et al., 2007). In addition, SiNWs-based sensors have various advantages such as biocompatibility, vast

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surface-to-bulk ratio, and fast response. To fabricate SiNWs for sensing applications, one can either follow a "bottom-up" or a "top-down" approach. Although most of the reported studies are based on the "bottom-up" approach, it is limited by the complex processes of transferring and positioning of SiNWs and making reliable ohmic contacts with them. Furthermore, the control of doping concentrations in self-assembled semi-conducting nanostructures remains a challenge, and the fabrication of high-density sensor arrays is very difficult (Li et al., 2005). In contrast, the "top-down" approach takes the full advantages of current semiconductor technologies which permit the production of hundreds of SiNWs in an array format simultaneously. It also creates a pathway for designing high-density, high-quality nanoscale sensors that can be fully integrated with the manufacturing processes of the SiNWs (Li et al., 2004).

To construct a SiNW-based biosensor that responds to calcium ions, a sensitive layer needs to be built on the surface of SiNWs. Up to dates, two types of sensitive layers for calcium sensing have been reported. The first type of sensitive layer is derived from calcium-binding protein such as calmodulin (Cui et al., 2001). However, the stability of protein may limit its application. The second type of sensitive layer is based on small molecules with functional groups which bind calcium ions with high affinity. For example, phosphate-containing molecules have been exploited in the past study for the design of calcium sensors (Moss et al., 1978; Jaffrezic-Renault et al., 1991). Unfortunately, to incorporate the phosphate moiety onto the sensitive layer requires multiple chemical reactions, which usually results in low yields and low sensitivities (Elbhiri et al., 2000). To overcome this problem, we select a nature-occurring amino acid phosphotyrosine (p-Tyr) to construct the sensitive layer for calcium sensing. Because p-Tyr also plays an important role in cell signaling processes, the proposed sensor may also give insights into the role of calcium in the cell signaling processes. To immobilize p-Tyr onto the surface of SiNWs, the oxide layer covering the SiNWs was first functionalized with *N*-(2-

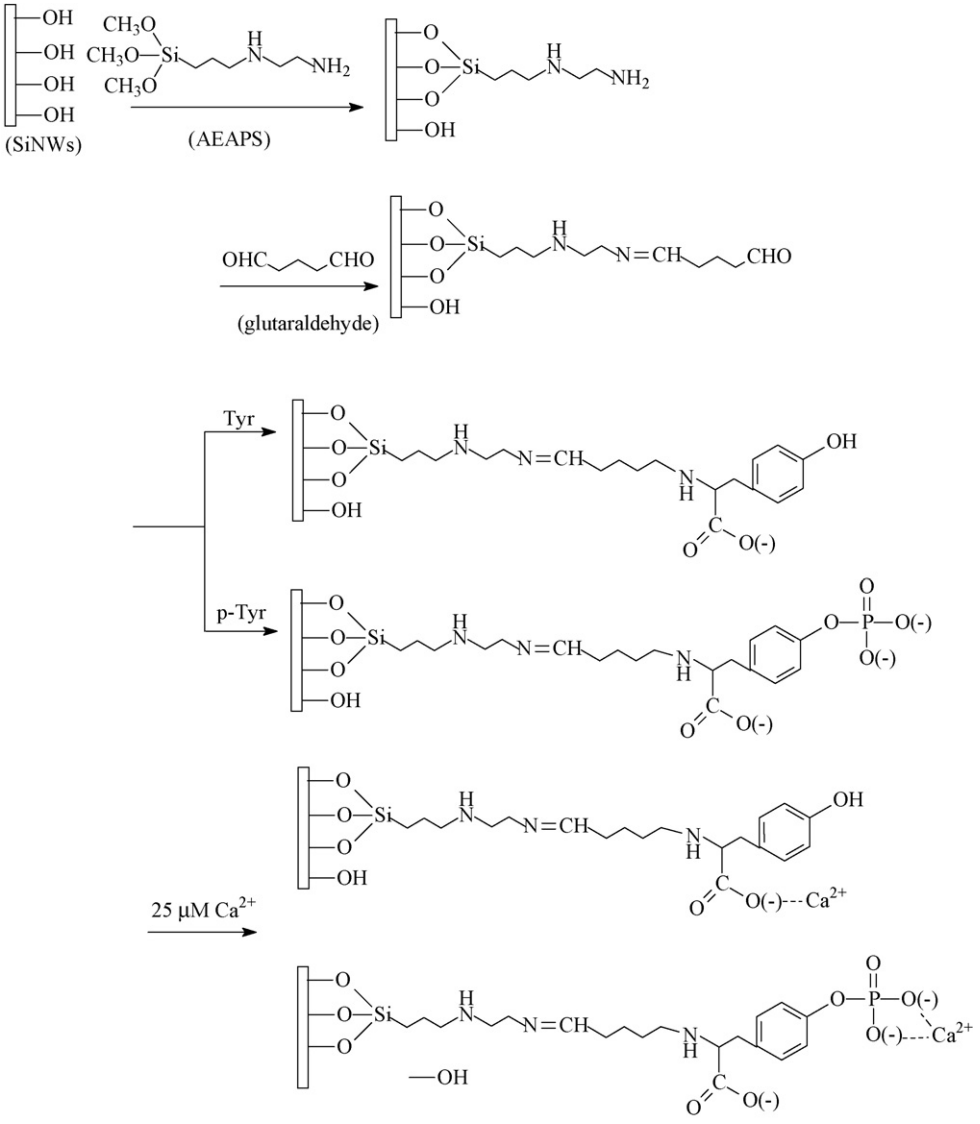

Fig. 1. Schematic illustration of the surface modification of SiNWs with AEAPS and glutaraldehyde, followed by Tyr or p-Tyr. The SiNWs modified with p-Tyr show high affinity for calcium ions whereas SiNWs modified with Tyr show low affinity for calcium ions.