Extended Abstracts of the 2007 International Conference on Solid State Devices and Materials, Tsukuba, 2007, pp. 308-309

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Electrical Sensing of Calcium Ions using Silicon Nanowire Array

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1. Introduction

The cell functions depend critically on the interactions among various proteins along specific signaling pathways, which are regulated by many factors including calcium ions and kinase activity. Measurements of calcium concentration and kinase activity in a single living cell will lead to better understanding of cell proliferation, gene expression and cell apoptosis. For the purpose, various techniques are being employed like calcium induced fluorescence change measurements [1], by detecting biotinylated calmodulin and calmodulin-binding proteins [2], by immobilizing calmodulin onto Silicon nanowire (SiNW) surfaces [3], etc. Silicon nanowire based chemical and biosensors have attracted wide attention because of their biocompatibility, vast surface-to-bulk ratio, fast response, good reversibility and SiO_2-coated or H-terminated surface, which allows easy attachment to various functional groups.

This paper reports highly sensitive, label-free and direct detection of Ca^{2+} ions by way of recording the change in SiNWs electrical conductance, when Ca^{2+} ions binds specifically to phosphotyrosine (p-Tyr), as compared to tyrosine (Tyr). Protein Tyr and p-Tyr that are important in cell signaling processes are covalently linked to SiNWs surface and the change in their conductance is monitored to assess concentration dependent binding of Ca^{2+} or Mg^{2+} ions. One order higher sensitivity for Ca^{2+} than Mg^{2+} ions is reported.

2. Experimental Detail

Calcium sensors are fabricated using CMOS compatible silicon technologies [4] having 500µm long SiNWs in array format. Fig. 1(a) shows an optical image of a biochip with two nanowire arrays having 100 nanowires each while zoom-in image shows one such array. Nanowires pitched at 2µm in fluid channel, contact metal lines and passivation layer are reflected in Fig. 1(b). A typical TEM image of a nanowire in Fig. 1(c) suggests rectangular cross-section with 30nm width and 45nm height.

Nanowire sensor surface having thin SiO_2 (<3nm) is suitably modified to attach Ca^{2+} ions specifically. For the purpose, DI water cleaned sensor surface is immobilized with N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane (AEAPS) followed by glutaraldehyde. The sensor surfaces are then coated by tyrosine or phosphotyrosine. The two different types of samples are then tested for change in electrical conductance of nanowires when exposed to calcium and magnesium ions (Fig. 2).

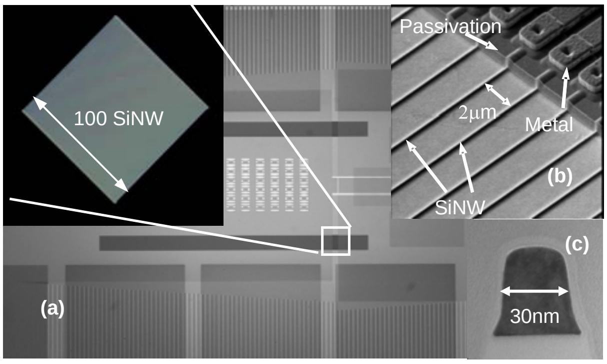

Fig. 1 (a) Optical image: Sensor chip with two 500µm long SiNW arrays; Zoom-in image shows 100 SiNW in an array (b) SEM image: SiNWs spaced at 2µm with metal lines and passivation layer (c) TEM image: Nanowire having rectangular cross-section.

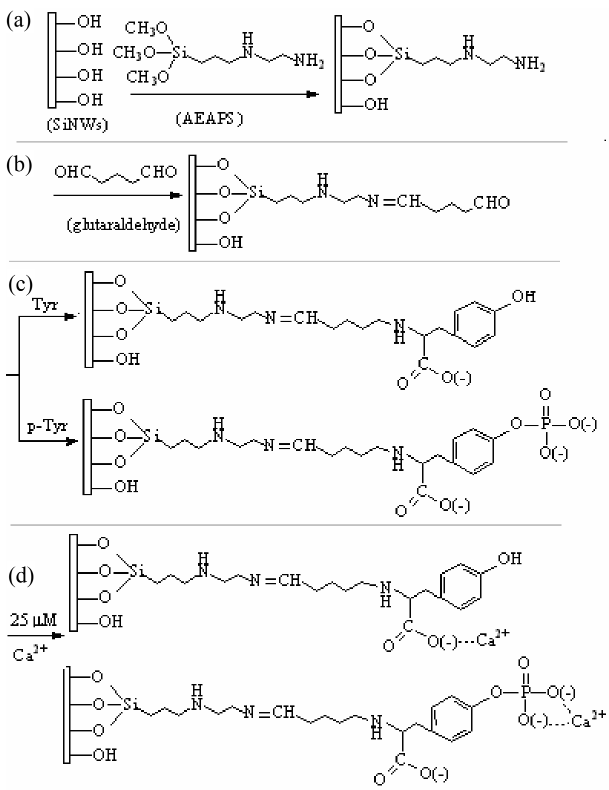

Fig. 2 Schematic of the surface modification of SiNW sensors: (a) silanization (b) attachment of gluteraldehyde (c) coating of tyrosine and phosphotyrosine and (d) immobilization of Ca^{2+} on tyrosine and phosphotyrosine modified SiNW sensors.

3. Results and Discussions:

Conductance for such an array of 500µm long n-type nanowires, before modification, shows normal distribution (Fig. 3). Significantly different conductance changes (samples t-test is 10.22) are recorded with respect to the conductance of phosphotyrosine and tyrosine modified SiNW, when 1M $CaCl_2$ solution contacts two arrays of sensors modified by Tyr and p-Tyr (Fig. 4). Tyr-modified SiNW shows a slight increase in conductance with the Ca^{2+} ions, which is attributable to weak binding of calcium to the surface carboxylate groups while more Ca^{2+} ions bind to the negatively charged phosphate and carboxylate groups on the p-Tyr modified SiNWs. The sensors with p-Tyr termination were also tested for Ca^{2+} and Mg^{2+} ion in wide concentration range (0.1 - 1000µM) (Fig.5). The results indicate maximum conductance change at 10µM Ca^{2+} ions while Mg^{2+} ions show maximum increase at 100µM. Further increase in Ca^{2+} concentration resulted in a decrease in the conductance that can be attributed to the compression of electrical double layer (EDL) caused by a high ionic strength of the aqueous solution [5]. After the surface phosphate groups saturated with Ca^{2+} further increase in their concentration no longer increases positive charges on the surface. Instead, more counter ions (Cl^-) are attracted to the interface and balanced the positive charges of attached Ca^{2+}. Therefore, the surface charge density decreases with the increasing of calcium concentration beyond the peak.

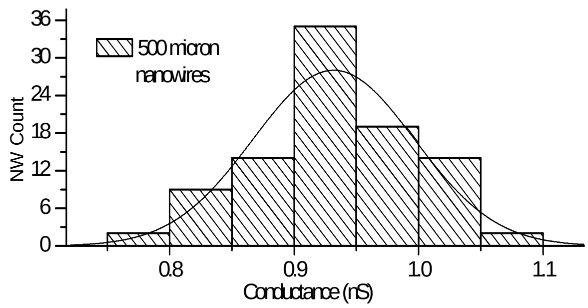

Fig. 3 Statistical conductance data for 500µm long (n-type) nanowire arrays showing normal distribution in an array.

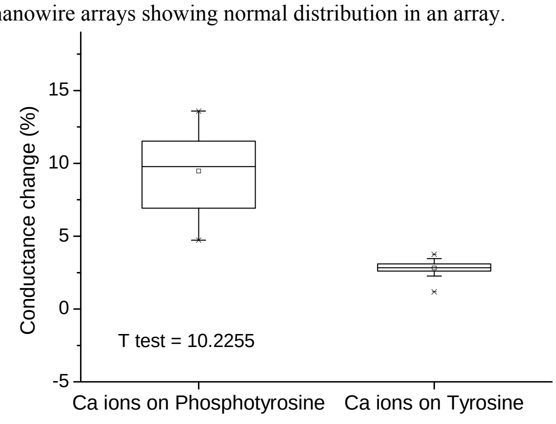

Fig. 4 Percent change in conductance of N-type nanowire sensors in arrays (modified by Phosphotyrosine and Tyrosine) when contacted with 1M $CaCl_2$. Box indicates data spread from multiple sensors in an array.

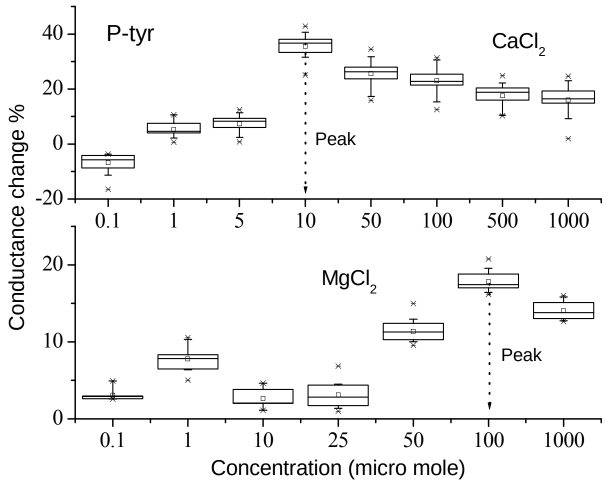

Figure 5: Percent change in conductance of N-type nanowire sensors modified by Phosphotyrosine, when contacted with $CaCl_2$ and $MgCl_2$ in concentration range of 0.1µM to 1000µM. Box indicates data spread from multiple sensors in an array.

4. Conclusions

SiNW modified with p-Tyr can be used as a channel in an FET configuration to detect Ca^{2+} ions in aqueous solution. SiNW's conductance increases with calcium-ion concentration up to 10 µM while the conductance decreases thereafter, which can be attributed to the compression of electrical double layer near the interface. In contrast, the conductance of Tyr-modified SiNWs is independent of Ca^{2+} ions concentration, indicating the importance of phosphate group in the sensing mechanism. SiNW-based sensor also responds to Mg^{2+} ions, but the threshold concentration to cause measurable change conductance is ~10 times higher than that of calcium.

The SiNW sensors integrated with micro fluidics can distinguish the affinities of distinct metal ions in real-time that are significant to the human body. Thus, could serve as a technology platform for understanding and curing of complex diseases and in drug discovery.

References

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