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Label Free Electrical Detection of Single Nucleotide Polymorphisms using Nanowire Biosensors

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1. Introduction

Novel genotyping methods amenable to high throughput analysis should be robust, highly sensitive and simple to use. To this end, silicon nanowires (SiNW) are being used as a sensor element in an array format [1]. We report silicon nanowires based sensors in array format that are used for label-free electrical detection of genotype single nucleotide polymorphisms (SNPs). With suitable surface modifications, peptide nucleic acid (PNA) probes were immobilized on SiNW and the charges due to PNA/ DNA interaction influenced NW conductance hence detecting target DNA up to femto-molar concentration range. The sensitivity and robustness of the system is illustrated by the accurate genotyping of SNPs (3435) based on conductance changes, which is >10 % for the complimentary sequences otherwise it is <3 % using heterozygous DNA target. Statistical electrical characteristics of such SiNWs in an array and the real time conductance changes during DNA hybridization, for 100 and 200 μm long nanowires is also reported.

2. Methodology

SiNW Fabrication

200 mm diameter silicon-on-insulator (SOI) wafers with device silicon: 200 nm thick, P-type and 1-10 ohm-cm resistivity; and 100 nm thick buried SiO_2 (BOX) were used to fabricate nanowires [2] in array format. Trenches were formed in silicon to realize fin/ beam structures up to the BOX, using DUV lithography to define ~100 nm wide fin patterns in an array format (pitched at 2 μm) followed by deep reactive ion etching to obtain silicon fins (width 70-80 nm). The silicon fins were then oxidized in dry O_2 ambient at 900ºC for two to four hours depending on the fin width and target nanowire dimension. Optical image of a biochip having two arrays of 500 μm long nanowires is shown in Fig. 1 (a) while zoom-in Scanning Electron Microscope (SEM) image in Fig. 1(b) indicates nanowires spaced at 2 μm with contact metal lines and passivation layer. Fig 1(c) depicts a Transmission Electron Microscope (TEM) image of nanowire showing rectangular NW cross-section.

Surface Functionalization

Thin silicon nanowire in array, covered with silicon oxide (~2 nm) were thoroughly cleaned and modified by N-[(2-aminoethyl)-3-aminopropyl] trimethoxysilane (silane) followed by succinimidyl 4-(4-maleimidophenyl) butyrate (SMPB) procured from Sigma Chemical Co. (USA), to realize covalent attachment of thiolated oligonucleotides [3] on the surface.

Probe Immobilization

SNP_3435 sequences, an exonic SNP in ABCB1 gene associated with differences in *MDR1* gene expression, plasma drug concentration, drug induced side effects and with drug response, was used to test the accuracy of the biosensor [4]. PNA oligonucleotide probes (18-mer) were designed to have all four possible single-nucleotide mismatches (i.e., A, T, G and C) in the middle of the probe sequence. The probes were modified with a mercapto group at the 5' end whilst the targets were label-free.

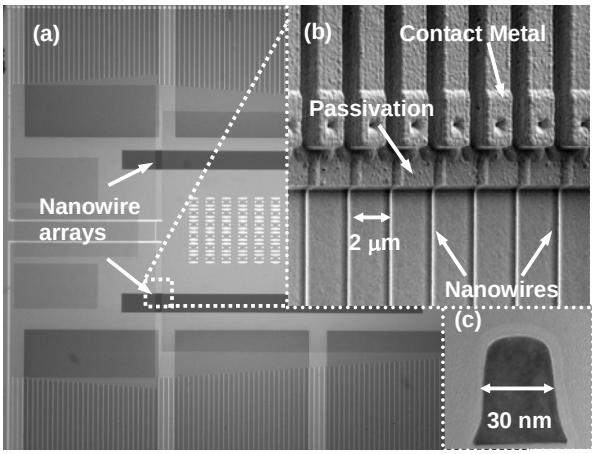

Fig. 1 (a) Optical image of a biochip (b) SEM image of nanowires spaced at 2 μm and (c) TEM image of nanowire showing rectangular NW cross-section.

3. Results

Statistical distribution of nanowires conductance

Each biochip has 100 SiNW sensors in an array format that are monitored for the conductance of each wire. Figure 2 illustrates the statistical conductance data of such an array having N-type 100 μm long nanowires, which follow a normal distribution. Such configuration provides built-in redundancy due to presence of sufficiently large number of sensors at any time, to acquire an accurate representation of the conductance changes due to the presence of DNA targets. The conductance changes of at least 20 nanowires were recorded each time.

Real-time hybridization monitoring

To optimize the hybridization time and to investigate the sensitivity of nanowire's length, real-time DNA hybridization was monitoring. The experiments were conducted on two sets of chips with different lengths of nanowires i.e. 100 μm and 200 μm. The surface of both sets of wires was modified as discussed earlier and termi-

nated with PNA probes followed by contacting the same concentration of target DNA (1 pM). Fig 3 depicts the change in conductance of the two different SiNW sensors as time increased.

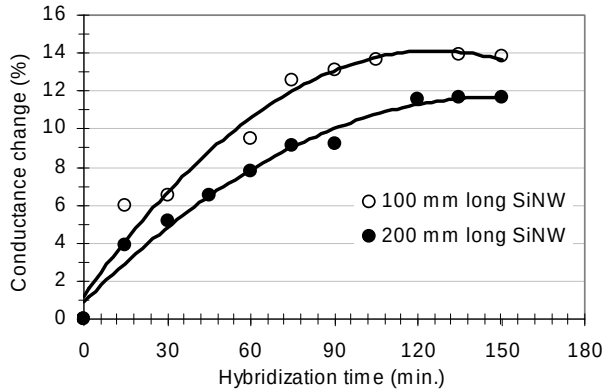

Fig. 3 Real-time conductance changes due to DNA (1 pM) hybridization on 100 μm and 200 μm long Si nanowire modified by complimentary PNA with time.

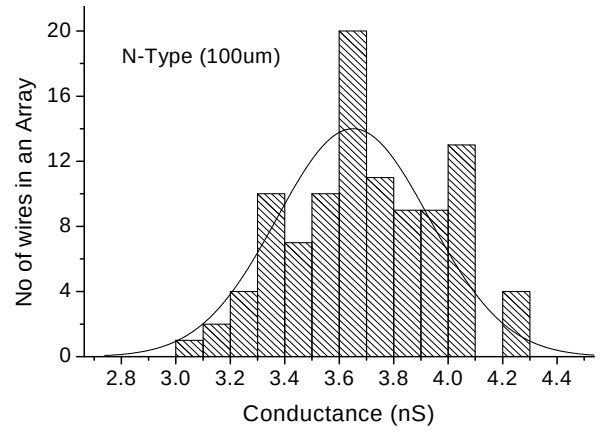

Fig. 2 Statistical conductance data of a SiNW array having 100 N-type nanowires (100 μm long), following a normal distribution.

The change in conductance was experienced for both sets of nanowires as the PNA/ DNA interactions progressed. There was a steady change in the conductance for the first 60 minutes then slowed down and saturated after about two hours; probably as most of the target DNA hybridized onto the immobilized PNA probes by that time. The 100 μm long nanowires indicated larger changes in conductance, suggesting more sensitive to the negative charges of the DNA molecules.

SNP genotyping analysis

The silicon nanowire sensors were also used for SNP genotyping analysis. Accuracy of the genotype determination using the biosensor platform relies on the discrimination ability between a perfect match (PM) duplex and duplex with single mismatch (MM) occurring at the middle position. The probe sequences that are PM duplex could cause the higher change in conductance. Four probes (3435G, 3435C, 3435A and 3435T) were immobilized on the NW surface. Blind heterozygous target complimentary to both 3435G and 3435T was used to simulate as SNP targets, having 1 pM concentration. Fig 4 shows the percentage change in conductance observed by four probes after two hours of hybridization process.

The probes 3435G and 3435T clearly show the much higher change in conductance (10 to 12%) as compared to the other two probes where change is about 3%. This is due to presence DNA sequences in the heterozygous target that are complimentary to 3435G and 3435T only. The capability to distinguish the presence of two targets in a single hybridization buffer solution clearly shows the high selectivity of these SiNW sensors, when suitably modified.

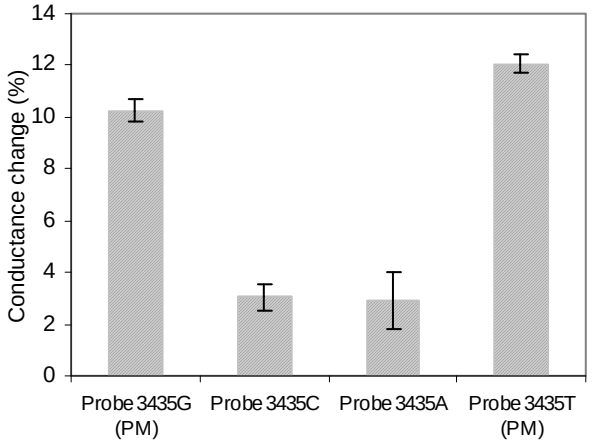

Fig. 4 SNP 3435 detection using label-free heterozygous DNA target; probes 3435G and 3435T being complementary to ssDNA strands in target indicate higher conductance changes.

4. Conclusions

Nanowire based bio-chemical sensors are demonstrated that are highly selective and sensitive. Single base pair sequence mismatches within the DNA target can be easily detected due to the strong PNA/ DNA interaction, making the SiNW sensors a viable detection tool. With such a promising and robust approach to SNP analysis, the future of genotyping in the medical sector or in the environmental sector looks bright and with the possibilities of automation and high throughput capabilities, large-scale associated studies will soon be possible.

The sensors are also significantly important for eliminating the PCR use and by label-free electrical detection extracted DNA can be directly identified; hence reducing experimentation time and operation costs.

References

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