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Highly sensitive covalently functionalised integrated silicon nanowire biosensor devices for detection of cancer risk biomarker

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ABSTRACT

In this article we present ultra-sensitive, silicon nanowire (SiNW)-based biosensor devices for the detection of disease biomarkers. An electrochemically induced functionalisation method has been employed to graft antibodies targeted against the prostate cancer risk biomarker 8-hydroxydeoxyguanosine (8-OHdG) to SiNW surfaces. The antibody-functionalised SiNW sensor has been used to detect binding of the 8-OHdG biomarker to the SiNW surface within seconds of exposure. Detection of 8-OHdG concentrations as low as 1 ng/ml (3.5 nM) has been demonstrated. The active device has been bonded to a disposable printed circuit which can be inserted into an electronic readout system as part of an integrated Point of Care (POC) diagnostic. The speed, sensitivity and ease of detection of biomarkers using SiNW sensors render them ideal for eventual POC diagnostics.

1. Introduction

Highly sensitive, reliable, low-cost, user friendly rapid diagnostic biosensor devices are required for a variety of biological and biomedical applications (Vu et al., 2009). Nanotechnology based biosensor devices have the potential to overcome many of the disadvantages of conventional health diagnostic and monitoring methods. For instance, electrochemical nanoscale biosensors offer the ability to measure biomedical parameters directly and rapidly, without using fluorescent labels. Nanoscale sensors also offer the potential for in vivo sensing.

Semiconducting silicon nanowire (SiNW) biosensor devices are capable of high sensitivity and label-free detection of biomolecular interactions at their surfaces (Ahn et al., 2010; Masood et al., 2010; Gao et al., 2011). SiNW biosensors have been developed for applications including characterisation of protein–protein interactions (Erhola et al., 1997), virus detection (Zhang et al., 2010c) and detection of nucleic acids (Zhang et al., 2010a; Gao et al., 2011) and biomolecules including the prostate cancer biomarker prostate specific antigen (PSA) (Zheng et al., 2005; Ansoon Kim et al., 2007). SiNW biosensors often consist of a conductive silicon channel – functionalised with a "bioreceptor" which is "gated" by the binding of a target disease biomarker to surface-attached bioreceptors. The gating effect results from changes in the surface charge density, which induce a depletion or accumulation region in the SiNW consequently modifying the electrical conductance of the functionalised SiNW sensor (Zhang et al., 2011). Electrochemical detection of even small numbers target biomarker molecules (Aoh et al., 2013) has been reported, with detection limits as low as 1 pg/ml (Zheng et al., 2005) and fg/ml (Ansoon Kim et al., 2007). The detection limit is highly dependent on the diameter of the SiNW devices and to a lesser extent by the SiNW doping.

Investigations of different diameter SiNWs (Elfström et al., 2008; Wu et al., 2009) concluded that the greater sensitivity of smaller diameter nanowires is related to their higher surface to volume ratio. Smaller SiNWs are more influenced by surface charges which induce a depletion or accumulation region in the SiNW, resulting in a greater effect on the conductance/resistance of the SiNW sensor device.

Consequently, many SiNW sensor fabrication processes used a tetramethylammonium hydroxide (TMAH) wet etchant nanowire thinning method to reduce the diameter of the nanowire (Vu et al., 2010; Gao et al., 2011; Kong et al., 2012).

Doping plays a relatively minor role in the sensitivity of the sensor, where the SiNW is lightly or moderately doped (Elfström

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et al., 2008), and fg/ml sensitivity has also been achieved using highly n-doped (3×10^{18} cm^{-3}) SiNWs (Ansoon Kim et al., 2007).

Most researchers use doping concentrations in the range 10^{13}–10^{19} cm^{-3} and are able to achieve detection of biomarkers at concentrations down to 10^{-12}–10^{-15} g/ml (Ansoon Kim et al., 2007; Zhang et al., 2009, 2010a, 2010b, 2010c, 2011; Vu et al., 2010; Gao et al., 2011; Kao et al., 2011; Kong et al., 2012).

Both bottom–up (Cui et al., 2000; Valavanidis et al., 2009) and top–down (Ansoon Kim et al., 2007; Wu et al., 2009) fabrication methods have been used to develop SiNW sensors. The "top–down" fabrication process provides a solution for manufacturing reliable biosensors on a wafer scale – because it is compatible with silicon-based complementary metal oxide semiconductor technology (Ansoon Kim et al., 2007; Zhang et al., 2009, 2010a, 2010b, 2010c, 2011; Vu et al., 2010; Gao et al., 2011; Kong et al., 2012). This is in contrast to the bottom up approach which can yield a more random arrangement of nanowires, but can be used to produce very small diameter nanowires – offering advantages in terms of sensitivity.

SiNW biosensors utilise functionalisation of the silicon surface with bioreceptor molecules. There are several well-known methods for covalent functionalisation of SiNWs including amino termination using (3-aminopropyl)triethoxysilane (APTES) linking chemistry, which has previously been applied to realise DNA and peptide nucleic acid (PNA) attachment to SiNW in DNA/PNA biosensors (Li et al., 2004, 2005; Zhang et al., 2004; Anon., 2005; Gao et al., 2007; Zhang et al., 2009; Ryu et al., 2010), and photochemical grafting using alkene derivatives (Stewart et al., 2004).

In this article we present an electrochemical method for functionalisation of SiNW surfaces (Fig. 1(a)) via an aryl amine linking molecule (Fig. 1(b)). Using chemical functionalisation of SiNW with nitrobenzene, via coupling with an aryl diazonium salt, and subsequent reduction of the nitro group to an amine, aniline can be attached to the SiNW. The amino group of the aniline molecule has been used to graft antibodies targeted against the oxidative stress biomarker 8-hydroxydeoxyguanosine (8-OHdG) onto the SiNW surface (Fig. 1(c)). 8-OHdG is formed through hydroxylation of the guanine base by radical oxygen species (ROS). Following oxidation, damaged DNA is repaired by cellular mechanisms, and the hydroxylated guanine is excreted in bodily fluids. Consequently, levels of 8-OHdG in the blood and urine correlate with the degree of internal DNA damage and 8-OHdG has been used as a marker for impaired metabolism, mitochondrial dysfunction (Cui et al., 2000) and disease modelling (Cui et al., 2001) with links to number of cancers (Cui et al., 2003).

Attachment of surface bound quantum-dot (QD) labelled anti-8-OHdG antibodies to SiNW channels has been verified using Laser Scanning Confocal Microscopy (LSCM). Interaction of the 8-OHdG target biomarker with the "bio-receptor" functionalised SiNW surface has been detected by monitoring conductance changes in response to concentrations of the target analyte as low as 0.1 ng/ml (0.35 nM), using current voltage (*I–V*) measurements. The generic sensor technology can be adapted to selectively and specifically detect other biomarkers – depending on the bio-receptor molecule attached to the SiNW. A hand-held, point of care (POC) system, where the SiNW chip is wire-bonded to a "bio-smartcard" and subsequently slotted in to an electronic readout device, has been developed. The SiNW sensor chip and electronic readout device have been used to detect binding of the 8-hydroxydeoxyguanosine (8-OHdG) biomarker with the SiNW surface within seconds of exposure of the sensor to 8-OHdG. The speed, sensitivity and ease of detection of biomarkers using SiNW sensors render them ideal for eventual POC diagnostics and monitoring devices.

2. Material and methods

2.1. Fabrication of SiNW biosensor array

SiNW arrays were fabricated on 10 mm^2 silicon-on-insulator (SOI) substrates. The substrates have boron doped top Si layers with thicknesses of 88 nm and a measured resistivity of 9–15 Ω cm, implying an approximate doping concentration of 10^{18} cm^{-3}. Lower doped silicon layers have also been investigated, but these yielded unreliable contacts – often Schottky in nature. In order to obtain a reliable Ohmic contact to the SiNW, with consistent and repeatable *I–V* characteristics, a higher doping concentration is desirable. In practice, we achieved low resistance, reliable Ohmic contacts using the 10^{18} cm^{-3} doping. Beneath the Si layer, there is a buried oxide layer with a thickness of 1400–1500 Å, which is supported by an 800 μm thick silicon substrate. The SOI samples were first cleaned using a standard RCA cleaning procedure consisting of solvent, acid and alkali cleaning steps, and incorporating a 10 s hydrofluoric acid (HF) immersion step after both the acid and alkali cleanse. The samples were then rinsed thoroughly in DI water. SiNWs were fabricated using a combination of electron beam lithography (EBL) (Raith E-Line Instrument, Raith) and optical lithography (Mask Aligner, MA/BA8 Gen 3 from SUSS MicroTec). PMMA-coated SOI substrates were spin-coated with PMMA (950 K PMMA:Chlorobenzene=1:3) using a spin speed of 4000 rpm for 40 s, to produce films 256 nm in thickness. The PMMA was subsequently soft-baked at 85 °C for 2 min before exposure to an electron beam for the direct-write EBL process. The SiNW device consists of two micro-sized contact pads at either end of a SiNW (Fig. 1(a)). The PMMA was patterned using EBL parameters: aperture size=30 μm, acceleration voltage=10 kV and beam current=0.20167 nA. The micro-contact pads of the device were patterned using a dose area exposure of 100 $\mu A/cm^2$, and the SiNW channel was patterned using a line exposure dose of

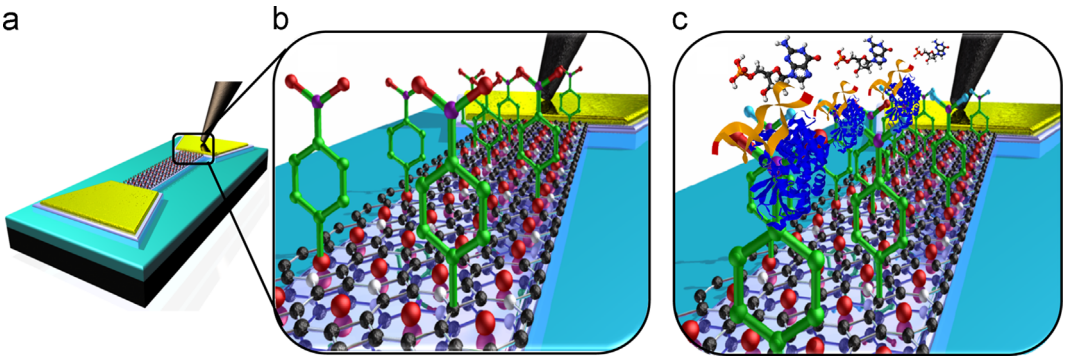

Fig. 1. Illustrations of a SiNW biosensor for detection of targeted 8-hydroxydeoxyguanosine (8-OHdG) biomarker onto the SiNW surfaces. (a) A schematic diagram of SiNW device, (b) thin film of covalently attached nitro-phenyl ($PhNO_2$) groups on the SiNW surface and (c) attachment of the "bioreceptor" antibody anti-8-OHdG to the amine terminated SiNW surface.