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Silicon nanonets for biological sensing applications with enhanced optical detection ability

P. Serre [a], V. Stambouli [b], M. Weidenhaupt [b], T. Baron [a], C. Ternon [a,b,*]

[a] Univ. Grenoble Alpes, CNRS, LTM, F-38000 Grenoble, France
[b] Univ. Grenoble Alpes, CNRS, LMGP, F-38000 Grenoble, France

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ABSTRACT

Optical sensors based on fluorescence methods are used in numerous areas of society, ranging from healthcare to environmental monitoring. But the race to elaborate portable and highly sensitive detection systems leads to the huge development of nanomaterial-based sensors. Here, we have fabricated a silicon nanonet, or silicon nanowire (SiNW) network, -based biosensor for DNA hybridization detection by fluorescence microscopy. We demonstrate that by leveraging the properties of the SiNWs such as their large specific surface and the high aspect ratio, these nanonet sensors have significantly enhanced sensitivity and better selectivity compared to plane substrates. The fluorescence signal shows an intensity increasing with the SiNW density on the nanonet and for the denser nanonets, the detection limit for DNA hybridization is 1 nM. The elaborated Si nanonet-based DNA sensors present more than 50% change in fluorescence intensity between complementary DNA and 1 base mismatch DNA which shows their high selectivity. Finally, we have integrated the Si nanonet-based sensor into a DNA chip and we have shown that this selective sensor can be reproduced on a large scale area.

1. Introduction

It is believed that combining nanotechnologies and biotechnologies will result in a new class of multifunctional devices and systems for biological and chemical sensing characterized by better sensitivity and specificity and higher recognition rates compared with current solutions based on bulk materials (Fortina et al., 2005). Indeed, molecular biologists operate in the domain of molecular and cellular dimensions ranging from several nanometers (DNA molecules, viruses) to several micrometers (cells) while engineers work on reducing material dimensions reaching feature sizes as small as several nanometers. Nano-objects with important analytical applications include nanoparticles, nanopores, nanotubes and nanowires. Among them, silicon nanowires (SiNWs) have attracted an increasing interest in the last decades. By considering their semiconducting properties, precise doping control during growth, high aspect ratio and superior specific area along with surface functionalities of the native oxide shell surrounding the SiNWs, they are indeed very interesting for integration into chemical and biological sensors (Chazalviel et al., 2011; Demami et al., 2012). Previously, individual SiNWs were reported as the sensing material for the detection of nucleic acids (Gao et al., 2011; Hahm and Lieber, 2004), proteins (Stern et al., 2007; Wang et al., 2005) and viruses (Ahn et al., 2010; Lin et al., 2009). However, despite the great potential of such nanowires, the above-mentioned unique SiNW-based sensors usually entail several limitations such as high-cost and time-consuming methods for the integration of the SiNW into functional devices. Indeed, this integration necessitates that an individual nanowire be positioned at a precise location on a substrate, which requires expensive, complex and numerous technological steps.

In the field of biosensing, there are two main classes of signal transduction for biochemical recognition: labeled signal transduction techniques measuring a particle or molecule attached to a receptor and label-free techniques directly measuring physical effects caused by specific biochemical binding events (Gervais et al., 2011). The optical detection using labels is the simplest and the most pervasive detection method used. Particularly, direct detection using fluorescence is simple and very sensitive. Constant improvements of technologies (labels, light sources, detectors, etc.) have contributed to the high sensitivity of fluorescence microscopy. One other possible way to increase further the fluorescence signal is to optimize the surface on which the probe is immobilized. First, it is possible to increase the number of available

* Corresponding author at: Laboratoire des Matériaux et du Génie Physique (LMGP)-Grenoble-INP, Minatec, 3 parvis Louis Néel, CS 50257, 38016 Grenoble Cedex 1, France.
E-mail addresses: pauline.serre@cea.fr (P. Serre), valerie.stambouli-sene@grenoble-inp.fr (V. Stambouli), marianne.weidenhaupt@grenoble-inp.fr (M. Weidenhaupt), thierry.baron@cea.fr (T. Baron), celine.ternon@grenoble-inp.fr (C. Ternon).

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sites for the labeled molecules while reducing steric hindrances and improving target accessibility. Second, working on the optical paths of the signal can increase the amount of excitation or emission light (Gervais et al., 2011).

Herein, we develop a material composed of randomly oriented SiNWs, that addresses these two issues. Also called “nanonets”, for NANOstructured NETworks (Gruner, 2007), such materials show several interesting properties arising either from their individual components, the NWs or nanotubes, or from the structural properties of the network itself (Ternon et al., 2013). First, due to the NWs, the surface area increases in comparison with thin films. As a consequence, it provides a higher probe immobilization capacity (Peterson et al., 2001) while reducing steric hindrance (Murthy et al., 2008). Second, due to the overall geometry, the fluorophore labeled targets are located at varying distances from the reflecting substrate, instead of a unique distance in case of flat surfaces. As a consequence, the optical path is more complex so that the reduction in fluorophore efficiency due to interference phenomena between excitation and emission light disappears (Murthy et al., 2008).

The nanonet elaboration can be carried out by various techniques such as direct growth (Kocabas et al., 2007) or self-assembly from solution for e.g. spray coating (Madaria et al., 2011; Scardaci et al., 2011), Langmuir–Blodgett (Acharya et al., 2006) or vacuum filtration (Mulazimoglu et al., 2013; Wu et al., 2004). The vacuum filtration method, used in this work, is particularly attractive since it enables low cost fabrication of nanonets that are homogeneous over large surfaces and present a wide range of thicknesses. Submonolayer coverage to over 1 μm thick with a precise control of the NW density in the nanonets (Serre et al., 2014, 2015) can be achieved.

In this paper, we describe the elaboration technique of silicon nanonets using the vacuum filtration method and we detail the integration of the silicon nanonet into DNA sensors by presenting the functionalization steps of the SiNW native oxide. We demonstrate the nanonet advantages for DNA hybridization detection by fluorescence, as well as their overall performances. For this purpose four parameters are studied: (i) the detection limit which is the smallest detectable target concentration, (ii) the sensitivity which shows a link between the signal variation of the sensor and the target concentration, (iii) the selectivity which describes the sensor's capacity to detect a target in presence of other species and (iv) the recyclability which expresses the possibility to reuse the sensor without deteriorating its properties. And finally, these sensors were integrated into a DNA chip on a large scale area.

2. Material and methods

2.1. Chemical reagents

APTES, (3-Aminopropyl)-triethoxysilane was bought from Carl Roth and glutaraldehyde 10% was bought from Sigma-Aldrich. Single-stranded DNA (ssDNA) was synthesized by Biomers or Apibio and the different DNA sequences used in this work are given in Table 1. The sensor was designed for the detection of ssDNA target hybridization with complementary ssDNA probes grafted on the sensor. The target was labeled with a cyanine (Cy3) fluorophore, and DNA hybridization was detected by fluorescence measurements. The DNA probes were diluted at 10 μM in a sodium phosphate solution (0.3 M, pH 9) and the target DNA sequences were diluted at concentrations between 0.2 nM and 30 μM in a hybridization solution composed of phosphate buffered saline 0.1 M and NaCl 0.5 M, in deionized water (pH 7).

2.2. Si nanonet fabrication

SiNWs were synthesized by reduced pressure chemical vapor deposition using the classical Vapor–Liquid–Solid mechanism (see Supplementary material). The obtained vertically standing SiNWs were then self-assembled by the vacuum filtration method forming the silicon nanonet (Wu et al., 2004). First, the SiNW solution was prepared by dispersing the SiNWs in 40 mL of deionized water using ultrasonic agitation for 5 min and this SiNW solution was characterized by absorption spectroscopy in order to qualitatively determine the SiNW amount in solution which is related to the SiNW density in the nanonet (Serre et al., 2013). The absorbance at 400 nm of the SiNW solution was fixed at 0.06. Then, the SiNW solution was filtered through a 0.1 μm porous nitrocellulose membrane (47 mm in diameter). As the solvent went through the pores, the nanowires were trapped on the membrane surface forming subsequently the Si nanonet with nanowires randomly oriented as shown on the SEM images of Figs. 3a and 4a. As described in Serre et al. (2015), a systematic SEM image analysis has been performed to determine surface coverage and NW density. Different volumes (20–160 mL) of the SiNW solution were filtered in order to prepare SiNW networks of controllable density, ranging from 10 to 120×10^6 NWs cm^{-2}.

2.3. Sensor design and use

The sensors designed in this work are based on a covalent ssDNA probe immobilization on the Si nanonet surface. First, the Si nanonets were transferred onto Si substrate by membrane dissolution in an acetone liquid bath for 30 min. Then, the DNA immobilization was conducted through a multistep procedure which was described in details in our previous study (Serre et al., 2013): (i) the hydroxylation of Si nanonets on Si substrate using oxygen plasma for 4 min; (ii) the functionalization of the SiNW surface and the Si substrate with an aminosilane (APTES) in vapor phase at 80 °C for 60 min followed by an annealing treatment at 110 °C during 60 min; (iii) the grafting of a cross-linker (glutaraldehyde, 10%) onto the APTES-functionalized surface for 90 min at room temperature and (iv) the immobilization of 10 μM ssDNA probes on the Si nanonet through covalent bonds and the $NaBH_4$, 0.09 M, treatment for 60 min in order to reduce imine bonds. After this

Table 1
ssDNA sequences used for the biochip fabrication (purchased from Apibio) and for the general study (purchased from Biomers).

	Function	Label	Sequence						
Biochip	*non-complementary Probe*	*nonCt-pDNA*	*5′-NH2-TTTTT*	*CCA*	*AGA*	*AAG*	*GAC*	*CCG*	*−3′*
	2 base mismatch Probe	*2bm-pDNA*	*5′-NH2-TTTTT*	*GAT*	*AAA*	***GA***C	*ACT*	*CTA*	*−3′*
	1 base mismatch Probe	*1bm-pDNA*	*5′-NH2-TTTTT*	*GAT*	*AAA*	***G***CC	*ACT*	*CTA*	*−3′*
	ssDNA Probe	**pDNA**	**5′-NH2-TTTTT**	**GAT**	**AAA**	**CCC**	**ACT**	**CTA**	**−3′**
	ssDNA Target	**Ct-tDNA**	**3′-AC**	**CTA**	**TTT**	**GGG**	**TGA**	**GAT**	**AC-Cy3-5′**
General study	*1 base mismatch Target*	*1bm-tDNA*	*3′-AC*	*CTA*	*TTT*	*G**C**G*	*TGA*	*GAT*	*AC-**Cy3**-5′*
	2 base mismatch Target	*2bm-tDNA*	*3′-AC*	*CTA*	*TTT*	*G**CA***	*TGA*	*GAT*	*AC-**Cy3**-5′*
	non-complementary Target	*nonCt-tDNA*	*3′-AC*	*TGG*	*CGC*	*AAT*	*CAC*	*TCT*	*AC-**Cy3**-5′*