Biosensors and Bioelectronics 35 (2012) 218–223

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Multiplexed detection of cardiac biomarkers in serum with nanowire arrays using readout ASIC

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ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form 8 February 2012
Accepted 24 February 2012
Available online 4 March 2012

Keywords:
Silicon nanowire array
Biosensors
Cardiac biomarkers
Serum
Multiplexed detection
Application specific integrated circuit

ABSTRACT

Early detection of cardiac biomarkers for diagnosis of heart attack is the key to saving lives. Conventional method of detection like the enzyme-linked immunosorbent assay (ELISA) is time consuming and low in sensitivity. Here, we present a label-free detection system consisting of an array of silicon nanowire sensors and an interface readout application specific integrated circuit (ASIC). This system provides a rapid solution that is highly sensitive and is able to perform direct simultaneous-multiplexed detection of cardiac biomarkers in serum. Nanowire sensor arrays were demonstrated to have the required selectivity and sensitivity to perform multiplexed detection of 100 fg/ml troponin T, creatine kinase MM, and creatine kinase MB in serum. A good correlation between measurements from a probe station and the readout ASIC was obtained. Our detection system is expected to address the existing limitations in cardiac health management that are currently imposed by the conventional testing platform, and opens up possibilities in the development of a miniaturized device for point-of-care diagnostic applications.

1. Introduction

Cardiovascular diseases, such as myocardial infarction, are the major cause of death among adults worldwide (Mensah and Brown, 2007). A heart attack takes place when the heart muscle is damaged and unable to fulfill its pumping role and distribute blood and oxygen throughout the body. Today the first test performed on a patient presenting himself to the Emergency Department (ED) with chest pain or other heart related suspicious symptoms is an electrocardiogram (ECG), which enables one to investigate whether the heart is beating in unison (Schlant et al., 1992) However, ECG is incapable of detecting cardiovascular diseases at the cellular level as it lacks the required sensitivity (McQueen et al., 1983). Injury to the heart muscle is usually synonym to the death of one or more cardiac cells. Upon their demise, their molecular contents are released onto the nearby tissues and eventually find their way into the blood stream. However, the reliance on a single biomarker for diagnostic purpose has in many cases proven unsatisfactory. It is noted that simultaneous assessment of multiple biomarkers would provide complementary information and enable doctors to stratify risk more effectively and rapidly among patients with acute coronary syndromes (ACS). Sabatine et al. (2002) reported that simultaneous assessment of troponin, C-reactive protein (CRP), and B-type natriuretic peptide (BNP) in ACS provided unique prognostic information. Clinicians could risk stratify patients over a broad range of short- and long-term major cardiac events by using a simple multimarker strategy in which patients were categorized based on the number of elevated biomarkers. Furthermore, Reichlin et al. (2009) found that the sensitive cardiac troponin assays could substantially improve the early diagnosis of acute myocardial infarction (AMI), particularly in patients with a recent onset of chest pain. The early detection of multiple protein biomarkers in the circulation has thus been recognized as the first, and most significant line of defense against this silent but deadly disease.

Challenges of early detection lie in the development of methodologies capable of rapid and simultaneous-multiplexed detection of biomarkers with high sensitivity and good selectivity. Standard assays to detect cardiac biomarkers, like enzyme-linked immunosorbent assay (ELISA) is time consuming (>6 h) and can only be performed in central laboratories of clinics and hospitals. Moreover, the sensitivity of ELISA is still low (~10 pg/ml), which may not facilitate early detection. Silicon nanowire (SiNW) based biosensing has shown promises as an analytical method for protein (Chua et al., 2009; Cui et al., 2001; Patolsky et al., 2004; Stern et al., 2007; Zheng et al., 2005) and DNA (Bunimovich et al., 2006;

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doi:10.1016/j.bios.2012.02.052

Gao et al., 2007; Hahm and Lieber, 2004; Li et al., 2004; Zhang et al., 2008a,b, 2009) analysis with the advantages of label-free, high sensitivity and direct electrical detection. Zheng et al. (2005) reported the SiNWs arrays for multiplexed detection of cancer biomarkers in serum. Very recently, real-time detection of troponin T in serum using the SiNW biosensor has been reported with a high sensitivity (Chua et al., 2009). In both cases, the serum had to be desalted to achieve the real-time detection for purpose of replacing the high ionic strength buffer in serum with the very low ionic strength buffer because a low ionic strength buffer possesses a long Debye length, preventing screening of the electrical signals in the SiNW field effect transistor (FET) biosensor. In addition, the currently developed SiNW biosensors depend on measurement with a probe station by monitoring the conductance/resistance changes between source and drain electrodes induced by recognition event between target molecules and the surface-bound receptors. The probe station is, however, big, expensive and only applicable for single sensor measurement at one time, which hinders the development of miniaturized device for simultaneous-multiplexed detection of biomarkers.

Human cardiac troponin-T (cTnT) is a key protein biomarker that is present in elevated concentrations in the bloodstream of a patient suffering from AMI. CTnT is released from the cell within 3–6 h following symptom onset. It remains elevated for 7–10 days after injury. Creatine kinase (CK) is a dimeric molecule composed of two subunits (M or B). CK was shown to exist in three molecular forms MM, MB, and BB. CK-BB is expressed in all tissues at low levels and has little clinical relevance. When the heart muscle dies during MI, it releases many molecules into the bloodstream, one of the more abundant being CK. Serial measurement of CK and CK-MB over the first 24 h after symptom onset has been suggested to have a high sensitivity and specificity for detecting myocardial injury (Storrow and Gibler, 1999). These biomarkers have been proven to be absolutely essential tools for the diagnosis of myocardial injures (Muller-Bardorff et al., 1997).

Here we present a label-free and simultaneous-multiplexed detection system for cardiac biomarkers, cTnT, CK-MM, and CK-MB, with femtomolar sensitivity in serum using an antibody-functionalized SiNW array biosensor and a readout ASIC. Apart from real-time measurement, a steady-state measurement is adopted in this work. The first resistance value of the SiNW sensor is measured in a low ionic strength buffer solution after immobilization of antibody. The SiNW sensor then undergoes incubation with serum and subsequent removal of unbound proteins by washing. Thereafter the second resistance value is recorded in the same measuring buffer solution. The sensing response is referred to as the resistance change before and after protein bound to the antibody anchored on the SiNW sensor surface. The new detection method is therefore independent of the ionic strength of the sample solution, allowing the SiNW sensor to directly analyze the biomarkers in raw samples. In addition, the readout ASIC allows the measurements of multiple SiNWs to be done simultaneously at a frame rate of kilo-samples per second. Compared to the conventional probe station measurement which is time-consuming (approximately one minute for one sensor measurement), the readout ASIC provides a simple, rapid and miniaturized solution. The good correlation results between the two measurements suggest that the novel detection approach enables simple and rapid multiplexed biomarker detection, pointing to an application for point-of-care disease diagnostics.

2. Experimental

2.1. Materials

3-Aminopropyltriethoxysilane (APTES, minimum 98%), glutaraldehyde solution (50 wt.% in H_2O), human serum and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. O-(2-aminoethyl)-O′-methylpolyethyleneglycol 750 (amino-PEG) was purchased from Fluka. The monoclonal antibody to CK-MM was bought from Abcam Inc. (Cambridge, MA). The monoclonal antibody to CK-MB was obtained from BioDesign International. Both their respective proteins CK-MM and CK-MB were purchased from RayBiotech Inc. (Norcross, GA). Both cTnT and its monoclonal antibody were obtained from HyTest Ltd. (Turku, Finland).

2.2. Fabrication of silicon nanowire biosensor array

SiNW biosensors array was fabricated on 8-in. silicon-on-insulator wafers using a top-down approach. First, lithography and reactive-ion etching were used to pattern fin-like structures on the wafer. Thereafter, oxidation was used to reduce the width of the fin. Then, a phosphorus (n-Type) implantation and activation was used to adjust the electrical properties. Next, metallization and annealing were used to realize metal contact pads and the metal lines connecting them to the SiNW. Finally, passivation layers of nitride and oxide were added to protect the metal lines, leaving only the metal contact pads and the active SiNW biosensors array areas exposed. A process flow diagram for the fabrication of the SiNW chip is shown in the Supporting Information (Fig. S1).

2.3. Antibody attachment onto nanowire array surface

Generally, a two-step procedure was employed to immobilize the antibodies onto the nanowire arrays as described previously (Chua et al., 2009). After the surface was treated with APTES and glutaraldehyde, solutions of monoclonal antibodies to CK-MB, CK-MM and cTnT (all of 100 μg/ml in 1× PBS, pH 7.4), were spotted on the SiNW arrays, and incubated in a moist environment for 2 h at room temperature. After the immobilization, the unbounded antibodies were removed by washing with buffer solution (1× PBS). Subsequently, passivation of the unreacted aldehyde surface groups was carried out with amino-PEG (1 mg/ml in 1× PBS) overnight under 4 °C. The SiNW chips were later washed with the same buffer solution, blow-dried, and immediately sent for electrical measurement. The protocol of the BSA-functionalized SiNW sensor is identical to that of the antibody attachment.

2.4. Protein binding on SiNW array surface

Target proteins in serum were applied to the SiNW surface and incubated for 30 min at room temperature to allow for protein binding. After which, the unbounded analytes were washed away with 1× PBS three times. To test for the selectivity and specificity of SiNW arrays, single protein of CK-MB, CK-MM and cTnT in human serum was separately added onto the SiNWs functionalized with the three different antibodies and BSA. For testing for the sensitivity, all three proteins were spiked into human serum to make various concentrations ranging from 1 ng/ml to 10 fg/ml. A control of non-spiked serum was also prepared and employed in the experiments. The samples were similarly added onto the antibodies-immobilized SiNW arrays for specific protein binding.

2.5. Electrical measurement

Electrical measurements with the probe station were conducted on the SiNWs by connecting a fixed voltage source (100 mV) across the source and drain electrodes of the SiNW using Alessi REL-6100 probe station (Cascade Microtech, Beaverton, OR) coupled with an Agilent 4156 parameter analyzer. Similarly, the same SiNWs were also measured using the readout ASIC. To allow a longer Debye length for detection, the sensing experiments were carried out in an aqueous environment of 0.01× PBS buffer solution, which was