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New insights for using self-assembly materials to improve the detection stability in label-free DNA-chip and immuno-sensors☆

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ABSTRACT

This paper examines reliable advancements in low-cost DNA- and immuno-chips. Capacitance detection was successfully chosen to develop label-free bio-chips. Probe immobilization was rigorously investigated in order to obtain reliable capacitance measurements. Protein probes immobilized by using usual alkanethiols or thiolated ssDNA probes directly immobilized on gold do not allow sufficient stable capacitance measurements. New alkanethiols improved with ethylene–glycol function are shown in this paper to be more suitable materials for capacitive bio-chip development. Atomic Force Microscopy, Quartz Crystal Microbalance, and Capacitance Measurements were used to demonstrate that ethylene–glycol alkanethiols allow high time stability, smaller errors in detection, and improved ideal behaviour of the sensing surfaces. Measured capacitance is in the range of 8–11 nF/mm^2 for antibody layers and close to 6 nF/mm^2 for DNA probes. It is in the range of 10–12 nF/mm^2 and of 4–6 nF/mm^2 for antigen and DNA detection, respectively. The percentage error in detection is highly improved and it is in the range of 11–37% and of 0,23–0,82% for antigen and DNA, respectively. The reproducibility is also improved and it is close to 0,44% for single spot measurements on ethylene–glycol alkanethiols. A molecular theory attributing these improvements to water molecules strongly coordinated by ethylene–glycol functional groups and to solution ions not entering into probe films is finally proposed.

1. Introduction

Label-free detection is a suitable technique delivering fast response and low-cost applications in point-of-care devices. Among them, capacitance transduction attracts researchers since it is easily integrated into CMOS technology. It was initially proposed for immuno-sensors development (Mirsky et al., 1997) and different alkyl thiols were tested for antibody immobilization (Riepl et al., 1999). It was proposed for the detection of gonadotropins (Berggren and Johansson, 1997), interleukins (Berggren et al., 1998), heavy metals (Corbisier et al., 1999), and DNA hybridization (Berggren et al., 1999). The achieved detection limit has been found in the femtomolar range (Bontidean et al., 2003). Detection of DNA was successfully demonstrated onto gold (Guiducci et al., 2004), normal silicon (Balasubramanian et al., 2005) and macroporus silicon (Betty et al., 2004) electrodes. It was also verified by using PNA (Peptide Nucleic Acid) probes (Macanovic et al., 2004). Recently, fully integrated CMOS bio-chips were developed for DNA detection (Stagni et al., 2006, 2007). Some good reviews describing details of this progress were published in the recent years (Berggren et al., 2001; Gabig-Ciminska, 2006; Daniels and Pourmand, 2007). However, despite this large effort in demonstrating the different possible applications of label-free impedance biosensors, the published data are not reliable enough for point-of-care developments. Published data usually present a large time drift (Riepl et al., 1999; Stagni et al., 2006), very large standard deviation (Berggren et al., 2001; Stagni et al., 2007), largely scattered data points (Mirsky et al., 1997; Berggren et al., 1999), poor reproducibility between electrodes (Berggren et al., 1998), and interfacial behaviour too far from that of an ideal capacitor (Carrara et al., 2007). Moreover, specificity is not very high (Berggren et al., 1998) and signals from non-specific molecules are often present (Stagni et al., 2006, 2007). The aim of this paper is to present improvements in terms of measurement stability and reproducibility by using new self-assembly strategies. The work is based on ethylene–glycol alkanethiols invented by George M. Whitesides. They were developed to decrease the interference of non-specific protein binding in Surface Plasmon Resonance biosensors (Ostumi et al., 1999; Horan et al., 1999; Lahiri et al., 1999). In the present article, we demonstrate that ethylene–glycol

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alkanethiols can be successfully used to highly improve impedance biosensors in terms of reliability of capacitance detection. Data from Atomic Force Microscopy, Quartz Crystal Microbalance, Capacitance Measurements are used to show that ethylene–glycol alkanethiols molecules eliminate data time drift and enhance the capacitive stability of DNA-chip and immuno-sensors. As a significant contribution in understanding the field of probe immobilization, we present a molecular theory attributing the stability improvement to glycolate groups of the alkanethiols monolayer.

2. Materials and Methods

2.1. Chemicals

Glycol alkanethiols differently terminated (alkanethiols terminated with $(OCH_2CH_2)_3OCH_2$—COOH; and terminated with $(OCH_2CH_2)_3$—OH) were purchased by Prochimia, Poland. 11-Mercaptoundecanoic acid, NaCl, Na_2HPO_4, KH_2PO_4, KCl, H_2O_2 50%, Standard sample of Bovine Serum Albumine (BSA), and absolute ethanol were obtained from Sigma–Aldrich. H_2SO_4, 96%, was purchased from Carlo Erba, Italy. All the chemicals were used without further purification. Antibodies against the "Squamous Cell Carcinoma Antigen" (SCCA) biomarker, and SCCA were purchased by Abnova GmbH (Heidelberg, DE).

2.2. Surfaces Preparation

DNA probe surfaces made with SH-terminated ssDNA were obtained by immobilizing oligonucleotides directly onto gold electrodes (for a schematic view, see drawing (A) reported in on-line available Supplementary file # 1). A well known protocol was used (Carrara et al., 2007). Probe surfaces made with alkanethiols were formed by using two different kinds of *Self Assembled Monolayers* (SAM) onto gold electrodes. Alkanethiols SAM without ethylene–glycol functionalization (from now on will be referred to as "non-EG-alkanethiols"-for a schematic view, see drawing (B) reported in on-line available Supplementary file # 1) were obtained from a 1 mM ethanol solution of 11-mercaptoundecanoic acid ($HS(CH_2)_{10}CO_2H$). Ethylene–glycol alkanethiols SAM (from now on "EG-alkanethiols"-for a schematic view, see drawing (C) reported in on-line available Supplementary file # 1) were prepared from an ethanol solution of $(HS(CH_2)_{11}(OCH_2CH_2)_3OH$ 1,96 mM and $HS(CH_2)_{11}(OCH_2CH_2)_3OCH_2COOH)$ 0,04 mM. The mixture was obtained with a ratio between OH-terminated and COOH-terminated thiols equal to 50:1. Both kinds of SAM were formed by using well known self-assembly procedures (Lahiri et al., 1999). Before measurements, the SAM samples were left in PBS (Phosphate Buffered Saline, water solution with 137 mM NaCl, 10 mM Phosphate, 2,7 mM KCl, at pH 7,4) buffer in dark for 24 h. This conditioning was necessary to further stabilize the capacitance measurements on the so prepared electrode chips. SAMs were formed onto template stripped gold (TSG) for AFM investigations, crystal quartz resonators for QCM investigations, and chips with interdigitated electrodes geometry for capacitance measurements. NH-terminated ssDNA probe molecules (Carrara et al., 2008) and antibodies (Lahiri et al., 1999) were immobilized onto the SAM monolayers by means of N-hydroxysuccinimide (NHS) and of Ethyl-Dimethyl-aminopropyl Carbodiimide (EDC) to form covalent bonds. Target DNA and antigen molecules were incubated following well established procedures.

2.3. Capacitance Measurements

10 μl of buffer drops were spread on top of each single sensing spot of the bio-chip for capacitance measurements. The used buffers were PBS and TE (water solution with 10 mM tris(hydroxymethyl)aminomethane, 1 mM ethylenediaminetetraacetic acid, and 0,1 M NaCl at pH 8) for measurements on proteins and DNA, respectively. Individual bio-chips were measured using an array biosensors measurement station developed in our lab. To improve the capacitance acquisition, the charge-based capacitance measurements (CBCM) (Stagni et al., 2007) technique was implemented in a PCB circuit.

2.4. AFM Measurements

Atomic force microscopy (AFM) imaging was performed in tapping mode with PointProbe nanocontact silicon probes mounted in a Nanoscope IIIa SFM system equipped with a multimode head and a type A piezoelectric scanner (Veeco, Santa Barbara, CA, USA). The images were acquired in ethanol using a 'liquid cell'. Raw images were processed only for background removal (flattening) using the microscope manufacturer's image processing software package.

2.5. QCM Mass Measurements

Functionalizations with alkanethiols, probe and target molecules were also checked using a quartz crystal microbalance (QCM). The used QCM apparatus was a home made instrument. The instrument was built by following a resonant circuit realized for the same purpose (Facci et al., 1993). Instrument calibration was done by drop casting of incremental amounts of standard Bovine Serum Albumine (BSA) onto the electrodes of crystal quartz resonators (Rickert et al., 1997). The single 5 MHz QCM crystals (catalogue code 151247–5) were purchased by ICM (International Crystal Manufacturing Co., Oklahoma City, USA).

3. Results and Discussion

3.1. Capacitance Increase in Probes Immobilization and Antigen Detection

Fig. 1 compares the behaviour of probe surfaces obtained with HS-terminated ssDNA immobilized directly onto gold and with NH-terminated ssDNA immobilized onto a film prepared by using

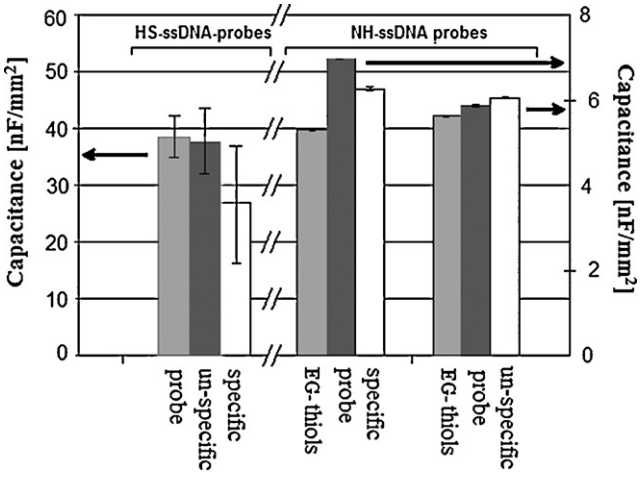

Fig. 1. Capacitance measurements acquired on probes DNA, non-specific, and specific DNA hybridized molecules. The comparison is between the usually considered immobilization technique by using HS-terminated ssDNA probes immobilized on gold (data on the left) and the new technique proposed in this paper based on NH-terminated ssDNA covalently bound to a preformed ethylene–glycol alkanethiols monolayer (data on the centre and left). The reported data errors are calculated as three times the standard deviation in measurement series acquired upon the time on the same chip spot. The errors correspond to the 99% of statistical data significance. All the measurements were carried out in TE buffer.