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Capacitance DNA bio-chips improved by new probe immobilization strategies

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ABSTRACT

Label-free DNA detection plays a crucial role in developing point-of-care biochips. Capacitance detection is a promising technology for label-free detection. However, data published in literature often show evident time drift, large standard deviation, scattered data points, and poor reproducibility. To address these problems, mercapto-hexanol or similar alkanethiols are usually considered as blocking agents. The aim of the present paper is to investigate new blocking agents to further improve DNA probe surfaces. Data from AFM, SPR, florescence microscopy, and capacitance measurements are used to investigate new lipoate and ethylene-glycol molecules. The new surfaces offer further improvements in terms of diminished detection errors. Film structures are investigated at the nano-scale to justify the detection improvements in terms of probe surface quality. This study demonstrates the superiority of lipoate and ethylene-glycol molecules as blocking candidates when immobilizing molecular probes onto spot surfaces in label-free DNA biochip.

1. Introduction

Medical diagnosis requires point-of-care biosensor arrays at the patient's bed. This requirement is due to new emerging demands for personalized therapies because therapeutic agents amount in tissue and blood serum is different on a patient-by-patient basis [1]. Therefore, the development of low-cost, point-of-care technologies for array biochips is a necessary step to introduce personalized therapies in clinical practice. The usually considered micro-array technology based on optical detection and molecular labeling is costly and time consuming. Thus, it is not adapted for applications to personalized therapy in hospital or at home. Label-free capacitance DNA biochips are a valid solution as they present many advantages. After the initial works of Mirsky [2,3], the application of capacitance detection for DNA [4], interleukin [5] and heavy metals [6] was extensively investigated in late 90s. After 2003, the capacitance detection was pursued by increased vigor as demonstrated by more recent works published by different research groups. It was showed that this detection principle can be applied to DNA hybridization by immobilizing single strand probe DNA molecules onto gold [7] and silicon [8], as well as PNA probe molecule [9]. Capacitive method has been used to reach femtomolar concentration range in metal ions detection [10], to check antibody affinity on macroporus silicon [11] and gold [12]. Finally, the possibility to develop fully integrated DNA biochip was demonstrated [13,14]. Good reviews about this large effort were published summarizing applications for pathogenic detection [15], and mechanisms of impedance change [16]. However, the usually considered probes immobilization does not present enough stable capacitance properties. Evident time drift [2,13], large standard deviation [14], scattered data points [4], and poor reproducibility [5] usually affect the detection signals. All these phenomena are related to electrode/solution interfaces which are not a perfect insulator [17]. Thus, the sensing capability is reduced. Special efforts are dedicated word wide to probes surface improvement in biosensors. New materials based on lipoamide (the lipa-DEA [18,19]) and on ethylene-glycol [20,21] were applied to improve surface plasmon resonance (SPR) biosensors. A large decrease of non-specific adsorption on probe surfaces was demonstrated for both lipoates [18] and ethylene-glycol alkanethiols [21]. The aim of this paper is to demonstrate that these new probe functionalizations assure an improved stability in DNA detection by using capacitive biochips, too. The paper presents original investigations on lipa-DEA and ethylene-glycol monolayers. The study was conducted by

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comparing with performances obtained with the usually considered mercapto-hexanol. Results from AFM, fluorescence, SPR and capacitance measurements on chip are used to demonstrate that these new functionalizations are innovative and suitable to improve label-free DNA biochip detectors.

2. Materials and methods

2.1. Biochip fabrication

A standard lift-off process is used to pattern the gold electrodes onto glass substrates. To improve adhesion between substrate and electrodes, first a 20 nm layer of chromium is deposited, followed by a 200 nm layer of Gold using thermal evaporation. The entire surface is covered by a thick (10 μm) layer of AZ1512 photoresist as passivation layer. Individual sensor spots are exposed by developing AZ1512. The chip contains a total of 32 square electrode arranged in groups of four electrode pairs. The electrode square side is 200 μm and the electrode's separation is 20 μm.

2.2. Capacitance measurements

Charge-based capacitance measurement (CBCM) technique is employed to improve capacitance estimation. With this method, capacitance values are estimated by measuring the current transient due to RC behavior of the electrodes/solution bio-interface driven by a square bias voltage. This bias is used to drive the equivalent capacitance between working and reference electrodes of each sensing area of the biochip. As shown in Fig. 1, the current at the electrochemical bio-interface may be written as

$$i(t) = I_{dc} + i_C(t) \tag{1}$$

by accounting for some leakages passing through the bio-molecular layer. The average charging-current and the interface capacitance are then related by [14]:

$$I_{AVG} = \frac{I_{dc}}{2} + \frac{1}{T}\int_0^{T/2} i_C(t)\,dt = \frac{I_{dc}}{2} + CV_{Step}f. \tag{2}$$

This equation shows a direct relationship between the average current of square driving signal and its frequency. The slope of this linear relationship returns the bio-interface capacitance. Fig. 1 also shows a possible CMOS implementation of this approach. In the design, a pulse generator is used to drive four switches required to generate the squared bias. The current emerging from the bio-interface drives other four switches to rectify the current transients which are used as input of an integrator. The integrator output is directly related to the current average flowing through the bio-interface. A sweep in frequency and the subsequent acquisitions of different average currents enable the estimation of the bio-interface equivalent capacitance by means of a simple computational algorithm. A VLSI biochip implementing the CMOS architecture reported in Fig. 1 has been realized [14]. However, a precise estimation of the equivalent bio-interface capacitance from Eq. (2) is possible only if that capacitance is not varying with the frequency. Unfortunately, it has already been demonstrated that the interface equivalent capacitance is varying in frequency both on bare gold electrodes [14] and on DNA probes directly immobilized onto gold electrodes [17].

Therefore, an alternative method for precise capacitance estimations has been proposed to overcome this problem. The frequency-to-capacitance measurement (FTCM) technique [13] is based on the relation between RC parameter and charging time at the bio-interface. In this case, a current generator charges the equivalent capacitance and the potential of that charging is used as input of a comparator, as shown in Fig. 2(A). Once the comparator threshold is overcome, then the comparator output switches up and the input polarity at the capacitance is reversed as well as that of the threshold generator. Thus, the bio-interface discharges towards negative potentials till the reversed threshold is reached, as shown in Fig. 2(B). Considering the exponential shape of the charging curves, the period of comparator switching may be related to threshold by the equation [13]:

$$T = 2RC\ln\left(\frac{1}{1-((V_{ref})/(I_{ref}R))}\right). \tag{3}$$

By adjusting current and voltage references, an approximated relation between the frequency of comparator switching and the capacitance of bio-interface is then possible:

$$f = I_{ref}(2V_{ref}C)^{-1}. \tag{4}$$

Therefore, an easy and precise measure of the frequency by means of usual counters returns a precise estimation of the bio-interface equivalent capacitance. Although this method avoids the problem of frequency-dependant capacitances, unfortunately it cannot address the problem of time-trends in frequency signals if the bio-interfaces are not enough stable [13]. The problem of bio-interfaces not enough stable-in-time may be addressed by developing special DNA probe immobilizations. In our laboratory, both the above mentioned methods for precise estimation of bio-interfaces capacitance have been tested by considering different kinds of DNA probe immobilization.

2.3. Bio-interface chemicals

Ethylene-glycol functionalized alkanethiols differently terminated ($SH–(CH_2)_{11}–(OCH_2CH_2)_3OCH_2COOH$, and $SH–(CH_2)_{11}–(OCH_2CH_2)_3OH$) were purchased from Prochimia, Poland. 11-Mercaptoundecanoic acid ($HS–(CH_2)_{10}COOH$), 6-mercapto-1-

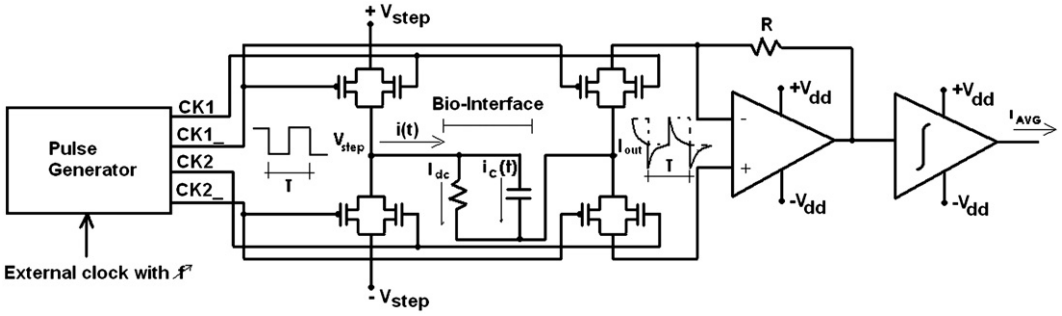

Fig. 1. Schematic drawing of a CMOS architecture implementing the charge-based capacitance measurement (CBCM) technique for precise measurements of bio-interface capacitance.