A Biosensor for Chromosome to Detect Genetic Disorder

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Abstract— The large demand for Chromosome analysis calls for the development of portable, low-cost, easy-to-perform assays. We developed and performed preliminary assessments of an integral biosensor for detecting genetic disorder and diseases. The device behaves consistently with a proposed electrical model and it reliably detects chromosome hybridization with high specificity for genetic disorder. We have also verified the reproducibility of the experimental results and the reusability of the Chromosome biosensor.

Key words: Chromosome, Portable, Easy-to-use Biosensor, genetic disorder

I. INTRODUCTION

Molecular detection has shown a great potential for rapid identification of diseases and for food and environmental monitoring. After the recent successes in sequencing the human genome, the detection of specific chromosome sequences in biological chromosome has been playing a fundamental role in genetic diagnostic and in the detection of patogens in cells.

The large demand for low-cost genetic assays has lead to the development of portable and easy-to-use biosensors. These systems should be able to perform the analysis in a very short time and with a very limited amount of specimen. Micro-fabricated structures, based on micro and nano-technology can satisfy these requirements and also allow a high degree of parallelism and sensitivity. The final aim of these micro-systems would be to integrate on the chromosome substrate the site of the reaction, the sensor, and the circuit for the conditioning and the amplification of the output signals. Two of the main desired characteristics of a bio-sensor are the close proximity of the biological recognition element to the transducer and the use of a label-free method to avoid lengthy and complex pre-treatments and unwanted alterations of the chromosome.

Our application of these principles has been focused on the direct detection of Chromosome from the measurement of the changes in the electrical parameters of a structure that interacts with the Chromosome molecules in solution. The possibility of detecting Chromosome at an electrode/solution interface through capacitance measurements has been demonstrated. However, the set-up used in previously reported experiments do not allow easy integration.

We implemented a prototype sensor whose elements and circuitry are standard and easy to integrate. The recognition takes place at the electrode/solution interfaces of a two-electrode cell. One or both the employed ultra-flat gold electrodes are functionalized with reactive layers that can capture single-stranded oligonucleotides or double-stranded chromosome molecules. The chromosome-sequence-specific binding to the electrode (the sequence recognition) was investigated with self-assembled monolayers (Chromosomes) of oligonucleotides probes that had been designed to capture oligonucleotides with complementary sequences thanks to base-pairing. The presence of layers and molecules on gold surfaces was verified independently by atomic force microscopy (AFM) imaging.

Cell capacitances were measured using capacitance- to-current transducers derived from the charge-based capacitive measurement circuits used to measure on-chip wiring capacitances in deep sub-micron ICs [5, 6]. A periodic pulse is applied to the cell, while measuring the average current needed to repeatedly charge the unknown capacitance. We have performed comparative capacitance measurements with cells equipped with bare gold electrodes, functionalized electrodes (with oligonucleotides) and functionalized electrodes that has been exposed to Chromosome. The device has shown high specificity for Chromosome hybridization detection and good reproducibility. We also verified the reusability of the Chromosome biosensor.

II. PREVIOUS WORK

According to the electrostatic model, a charged surface/electrolyte solution interface can be modelled as a series of capacitors. The surface charge is balanced by a region of oppositely charged ions (counterions) known as the diffuse electrical double layer. The layer closest to the surface, made up of the counterions bound to the surface and water dipoles, is called Stern or Helmholtz layer; the second layer consists of a diffused atmosphere of hydrated counterions, whose characteristic length corresponds to the Debye length and is known as the Gouy-Chapman layer.

If a metal electrode surface is functionalized, any charged molecule captured at the interface with the electrolyte produces a change both in the capacitance structure and its electrical behavior.

Consequently, the presence of molecules bound to the surface transduces into a capacitance change. Recent works presented several methods for the analysis of the electrical parameters of composite interfaces. The most relevant are chronoamperometry [3] and impedance spectroscopy [8]. Both of them require a three electrode cell and a potentiostat.

Chronoamperometry consists in applying a single step to the cell and measuring the relaxation constant of the structure. Impedance spectroscopy is based on impedance frequency measurement and parameters fitting of an empirical electrical model. These are electrochemical analysis systems, they need a reference electrode and an experimental set-up which is not easy to integrate. Fortunately, for the sole purpose of detecting molecules bound to the surface, full-blown electrochemical interface characterization techniques are not strictly needed. We implemented a system which measures the changes in the total charge stored on a couple of functionalized electrodes after a voltage step. Perkinsetal. [9] Applied a similar

rationale transducing charge changes through a field effect transistor. Our setup is fully integrable and made up of standard components.

III. EXPERIMENTAL METHOD

A. Surface Functionalization

The gold electrodes were obtained by the template stripped technique [10]: a 200 nm thick gold layer was evaporated on freshly-cleaved ruby mica in high vacuum, subjected to prolonged heating in vacuum and glued on glass cover slips (approximately 1 cm in diameter) with high-performance epoxy-glue. At the time of use, the mica strip is peeled off and the freshly exposed ultra-flat gold surface is chemically functionalized (or used as a reference).

Two kinds of CHROMOSOME targets were chosen to hybridise with the CHROMOSOME: short oligonucleotides or longer double- stranded CHROMOSOME molecules. Two oligonucleotides have been tested: the first is 26-base long and complementary to the surface-bound probe oligonucleotide (5'-GATG-z; the second is 27- base long and it is not complementary to the probe sequence. As a long double-stranded CHROMOSOME target, we used pBR322, a 4361-base pair long CHROMOSOME that has a 15-base tract complementary to the oligonucleotide probe. The hybridization was implemented by heating the functionalized gold disc up to 90°C, covering its surface with hot oligonucleotide solution and slowly cooling it down to room temperature. AFM imaging was performed to verify the presence of a self-assembled monolayer (CHROMOSOME) of oligonucleotides immobilized on the gold surface: as shown in Figure 3,the presence of a flat layer was evidenced through the application of a local high vertical force by the scanning probe, which removed a quadrangular portion of the layer, thus exposing the underlying gold surface [11].

B. Capacitance Measurement

To fully understand the measurement technique, we describe a simplified electrical model of the structure (see Figure 4). The parallel capacitance between the two gold electrodes is negligible. The capacitive behaviour of the structure is determined by the two interface capacitances, which consist of the biological recognition element and the electrical double layer. We measure the capacitance variation of the electrical double layer at the electrode/solution interface. According to the equivalent circuit of Figure 4, when electrodes A and B are kept at the CHROMOSOMEe voltage level there is no quiescent current flowing across the cell and no voltage drop across the capacitors. On the other hand, when V(A) = V(B) + ΔV aquiescentcurrent IDC = ΔV/(2Rp+Rs) appears that gives rise to a voltage drop Δ□1= RpIDC across each capacitor.

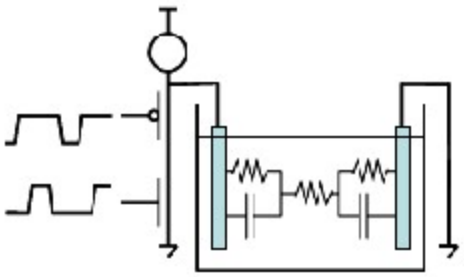

Fig. 1Capacitance Measurement

If a pulse is periodically applied to electrode A, and the period is long enough to allow the capacitors to be completely charged/discharged at each cycle, then the average current drawn by the cell during the pulse is the sum of two contributions: the quiescent current IDC and the charging current that provides the amount of charge needed by the two (unknown) capacitors to sustain the voltage drop. The system employed an HP8011A pulse generator, an Agilent34401A multi-meter to measure the average current and a TektronixTDS3032 oscilloscope to monitor the pulse frequency.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

Capacitance changes can be monitored by measuring the average current flowing through the cell during discharge at different frequency. The current measurements have been made with a pulse sequence with 500 mV step amplitude. In our set-up, one of the two electrodes is always bare gold, while the other is the one subjected to surface modifications. The total capacitance between the two gold electrodes is proportional to the slope of the current-frequency linear regression (Equation 2).

A. Surface Functionalization with A CHROMOSOME of Oligonucleotide

As described in the methods section, we have prepared a gold electrode that carries a CHROMOSOME of thiol-modified oligonucleotides. The presence of the CHROMOSOME causes a reproducible decrease of the capacitance of the structure. An independent verification of the presence of the CHROMOSOME has also been obtained by "nanoshaving" with the AFM (see Figure 3). In Table 1 are reported the results on current measurements throughout the experiments. The last row reports the calculated values of the slope of the linear regressions. This behaviour is in accordance with the proposed model of an increase of spacing between the charges in the electrical double- layer induced by the oligonucleotide film, thus leading to a reduction of the capacitance [3].

B. Detection of CHROMOSOME Hybridization and De-Hybridization

The oligonucleotides of the CHROMOSOME on the electrode surface have been hybridized with the 26-base complementary oligonucleotides, in order to verify if hybridization could be detected through a capacitance variation. An additional reduction in the measured capacitance was found. This experimental result is in accordance with previous findings [3] and it could be explained with the CHROMOSOME rationale of an increased spacing between charged layers in the capacitor.

To verify the reversibility of the measuring technique, we removed the oligonucleotides that bound to the probe-oligonucleotides thanks to a heat treatment (heating above oligonucleotide melting temperature and rinsing in the CHROMOSOME conditions to remove the detached oligonucleotides). Such de-hybridization method is not expected to disrupt the CHROMOSOME thanks to the stability of the sulphur-gold bonds. The measure (see Table 1) shows a good reversibility (an increase in capacitance after de- hybridization). Un additional cycle of hybridization confirms that the reset device can be reused.

C. A Demonstration of the Sequence-Selectivity of the Biosensor:

A different oligonucleotide (with a base-sequence non complementary to the probes) has been employed to test for a capacitance change due to a specific target binding to the CHROMOSOME surface. The measurements have demonstrated that the device is not sensitive to non-complementary oligonucleotides (see Figure 5) confirming his suitability as a hybridization detector.

D. Detection of the Hybridization of a Long Double-Stranded CHROMOSOME

The 4361 base-pair long pBR322 CHROMOSOME has been used for preliminary tests of the detection of a specific short CHROMOSOME sequence in a long molecule in solution. A nanomolar concentration of pBR322 has been laid on the surface and subjected to a similar heating-cooling cycle as the oligonucleotides of the previous experiments. The experimental results are consistent with the measurements of the oligonucleotides.

V. CONCLUSIONS

We have designed and tested a prototype of an integrated biosensor for the direct detection of CHROMOSOME sequences. The sensor has proved to function reproducibly, to be insensitive to aspecific sequences, and to be reusable after desorption of analyte CHROMOSOME.

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