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Evaluation of capacitive surface stress biosensors

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ABSTRACT

The evaluation of a capacitive type biosensor array, consisting of a total of 256 biosensing elements, in the detection of single oligonucleotide mutations is presented. The biosensor takes advantage of surface stress changes during biological interactions and is able to translate them into a capacitive signal. The array is organized in a 16 × 16 matrix of distinct biosensing elements thus allowing for the concurrent sensing of multiple biological targets. In this work the sensing elements of the array are spotted with three different oligonucleotides (CD8, CD17 and CD19) and their hybridization is detected using 36 nM PCR. Moreover tests with CD19 revealed the ability of the biosensor to detect the hybridization of the oligo with sample concentrations of 36, 18 and 9 nM.

1. Introduction

Miniaturized and label-free systems such as electrical biosensors and surface stress based biosensors have recently received a lot of attention due to the potential they have to be integrated into portable point of care (PoC) systems. The electrical biosensors can be amperometric, voltametric, impedance or capacitive sensors [1–3]. Surface stress based biosensors are usually bimorph microcantilevers with optical or piezoresistive readout [4] or alternatively capacitive micro-membranes [5–8].

Surface stress based biosensors require flexible structures, microcantilevers or membranes, where one of their surfaces is functionalized with the probe biomolecules. The interaction with the appropriate target molecules induces surface stress variations and finally changes in the deflection of the structure. Microcantilever biosensors provide high sensitivity and the ability of parallelization into arrays for simultaneous multiple sensing through the use of differently functionalized cantilevers. In most cases, cantilever bending is detected optically [9–11] or by utilizing the piezoresistive effect [12]. Capacitive membranes, similarly to cantilevers, exploit the variations of the surface stress induced when the probe molecules on the functionalized surface interact with their target counterparts. The sensing element of a capacitive membrane biosensor consists of a rigid fixed electrode implemented on the substrate, a cavity and the flexible membrane electrode overhanging the cavity. The probe molecules are immobilized on the membrane surface and upon interaction with the appropriate target molecules, the membrane deflects due to surface stress variations. The deflection is translated into a change in capacitance between the flexible electrode and the substrate.

In the present work, the capacitive sensing elements, shown in Fig. 1, are parts of a 2D array (16 × 16) and are composed of an ultrathin silicon membrane passivated by a 0.5 um thick low temperature oxide (LTO) layer that also serves for the functionalization of the biosensors. The sensor array fabrication and its response to the detection of protein interactions have been presented in [6,7]. The evaluation of the devices for effective DNA sensing is presented in the following sections.

2. Experimental

2.1. Sensor array description

The capacitive biosensor incorporates an array of 16 × 16 sensing elements that occupy an area of 5 mm × 5 mm on a silicon die. The operation principle of the sensors relies on the deflection capability of an ultrathin silicon membrane and the consequent translation of the deflection changes into capacitance changes.

The basic capacitive sensing element is depicted in Fig. 1. The capacitor plates are a highly boron doped flexible silicon membrane and a fixed phosphor doped counter electrode realized in the underlying substrate. The spacing between the capacitor plates is determined by the thickness of a silicon dioxide layer that supports the membranes in their periphery. A cavity etched in the SiO_2 layer allows for the membrane deflection. On the membrane surface, prior to testing, probe molecules are immobilized and waiting for binding with their respective target counterparts.

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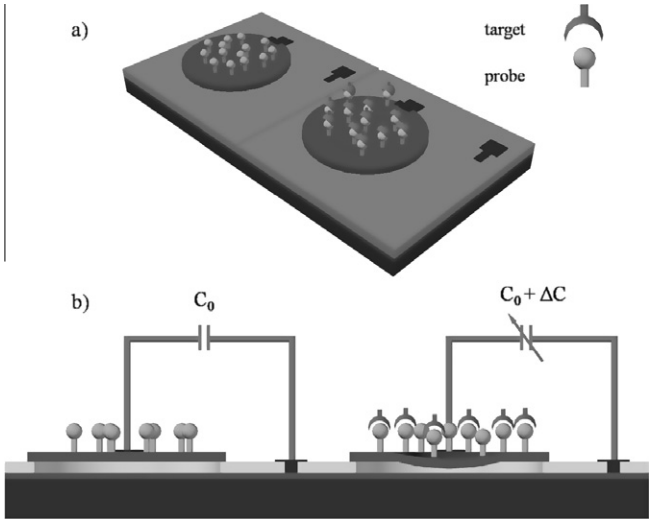

Fig. 1. Schematic of the capacitive sensing element: (a) top view, (b) cross section view.

When this happens, the stress that arises on the membrane surface causes the membrane to deflect, effectively changing the capacitance between the flexible electrode and the substrate.

In order to form the sensor matrix, each sensing element is aligned onto thin stripes (rows) cut out of the device layer of an SOI wafer. Aluminum (Al) lines running perpendicular to these stripes connect the sensors in the other direction (columns) as shown in the close-up photograph of the array in Fig. 2. Thus each capacitor can be addressed by its corresponding column and row. Finally, in order to protect the sensor array from the reaction solutions the whole area of the sensor die is covered by a passivating low temperature oxide (LTO). The complete 16 × 16 biosensor array die measures 12 mm × 12 mm to allow for integration with the hybridization chamber.

2.2. Experimental setup

The sensor array was mounted onto a printed circuit board (PCB), which provides the necessary electrical connections to the measurement setup and is hosted within a hybridization cassette. Within the hybridization cassette, a polydimethylsiloxane (PDMS) gasket restricts the biological reaction over the active area of the

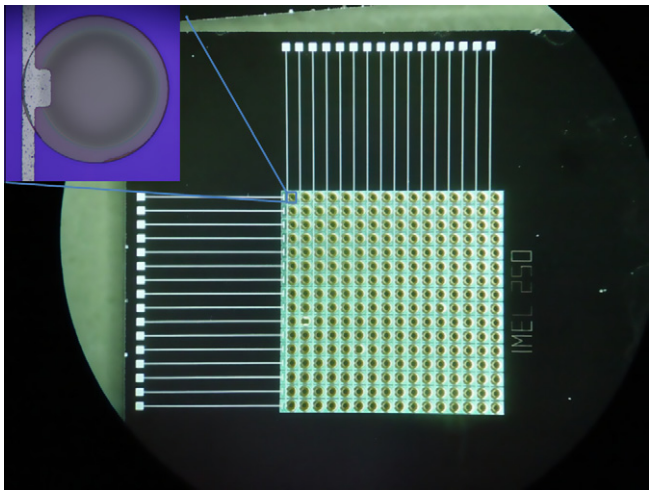

Fig. 2. Fabricated biosensor array. In the inlet a biosensing element is presented.

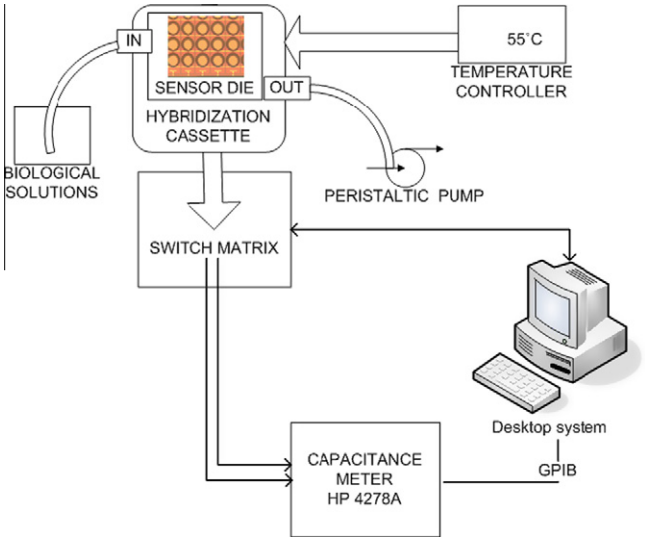

Fig. 3. Experimental setup. In a typical experiment the following solutions are inserted in the hybridization chamber: (1) tetramethylammonium chloride buffer (TMAC); (2) ddH_2O; (3) the PCR product of the complementary strands, which remains into the chamber for several hours; and (4) TMAC in order to wash away the nonspecifically bound molecules.

array while at the same time accommodates one fluidic input and one output to allow for the controlled insertion of reaction fluids and necessary buffers. In order to allow for the scanning of the whole array, the PCB edge was designed to be compatible to Peripheral Component Interconnect (PCI-Express) plugging directly in a standard connector on a 16 × 16 switch relay matrix. Control of the whole system is performed via a Labview program running on a PC, able to control the switch matrix and an LCR bridge, and thus enabling the concurrent measurement of multiple sensors.

To operate the system, 0.5 μm inside diameter (ID) silicone tubing is used to implement the fluidic ways and a peristaltic pump is introduced at the exit of the microfluidic system. The pump draws the solution out of the tank and into the hybridization cassette and chamber and then out to an external waste tank. The time needed for the fluids to enter the hybridization cassette is measured during each experiment and is taken into account in order to know exactly when the solutions flow above the sensors. Finally, temperature is controlled within 1 °C using a temperature controller and a hotplate, onto which the hybridization cassette is positioned. The schematic view of the experimental setup is shown in Fig. 3.

3. Results

The sensor arrays used in the experiments were composed of sensing elements with Si membrane diameter of 250 μm and 0.5 μm thickness, while the thickness of the SiO_2 onto which it stands and separates the two electrodes of the sensor is also 0.5 um. The DNA hybridization experiments were performed using beta-thalassaemia oligonucleotides. Namely: oligonucleotides CD19, CD17 and CD8. To immobilize the oligonucleotide probes on the sensor surface, the top LTO layer of the capacitive micromembrane was functionalized by coating its surface with (3-Aminopropyl)tris(trimethylsiloxy)silane (APTTMS) and activating it with 1,4-Phenylene diisothiocyanate (PDITC). Then, in order to immobilize the probes, oligonucleotides which contain an amino group at their 5′ end were used.

In order to explore the sensitivity limits of the array, biosensor dies were spotted with the CD19N and the CD19M oligonucleotides. In particular, some sensing elements were covered with

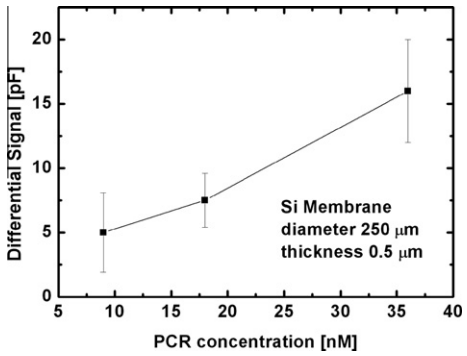

Fig. 4. Average response of the CD19N functionalized sensors, in respect to the mutated, to 36, 18 and 9 nM PCR concentration.

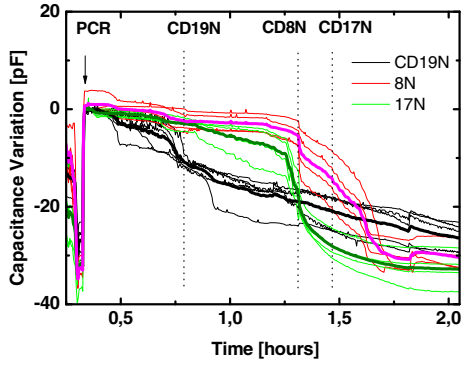

Fig. 5. Response of individual sensors and average response (thick lines) for 36 nM PCR. Three different oligos (CD19N, CD17N and CD8N) are depicted.

CD19N normal probe molecules and some with CD19M mutated probe molecules. On the remaining elements neither probe was immobilized in order for them to be used as reference. Out of the two types of probe molecules, only CD19N were fully complementary to the target molecules.

The hybridization itself took place at 55 °C. This temperature is determined by the design of the oligonucleotides (length, GC content) and on the hybridization buffer. For these experiments tetramethylammonium chloride (TMAC) containing buffer was selected in order to eliminate the dependence of the melting temperature on the G–C content of the probe, thus allowing the stringency of the hybridization to be controlled as a function of probe length only. Overall, the hybridization experiments included the sequential insertion into the hybridization chamber of the following solutions: (1) TMAC buffer; (2) double distilled water (ddH_2O); (3) the PCR product of the complementary to CD19N strands, which remained into the chamber for several hours; and (4) TMAC buffer in order to wash away the non-specifically bound molecules.

During the experiments, the capacitance values of sensing elements containing both types of probes and also of the reference membranes were monitored simultaneously during the hybridization process. Three different sample concentrations (36, 18 and 9 nM) were used to hybridize the immobilized probes on the sensor surface. In all three cases the hybridization was detected successfully (Fig. 4).

In another array, two more probes (CD17 and CD8 normal) were immobilized on different sensors in the same array die and measured in 36 nM PCR. The hybridization of the three oligonucleotides was successfully detected and monitored in real time with each of the three oligos being hybridized on its own time (Fig. 5).

4. Conclusions

Capacitive detection of DNA hybridization using a Si micromechanical biosensor array was demonstrated. The deflection variations of the ultrathin Si membranes are transformed into capacitance changes between the membrane and the substrate. The array proved able to distinguish between different species when it was spotted with three different oligonucleotides (CD19, CD17 and CD8). Moreover, it has been tested in the detection of oligonucleotide hybridization with concentrations down to 9 nM using CD19 probes. Finally, it should be stressed out that one of the main advantages of the device is that it does not require labeling thus reducing sample preparation while at the same time it enables capacitive detection of biological interactions. Capacitive type of sensors are passive devices and therefore are low power and thus are very suitable for portable type devices like for instance for Point-of-Care diagnostics.

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