FABRICATION OF A LABEL-FREE MICROMECHANICAL CAPACITIVE BIOSENSOR AND INTEGRATION WITH μPCR TOWARDS A LoC FOR DISEASE DIAGNOSIS

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ABSTRACT

A label-free micromechanical, capacitive type, biosensor is presented and evaluated. The sensor is integrated together with a continuous flow μPCR on a common printed circuit board (PCB) platform. In this scheme, the sensor is placed into a cavity formed on the PCB to provide the hybridization chamber, while the μPCR channel is implemented in thin photo-patternable polyimide films to maintain biocompatibility, and lies directly on top of the microheaters formed on the PCB Cu layer to provide the three thermal zones necessary for PCR.

KEYWORDS

Lab-on-Chip, μPCR, capacitive biosensors, microfluidics, μTAS

INTRODUCTION

Surface stress based biosensors are of great interest, as they allow label-free sensing resulting in simplified sample preparation, small size and ability for parallelization into arrays for high throughput analysis [1-2]. However, for any biosensor implemented for successful DNA detection, it is necessary to multiply beforehand the DNA in the sample under examination, so as to bring the concentration within the limits of detection of the device. This task is routinely achieved using the polymerase chain reaction (PCR) in order to replicate DNA and is widely used in bio-analysis such as microbial detection and medical diagnosis. Recently, the development of miniaturized PCR (μPCR) devices has led to faster process and decreased cost for fabrication and use, while it promotes integration of such devices with biosensors into portable lab-on-a-chip (LoC) systems.

In this work, we present a micromechanical 64 element capacitive biosensor array fabricated using an improved (over the one used in [3]) self-aligned process and which targets the detection of β-thalassemia mutations - a wide spread condition around the Mediterranean Sea. Each element of the array is an ultrathin round Si membrane on the surface of which receptor probes are immobilized and which is able to deflect upon binding events between the probes and target molecules in its vicinity. The sensor is integrated with the necessary μPCR in one solid, integrated platform implemented on a PCB. Use of a PCB as an integration platform allows for the implementation of both the microfluidic and electrical circuit necessary for sensor connections as well as of a continuous flow μPCR device on the same chip. The DNA-containing sample will enter the system and first go through the μPCR part, where the DNA will be multiplied. Next, the sample will be carried through microfluidic channels to the biosensors silicon chip, where the detection will take place.

EXPERIMENT-RESULTS

To fabricate the sensor array, direct bonding between a Si wafer holding the membranes and a substrate wafer is required. The process begins by first forming a 5000Å thick thermal SiO_2 layer on the membrane wafer. Circular sensor cavities are then formed in the oxide using optical lithography and CHF_3 anisotropic dry etching. Next, a boron ion implantation follows through the openings in the thick oxide. This step creates a highly boron doped region which subsequently forms the flexible membrane after wet etching. The whole procedure, effectively, aligns the sensor membrane placing it over the sensor cavity in which it is allowed to deflect. Next, the membrane wafer is bonded with the substrate wafer which has gone through phosphor implantation followed by thermal annealing to render it conductive and form the sensor fixed substrate electrode. The wafer stack is then grinded mechanically, from the membrane wafer side, down to about 50μm while the rest of the wafer is etched in EDP solution until the ultra-thin Si membranes are revealed. The process then goes on with Al metallization and low temperature oxide (LTO) to passivate the device (Fig. 1).

To test the array performance, 15mer synthesized thiol-modified oligonucleotide probes and corresponding targets were used. In order to immobilize the probes on the arrays, the latter were first functionalized with 3-glycidoxypropyl-tri-methoxy silane (GOPTS). Selective spotting of the probes on each membrane was then performed using the Laser Induced Forward Transfer (LIFT) technique. Three kinds of probes were used in this experiment: probes which were fully complimentary (FC) to the target, non-complimentary (NC) to the target and probes having one discrepancy to the target. All three kinds were immobilized on various sensors of the array as well as the Al reference capacitors. First experimental results indicate that the sensors are able to detect the hybridization of 10uM synthesized 15mer oligos (Fig. 2).

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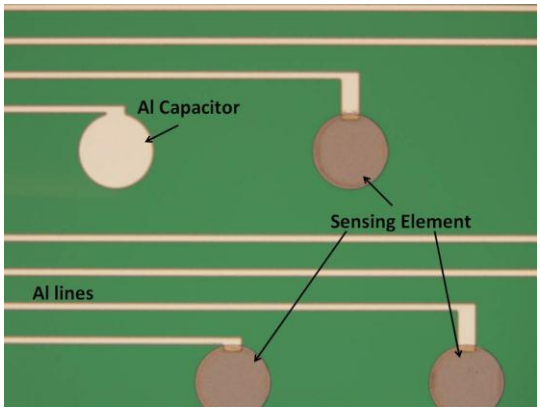

Figure. 1 Image of part of the sensor array consisting of membranes (sensing elements) and an Al capacitor.

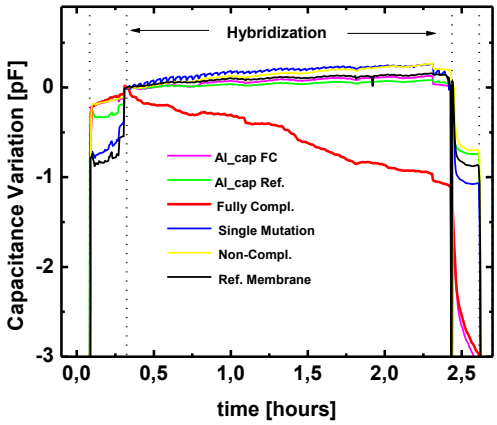

Figure. 2 The average response of several sensing elements during DNA hybridization of 15mer model oligos.

The complete design of the LoC including the integrated μPCR and biosensor system is shown in Figure 3 and is compatible with an instrument which will be responsible for flow and temperature control of the LoC, where necessary. The μPCR microfluidic channels are placed directly over the heaters (depicted in red) which are designed to form three heating zones [4], so that by means of cycling the sample through them, in continuous flow, DNA multiplication can be achieved. Heat transfer computations for one thermal cycle of the whole μPCR [5] indicate good temperature uniformity within each thermal zone and are in agreement with the experimentally determined small power consumption for the device operation. The sensor itself sits in a chamber (for hybridization) in the right hand side of the chip which accommodates two microfluidic inputs and two outputs to ensure even transfer of the sample and complete coverage of the chamber area. Finally, the chip is completed with a pre-concentration chamber placed before the sensor and a waste chamber at the end of the microfluidic circuit, where pumping is implemented.

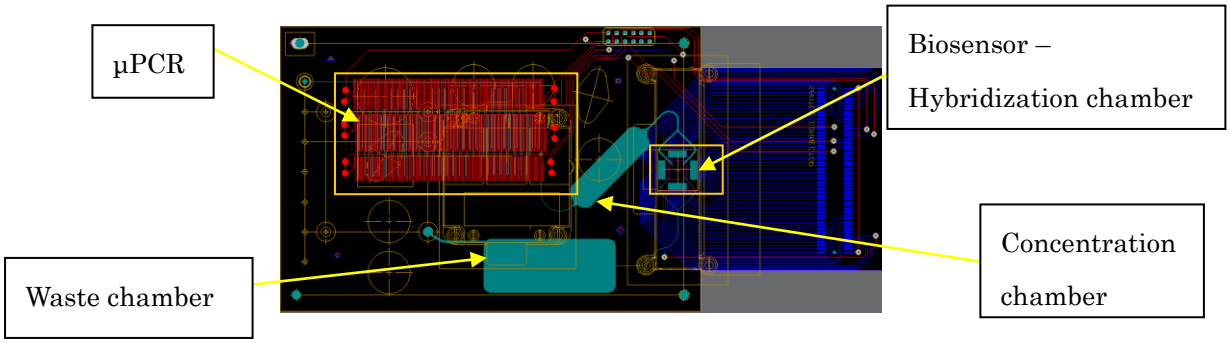

Figure 3. Complete design of all LoC layers

The fabrication process of the integrated system, shown schematically in Fig. 4, starts with a two-side copper cladded standard PCB. First the cavity into which the sensor sits is carved out of the PCB using mechanical drilling (LPKF ProtoMat S62). Next, the electrical connections and the three microheaters are formed on the copper layers of the PCB using standard PCB processing (photolithography and Cu etching).

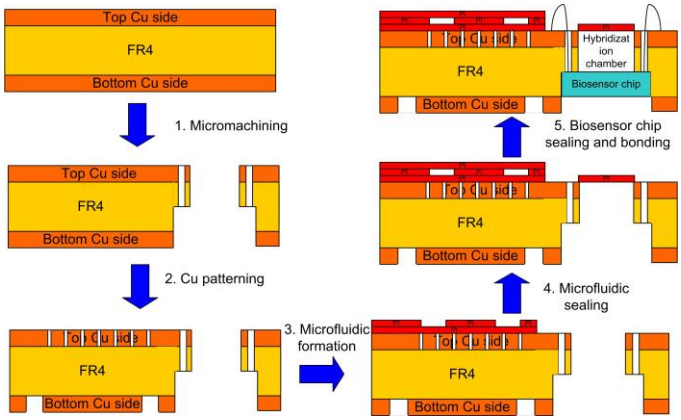

Figure 4. LoC fabrication process flow

After this step, the microfluidic channels and chambers are formed on the top side of the PCB. The μPCR

channels are placed directly on top of the microheaters, so as to minimize the power losses of the heated device. In order to form the microfluidic circuit, a first thin polyimide film is laminated onto the PCB to provide a biocompatible bottom surface for the channels. Then, another thin photo-patternable polyimide film (Dupont PC1015) is laminated over the first polyimide film, exposed to UV radiation through a mask and developed to form microchannels 150um wide and 30um deep. At the same step, the waste chamber and a concentration chamber is formed at the microfluidic circuit end, and between the μPCR and the biosensor, respectively. In the following step, the Si biosensor chip is fixed in the carved cavity formed at the beginning of the process and its Al pads are wire-bonded to the Cu PCB pads, so that the electrical connections to the external read-out equipment can be established. In the final step, the microfluidic circuit and sensor chamber are sealed with a third laminated film and microfluidic ports in the sample inlet are attached. A view of the chip after the formation of the microfluidic circuit on the polyimide is shown in Fig. 5 and 6.

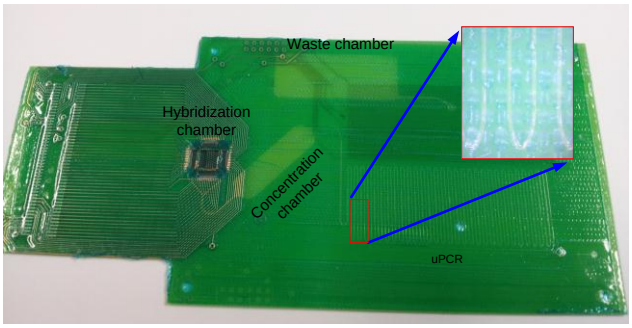

Figure. 5 Fabricated microchannels.

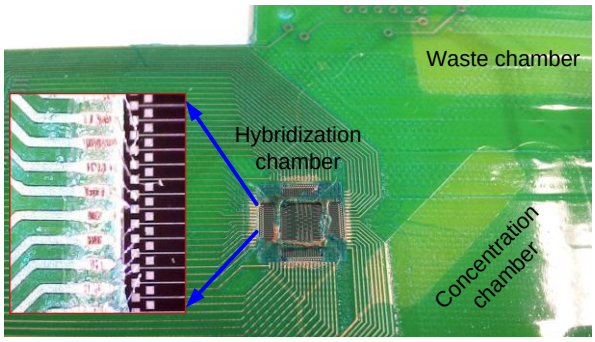

Figure. 6 Hybridization chamber with biosensor chip wire bonded to the Cu pads of the PCB to provide electrical connections.

CONCLUSIONS

A label-free micromechanical, capacitive type biosensor has been presented and evaluated. A sensor integration strategy with a microfluidic circuit which also includes a μPCR implemented within a thin polyimide film laminated on a PCB has been drawn and key processing steps were demonstrated. The whole process has the potential of integrating on one chip both microfluidic as well as electrical function required for biosensor-based disease diagnosis on an integrated LoC system.

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