A Temperature-Controllable Microelectrode and Its Application to Protein Immobilization

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ABSTRACT—This letter presents a smart integrated microfluidic device which can be applied to actively immobilize proteins on demand. The active component in the device is a temperature-controllable microelectrode array with a smart polymer film, poly(N-isopropylacrylamide) (PNIPAAm) which can be thermally switched between hydrophilic and hydrophobic states. It is integrated into a micro hot diaphragm having an integrated micro heater and temperature sensors on a 2-micrometer-thick silicon oxide/silicon nitride/silicon oxide (O/N/O) template. Only 36 mW is required to heat the large template area of 2 mm×16 mm to 40 °C within 1 second. To relay the stimulus-response activity to the microelectrode surface, the interface is modified with a smart polymer. For a model biomolecular affinity test, an anti-6-(2, 4-dinitrophenyl) aminohexanoic acid (DNP) antibody protein immobilization on the microelectrodes is demonstrated by fluorescence patterns.

Keywords—BioMEMS sensor, microelectrode, micro hot plate, smart polymer, immobilization.

I. Introduction

The microelectromechanical system (MEMS) technique in biosensors offers important new opportunities due to advantages including the possibility of mass production, reduced unit costs, and good performance [1]. In particular, temperature control in microdevices with components for heating and sensing is of practical importance, enabling accurate control of required functions or reactions. Several research groups have demonstrated that resistive microheaters on silicon chips can be utilized to give localized temperature control in microfluidic devices [2], [3]. Microfabricated electrodes have also been used for detection and microfluidic control [4]. When a microelectrode is laid down near microheater lines, the heat is very advantageous for controlling surface temperature precisely at the micrometer-scale electrode surface. As device scales become smaller, interactions at the sensing surface begin to dominate overall sensor performance [5]. Recently, passive self-assembled monolayers (SAMs) have been widely used in microfluidic-based biosensors; however, it is becoming evident that greater functionality would be added to devices with the use of active interfacial coatings [6]. Smart polymers have attracted much attention for biomedical applications due to their capability to change their surface chemistry, such as hydrophilic-hydrophobic phase transition in response to stimuli including heat and pH. One of the most representative thermo-responsive polymers is poly(N-isopropylacrylamide) (PNIPAAm). To combine this function into the microdevice, in which rapid and precise heating and cooling can be obtained, is crucial for the development of the next generation of biomedical microdevices [6], [7].

In this letter, we present a novel temperature-controllable microelectrode array incorporated with a detection part on a single chip and its applications for active biomolecular immobilization.

II. Experiment

The proposed device consists of a Si bulk-micromachined component and a hot-cast poly(dimethylsiloxane) (PDMS) component. The Si component consists of a heater, temperature...
sensors, and an electrode array for immobilization and patterning of proteins and electrochemical sensing integrated on a thin, freestanding layer consisting of a silicon oxide (0.8 µm)/silicon nitride (0.4 µm)/silicon oxide (0.8 µm) membrane over a silicon frame designed for good thermal isolation, low thermal mass, and stress-reduction, as shown in Fig 1(a). The fabricated microfluidic device with a chip size of 12 mm×28 mm×2 mm is shown in Fig. 1(b). The fabrication process was designed to be simple and suitable for reliable mass production, using five-inch wafer-level silicon CMOS protocols with 4 photomasks. Creating a silicon bottom component and a PDMS top component are part of the fabrication process [8], [9], as shown in Fig. 2.

A smart polymer, NHS-PNIPAAm, was chemically synthesized based on radical polymerization. After carrying out the surface modification along with the self assembly of the monolayer and the G4 poly(amidoamine) dendrimer nanoscale layer on the electrodes [8], thin film gold surfaces in the microfluidic device were chemically modified with the heat-sensitive bio-recognition layer. Then, the electrode surface was manipulated based on the structural transition of PNIPAAm by changing the surface temperature of the electrodes across its lower critical solution temperature (LCST).

III. Results and Discussion

The resistive thermal device (RTD) was designed to enable rapid and accurate temperature measurements, through the calibration curve for the heater-RTD element. The temperature coefficient of resistance in the RTD was approximately 2,450 ppm/℃ in this device.

The temperature-consumption power performance and thermal response curve of the temperature-controllable microelectrodes are shown in Fig. 3. The power consumption versus the temperature measured at the RTD highlights the extremely low electrical power consumption, which is critical for application in portable battery-powered microsystems. Only 36 mW was required to heat the large diaphragm area of 2 mm×16 mm to 40℃ within 1 second. Passive cooling was enough to decrease the membrane temperature to an environmental level due to the low thermal mass, despite its relatively large area. Temperature ramping cycles were examined between 18℃ and 35℃ across an LCST of about 26℃, and satisfactory heating and cooling results were observed. The thermal response for heating was as fast as 25℃/s due to the low thermal mass and good thermal isolation from the use of the thin diaphragm configuration of the chip.

To observe the micropatterns of differentiated protein immobilization with fluorescence microscopy, a sequence of affinity reactions with the anti-DNP antibody was employed with the scheme shown in Fig. 4. An FITC-conjugated anti-DNP antibody immobilization was then conducted under two conditions, using reduced and extended forms of PNIPAAm above and below the LCST, respectively. The two modes were named A and B for simplicity. With the protein-immobilized and FITC-labeled surfaces, Figs. 5(b) and (c) show the images of the two micropatterned microelectrode arrays under modes A and B. The testing DNP amount was 0.02 mg/ml, and the total reaction time was 10 minutes. The two distinct images appear to be positive (A mode) and negative (B mode).
IV. Conclusion

A novel temperature-controllable microelectrode for active biomolecular immobilization was developed through microfabrication protocols by using a heat-sensitive polymer, PNIPAAm, in a microfluidic biosensor. The temperature-addressable microelectrode served as a temperature-modulation platform and biosensing surface. The micro hot membrane showed a power efficiency of $1.1 \, ^\circ C/mW$ and a ramping rate of $25 \, ^\circ C/s$. Only $36 \, mW$ was used to heat the hot diaphragm area of $2 \, mm \times 16 \, mm$ to $40 \, ^\circ C$ within 1 second. NHS-PNIPAAm was synthesized, and the surface modification along with the polymer and dendrimer nanoscale layer on the electrodes was achieved. As a model affinity test on the microelectrodes, an anti-DNP antibody micropatterning on the microelectrodes was carried out and evaluated by patterns generated from fluorophores-tagged antibodies.

References