온도 조절이 가능한 트립신 전처리 반응칩의 제작

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Fabrication of the temperature controllable microreactor for trypsin treatment

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Abstract - In the research of proteomics, mass spectrometry analysis is the essential method for identification of the unknown proteins. Trypsin treatment for the sample preparation of mass spectrometry is the inevitable procedure [1]. However, sample preparation procedure is cumbersome and time consuming. To resolve these problems, Temperature controllable microreactor was designed and fabricated. It consists of metering chamber, micro channel, reaction chamber, platinum (Pt) thin film heater and a temperature sensor so that micro metering and mixture of reagent with temperature control can be done on the same chip. The total size of the fabricated microreactor was 37×30×1 mm^3 and the size of channel cross section was 200×100 μm^2. PID temperature controller was realized using NI DAQ, PCI-MIO-16E-1 board and LabVIEW program.

1. Introduction

Although DNA/RNA is easier to work with, there are limitations to the information that can be derived from DNA/RNA analysis. DNA sequence analysis can not predict if a protein is in an active form. RNA quantitation can not always reflect corresponding protein levels. With the completion of the Human Genome Project, the emphasis is shifting to the protein compliment of the human organism. This has given rise to the proteomics, the study of all the proteins produced by cell type and organism. There are several major technologies used in proteomics. Common analytical techniques incorporated in proteomics include mass spectrometry, electrophoresis, and liquid chromatography. Since the mass spectrometry analysis is the only method for identification of the acquired protein samples, it is essential for the research of proteomics. In this essential analysis, sample preparations such as purification and segmentation are also inevitable key procedure. Since proteins can not be amplified, sample preparation is critical to the outcome of proteomics experiments [1]. Trypsin is the most discriminating of all the proteolytic enzymes in terms of the restricted number of chemical bonds that it will attack, Good use of this fact has been made by chemists interested in the determination of the amino acid sequence of proteins. However, the current limitation of the trypsin treatment is that it is a cumbersome and time consuming work. Thus, it is worthwhile to make trypsin treatment easy, simple and fast. Therefore, there is needed to be improved. The proposed microreactor can meter and mix reagent with the precise temperature control in sequence on the same chip (Fig 1). And, it needs only small volume of reagent so that the reaction time can be reduced. Since the trypsin treatment is one of the common enzym reactions, the accurate temperature control also can reduce the reaction time. For manual fluidic operations such as metering, moving and mixing, two inlets for air injection were added to micro channel. And, the metering chamber which is fabricated accurately with MEMS technology acts as a volume indicator. For high performance temperature control, we constructed PID feedback controller using LabVIEW program and data acquisition board.

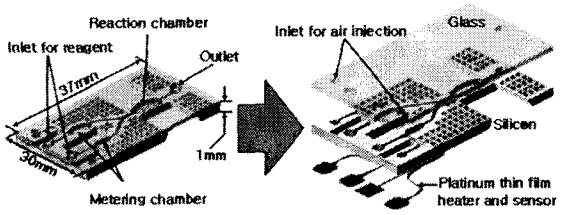


Fig 1. Schematics of the proposed temperature controllable microreactor.

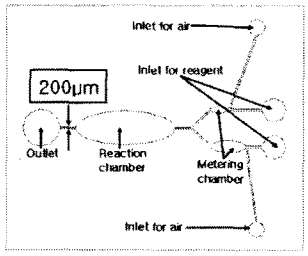
2. Design and fabrication

Fig. 2 shows layouts of the temperature controllable microreactor. the chambers has an elliptical shape to prevent air bubble generated during injection. the area of the chambers was

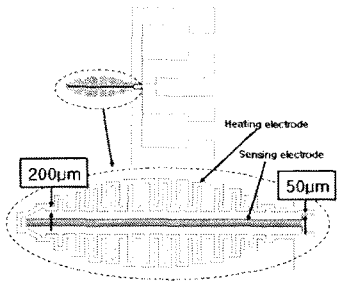
designed with respect to the reagent volme for the fixed depth of 100 μm. The volume of two metering chamber are 3 μl and 1 μl and that of reaction chamber is 4 μl. Pt was chosen for the material of temperature sensor because Pt has the optimum characteristics for service over a wide temperature range and Pt resistance thermometers are the international standard for temperature measurments [2, 3]. By the following equation for the resistance of a bulk material, the resistances of heater and sensor were designed to be around 1 KΩ and 0.5 KΩ, respectively.

$$R = \frac{\rho L}{A} \quad (1)$$

where R is the resistance of Pt (Ω), ρ is the resistivity (Ω · cm), L is the length of conductor (cm) and A is the cross-section area of conductor (cm^2). Pt was deposited to the depth of 1000Å. The widths of Pt thin film metal lines for heater and sensor are 200 μm and 50 μm, respectively.



(a)



(b)

Fig 2. Layouts of a temperature controllable microreactor: (a) layout of a deep RIE pattern (b) layout of a heater and sensor.

Fig. 3 shows fabrication process. The starting wafer is a double-side polished silicon (100) substrate with a diameter of four inches and a thickness of about 500 μm. At first, micro channel and chambers were patterned by photolithography then the silicon was etched to the depth of 100 μm by deep reactive ion etching (DRIE). With the removal of the photoresist, Si_3N_4 layer was deposited with a thickness of 5000 Å. The Si_3N_4 layer was patterned by photolithography and dry etched down to the silicon on the opposite side. With the removal of the photoresist, the silicon was etched to the depth of the 490 μm using KOH (30 wt%, 80 ℃, 6.5 hours). After this, Si_3N_4 layer was etch away which is on the side of DRIE pattern. The glass substrate, with a diameter of four inches and a thickness of 500 μm, was cleaned with H_2SO_4 and laminated by a BF410 film photoresist. The BF410 photoresist was patterned by photolithography to form inlet and outlet holes. By using a sand blast, holes were formed through the glass. After alignment, the etched silicon wafer was anodically bonded with the glass substrate. After anodic bonding, the remainder of silicon was etched by DRIE for the wet etched holes to penetrate the silicon substrate. A Ti film of 300 Å and a Pt film of 1000 Å were deposited on the bottom side of wafer by thermal evaporator. Using NP-9 negative photoresists, patterns for heater and sensor were developed. After etching Pt with the etchant ($7HCL+HNO_3+8H_2O$ at 85 ℃), the completed wafer was diced into individual temperature controllable microreactors (Fig 4).

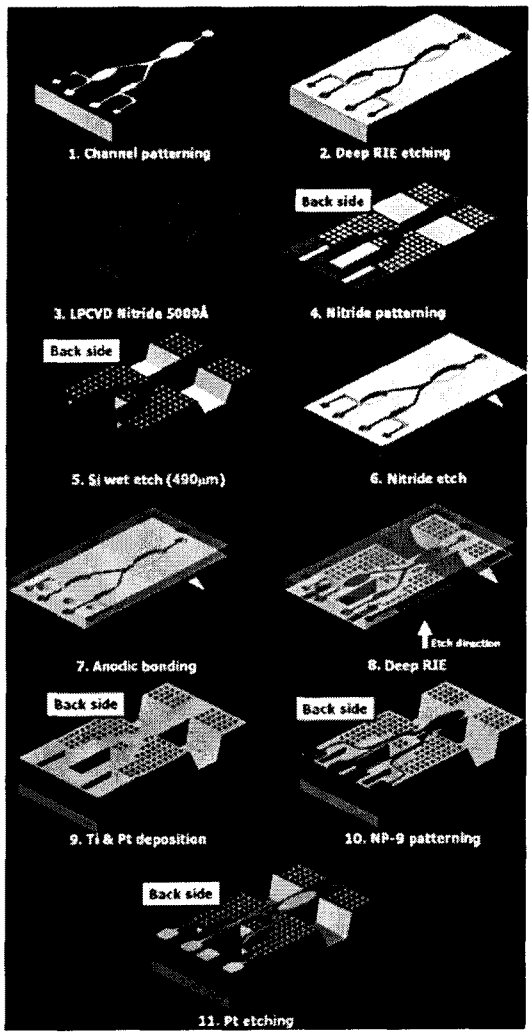


Fig 3. Fabrication process of the temperature controllable microreactor.

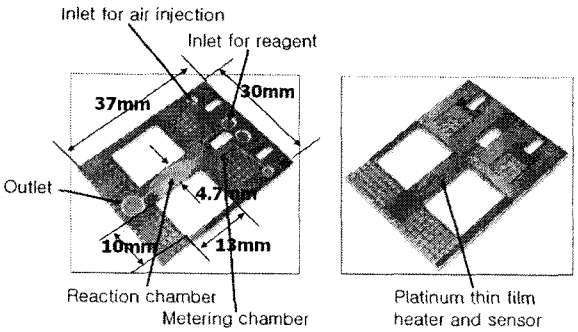


Fig 4. Pictures of the fabricated temperature controllable microreactor: (a) top view, (b) bottom view

As shown in Fig 5, A temperature control system was constructed to control the temperature of reaction chamber. Wheatstone bridge was used to transduce the resistance variation of the Pt thin film sensor to voltage variation. Instrumentation amplifier (AD524) was connected to the output of wheatston bridge circuit for precise amplification without loss. OPAMP (Trek 50/750) was used to amplify the power which is supplied to the heater.

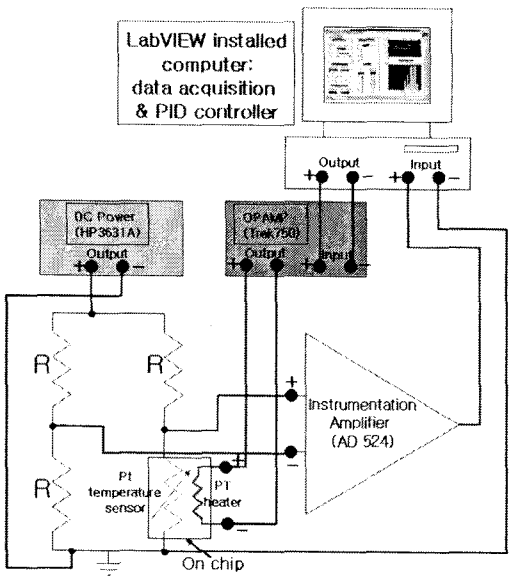


Fig 5. Schematic diagram for the interface of temperature controllable microreactor and temperature control system.

3. Measurement

3.1. Performance of the thin film temperature ensor

The resistance of Pt temperature sensor was measured for the various steady state temperature in the environmental chamber five times. Fig 6 shows the stactistical measurement results of sensor resistance. Since the trypsin treatment is done at 37 ℃ and 55 ℃, the measurement was done within the range from 10 ℃ to 70 ℃. The resistance of the sensor with respect to the temperature showed a good linearity and a repeatability in the whole temperature range without hysterysis. The standard deviations are so small that it is hard to notice the standard deviation bar in Fig 6. The standard deviations did not exceed ±1 Ω.

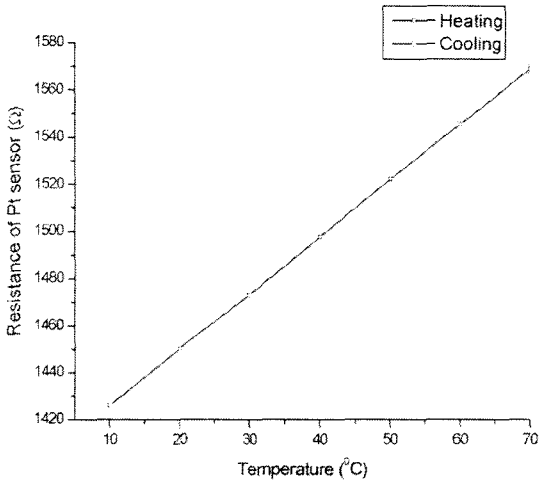


Fig 6. Statistical graph of sensor resistance with respect to the temperature.

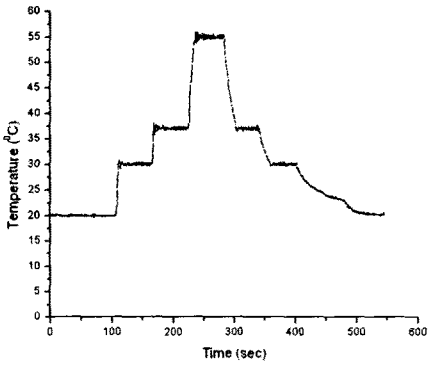
The linear property of the sensor can be represented by the following equation

$$R = R_0 \times [1 + \alpha(T - T_0)] \quad (2)$$

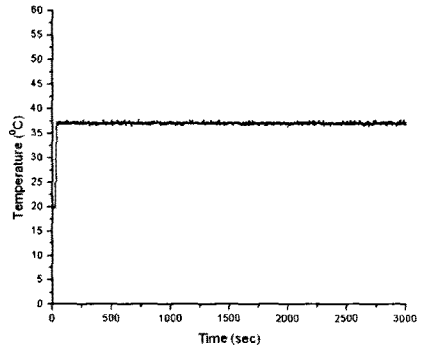
where R is the resistance of the sensor (Ω) at temperature T (℃), R_0 is the resistance of the sensor (Ω) at temperature T_0 (℃) and α is the temperature coefficient of resistance (TCR) of the Pt thin film. The TCR of the Pt thin film was estimated to be 1.68×10^{-3} ($℃^{-1}$). Once the TCR of the Pt thin film was estimated, temperature control system can measure the temperature of reaction chamber based on the output voltage of wheatstone bridge circuit [4, 5].

3.2. Performance of the temperature control system

The reaction chamber was heated to 30 ℃, 37 ℃ and 55 ℃ for about 1 minute and then cooled to 20 ℃ in reverse sequence. As shown in Fig 7. (a), the temperature of reaction chamber reached the desired temperature within 10 seconds at the rising step. the overshoots were less than 1.1 ℃ and the steady state errors did not exceed ±0.3 ℃. However, in the case of falling step, it took more time to reach the next temperature. An external cooling part is needed to reduce cooling time. To confirm the stability of the temperature control system, temperature was controlled for over 50 minutes at the fixed target value (37 ℃). As shown in Fig 7. (b), the overshoot and the steady state error were also less than 1.1 ℃ and ±0.3 ℃, respectively.



(a)



(b)

Fig 7. Results of the temperature control using PID controller: (a) short time for various temperature (b) long time for fixed temperature.

4. Conclusion and discussion

We have fabricated and tested a Si-based micromachined temperature controllable microreactor which is applicable to trypsin treatment. Although the resistances of the fabricated Pt thin film temperature sensor and heater did not accord with the designed value, accurate temperature control could be realized with the temperature control system by calibrating wheatstone bridge circuit. However, it was revealed that cooling time is much slower than heating time. Thus it seems that an external cooling part is needed to reduce cooling time. The stability of the temperature control system was also verified. For over 50 minutes, the temperature of the reaction chamber was successfully controlled with the steady state error less than ±0.3 ℃. Therefore, the fabricated temperature controllable microreactor and temperature control system can be applied to the trypsin treatment with the merits such as accuracy, rapidity and stablility. In conclusion, the experiment of trypsin treatment with the fabricated microreactor will be done to confirm the availability of the proposed temperature controllable microreactor.

[Reference]

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