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Fabrication of a polymerase chain reaction micro-reactor using infrared heating

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Abstract

A silicon-based micro-reactor to amplify small amount of deoxyribonucleic acid (DNA) has been fabricated using micro-electro-mechanical systems (MEMS) technology. Polymerase chain reaction (PCR) of DNA requires a precise and rapid temperature control. A Pt sensor is integrated directly in the chamber for real-time temperature measurement and an infrared lamp is used as external heating source for non-contact and rapid heating. In addition to the real-time temperature sensing, PCR needs a rapid thermocycling for effective PCR. For a fast thermal response, the thermal mass of the reactor chamber is minimized by removal of bulk silicon volume around the reactor using double-side KOH etching. The transparent optical property of silicon in the infrared wavelength range provides an efficient absorption of thermal energy into the reacting sample without being absorbed by silicon reactor chamber. It is confirmed that the fabricated micro-reactor could be heated up in less than 30 sec to the denaturation temperature by the external infrared lamp and cooled down in 30 sec to the annealing temperature by passive cooling.

Key Words : polymerase chain reaction, PCR, micro-reactor, MEMS, IR heating

1. Introduction

Since Northrup *et al.* have realized a PCR chip using MEMS technology in 1993[1], a great deal of efforts has been dedicated to the development of miniaturized PCR chips by a number of laboratories. In general, micro-PCR chips have been classified into the continuous-flow type[2] and micro-chamber type[1,3]. A continuous-flow type has well-defined three individual temperature zones, kept constant at different temperatures over time, and has a long channel delivering DNA sample. On the other hand, a micro-chamber type has one reactor filled with the DNA sampleand heat source that controls the temperature. The latter has more advantages, such as simple structure and small size that give suitability for portable devices with low cost. In addition, the micro-chamber type allows more flexibility in the temperature cycling for DNA amplification.

A wide variety of materials, such as silicon, glass, poly-dimethylsiloxane (PDMS), polyimide and so on, has been used to realize PCR chips. Most of the polymer materials, such as PDMS and polyimide, are frequently considered desirable due to the easiness in manipulation, the biocompatibility and transparent property[4,5]. However, silicon is preferable material than others for realizing an intelligent PCR chips with integrated circuit as a part of a lab-on-a-chip (LOC) or system-on-a-chip (SOC), which we are finally aiming at. Furthermore, well-developed processing technologies with silicon give more chances to realize micro-scaled devices that have complex structures. For these reasons, silicon is chosen for the bottom wafer where the PCR micro-chamber is fabricated. The transparent top glass opens a channel to the direct observation of the sample injected into the PCR chamber. In this paper, a MEMS-based silicon micro-reactor for DNA amplification and its IR-mediated thermal cycling are demonstrated.

2. Fabrication

The schematic view of the PCR micro-reactor is illustrated in Fig. 1. The starting material is 490 μm-thick and double-side polished (100) silicon wafer, coated on both sides with a low-stress LPCVD silicon nitride layer. The nitride layers on both sides are patterned using photolithography and following reactive ion etch-

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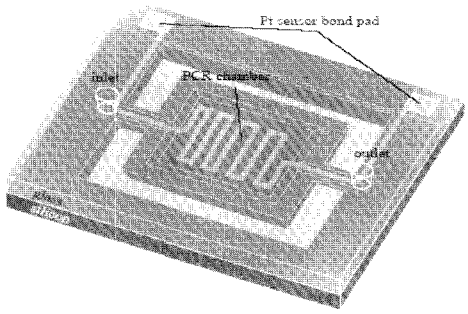
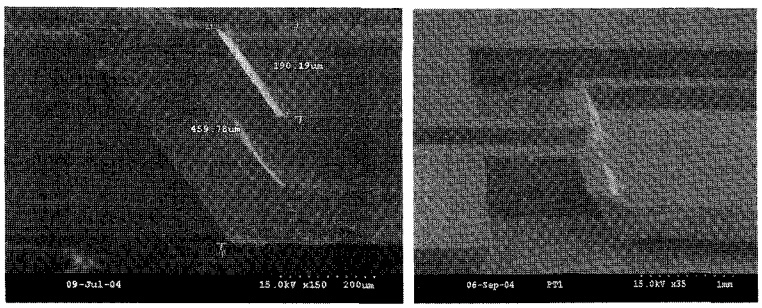


Fig. 1. Structural design of the PCR micro-reactor.

ing (RIE). Using the patterned nitride layers as the mask, anisotropic wet etching through the silicon wafer is performed in aqueous potassium hydroxide (KOH, 30 wt%, 90 °C). In order to define the reaction chamber the silicon is etched from the top side to a depth of 190 μm in advance and then the bulk-silicon parts underneath and around the chamber are etched away from the back side to minimize the thermal capacity of the reaction chamber[3,6]. This results in a suspended PCR chamber by two silicon bridges that contain the inlet and outlet channels, as shown in Fig. 2. The well size is $3 \times 3 \times 0.19$ mm^3 so that the total volume of the well is 1.71 μl.

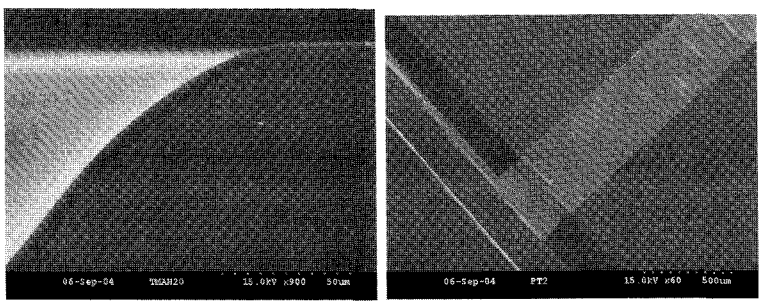
A successful patterning of the Pt temperature sensor in 190 μm-deep reactor chamber is achieved by applying additional process steps to improve the uniformity of photoresist coating at the sharp convex corners. The sharp corners are rounded off using two-step KOH etching, followed by maskless tetramethyl ammonium hydroxide (TMAH, $(CH_3)_4NOH$) etching step. Fig. 3 shows the SEM photos of rounded corner and Pt temperature sensor fabricated in the PCR well.

For PCR-compatible surface coating, a silicon dioxide (350 nm) was deposited by plasma enhanced chemical vapor deposition (PECVD) on the silicon wafer. The most used materials for the realization of biomedical coatings are based on carbon, in the form of amor-



(a) (b)

Fig. 2. Suspended PCR micro-reactor by in/outlet channels for minimizing the thermal mass; (a) Cross section of PCR micro-reactor and (b) SEM photo of suspended PCR micro-reactor.



(a) (b)

Fig. 3. Rounded convex corner and Pt temperature sensor fabricated through the non-planar in/outlet channels; (a) Cross section of the convex corner rounded in TMAH, coated with AZ9260 photoresist and (b) Pt patterning through the rounded concave corner.

phous carbon (a-C), hydrogenated amorphous carbon (a-C : H) and diamond like carbon (DLC). Also silicon carbide (SiC), both in its amorphous and microcrystalline form, shows good possibilities for its use in the biomedical field, especially thanks to its properties of great hardness and resistance to degradation. Another material of great interest is silicon oxide in its amorphous form (a-SiO), which shows characteristics of hardness and mechanical resistance similar to the ones of glass. There are very few scientific information on the biocompatibility of a-SiO_2 thin films, but some studies exist, that deal with the use of bioactive glasses, which include SiO_2 in their components[7,8]. Also of interest are the amorphous alloys based on silicon and nitrogen (a-SiN), which show characteristics that are quite similar to a-SiO.

Soda-lime glass wafer, which has inlet and outlet holes prepared by sand blasting, is finally bonded with the completed bottom silicon wafer using dry film photoresist Ordyl BF 410 (manufactured by Tokyo Ohika Kogyo co., Ltd.)[9-11]. The bonded PCR chip is shown in Fig. 4.

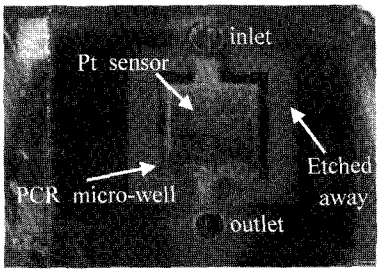
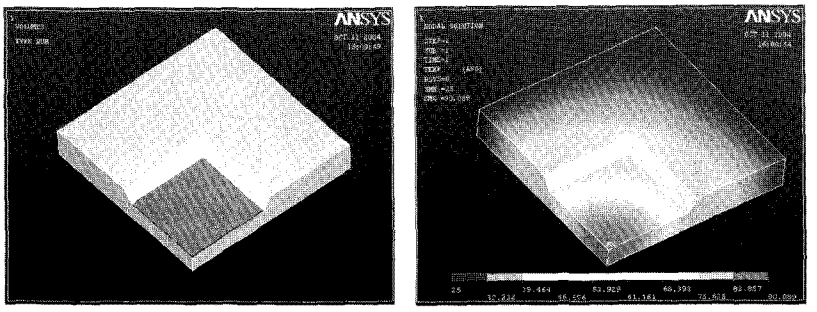


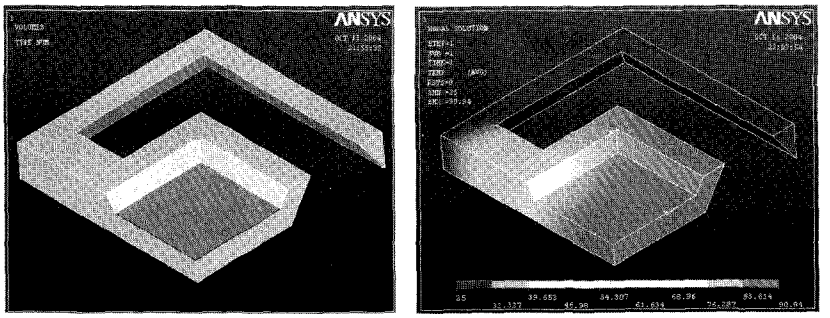
Fig. 4. The fabricated PCR micro-reactor.

3. Finite Element Analysis (FEA) of the PCR micro-reactor

Finite element analysis (FEA) is used for investigating the steady-state temperature distribution of the proposed PCR micro-reactor with fully-reduced mass, minimized heat capacity around the reactor chamber. Because of symmetry considerations, it is sufficient to model only a quart of the proposed device for a simplicity. As the boundary conditions of simulation, the temperatures at the bottom plane and two side-planes of the proposed device are fixed to the ambient tempera-



(a) PCR micro-reactor with full mass.



(b) PCR micro-reactor with fully-reduced mass.

Fig. 5. Thermal analysis of the proposed structures.

ture. Fig. 5 shows the simulated temperature distributions on (a) the reactor with full mass and (b) the reactor with fully-reduced thermal mass around the reactor chamber, when the heat generation is imposed upon the bottom plane of the reactor chamber until it reaches at 90 °C. A uniform temperature distribution is observed in the reactor chamber with fully-reduced thermal mass.

4. Measurements

The measured resistance of the fabricated Pt sensor was about 200~250 Ω. The linear property of the sensor can be represented as follows

$$R = R_0 [1 + \alpha (T - T_0)],$$

where R is the resistance of the sensor at temperature T (°C), R_0 is the resistance at reference temperature T_0 and α is the temperature coefficient of resistance (TCR) of the Pt sensor. The resistance change of the fabricated Pt sensor was measured over the temperature range 20 ~100 °C in a temperature/humidity-controlled chamber and shown in Fig. 6, the measured TCR was about 2.92×10^{-3}/°C that is close to that of bulk Pt (3.92×10^{-3}/°C)[12].

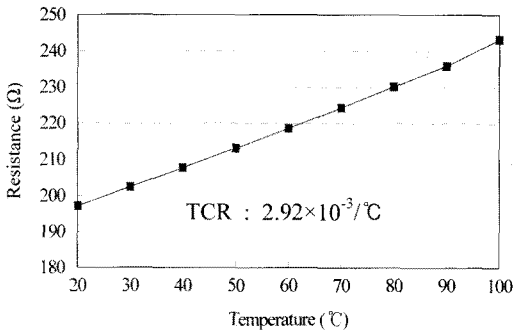


Fig. 6. Measured resistance of Pt sensor as a function of temperature.

The measurement setup to measure the heating performance of the fabricated device is illustrated in Fig. 7. The actual heating performance has been demonstrated with de-ionized water of 1.7 μl filled in the micro-reactor instead of real DNA sample. A 100 W tungsten lamp illuminates the sample filled in the fabricated whole micro-reactor chamber and Pt sensor formed in the micro-reactor chamber detects the real-time temperature of the sample by observing the change in its resistance[13]. The measured heating and passive cooling profiles are shown in Fig. 8. As soon as the lamp illuminates the PCR micro-reactor, the temperature rapidly increases. As can be seen in Fig. 8, it takes less than 30 sec to reach to the denaturation temperature (90~96 °C) from the room temperature and requires another 30 sec to reach into the annealing tempearture (50~65 °C) in PCR process. These results demonstrate that the fabricated PCR reactor is applicable to the PCR process.

5. Conclusions

A MEMS-based PCR micro-reactor for amplifying DNA has been fabricated and characterized. A new technology, rounding sharp convex corners, has been devised to overcome the difficulty to form a Pt sensor in the reactor chamber along the non-planar inlet/outlet channel.

Differently from conventional PCR chips that have micro-heater and temperature sensor on the back side of the reactor chamber with a distance through bulk silicon, the device reported in this paper has a Pt temperature sensor integrated directly in the reactor chamber

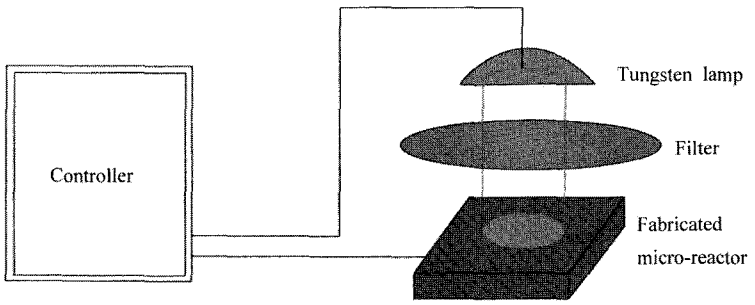


Fig. 7. Experimental Set-up.

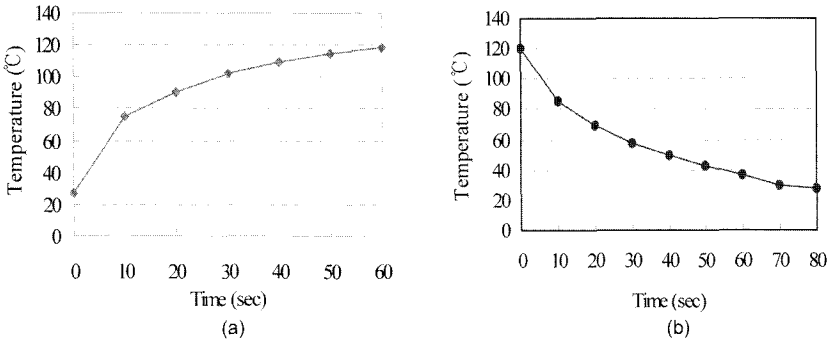


Fig. 8. Heating and cooling profile.

for improved real-time temperature measurement and uses non-contact infrared-mediated heating for more effective PCR amplification. In fact, the heating method using IR source results in more efficient and uniform heat transfer to the sample because the silicon micro-reactor does not absorb most part of IR wavelength range[14], while only the sample does due to the fact that a specific IR-active absorption band for the sample is limited.

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References

[1] M. A. Northrup, M. T. Ching, R. M. White, and R. T. Watson, "DNA amplication with a microfabricated reaction chamber", *Proc. Int. Conf. Solid-State Sensors and Actuators*, pp. 924-926, 1993.

[2] M. U. Kopp, A. J. de Mello, and A. Manz, "Chemical amplification: Continuous-flow PCR on a chip, Science", *Science*, vol. 280, pp. 1046-1048, 1998.

[3] J. H. Daniel, S. Iqbal, R. B. Millington, D. F. Moore, C. R. Lowe, D. L. Leslie, M. A. Lee, and M. J. Pearce, "Silicon microchambers for DNA amplification", *Sensors and Actuators A*, vol. 71, pp. 81-88, 1998.

[4] A. Fuchs, H. Jeanson, P. Claustre, J. A. Gruss, F. R.-Cavalier, P. Caillat, U. Mastromatteo, M. Scurati, F. Villa, G. Barlocchi, P. Corona, and B. Grieco, "A silicon lab-on-chip for integrated sample preparation by PCR and DNA analysis by hybridization", *Int. Conf. on Microtechnologies in Medicine & Biology*, pp. 227-231, 2002.

[5] Y.-K. Kim, "The Trend of MEMS Technology", *J. Sensors Soc.*, vol. 8, no. 5, pp. 354-358, 1999.

[6] D. S. Yoon, Y. S. Lee, H. J. Cho, S. W. Sung, K. W. Oh, J. H. Cha, and G. B. Lim, "Precise temperature control and rapid thermal cycling in a micromachined DNA polymerase chain reaction chip", *J. Micromechanics and Microengineering*, vol. 12, pp. 813-823, 2002.

[7] S. L.-Esteban, E. Saiz, S. Fujino, T. Oku, K. Suganuma, and A. P. Tomsia, "Bioactive glass coatings for orthopedic metallic implants", *J. of the Europ. Ceramic Soc.*, vol. 23, pp. 2921-2930, 2003.

[8] J. Ballarre, J. C. Orellano, C. Bordenave, P. Galliano, and S. Cere, "*In vivo* and *in vitro* evaluation of vitreous coatings on cobalt base alloys for prosthetic devices", *J. of Non-Cryst. Solids*, vol. 304, pp. 278-285, 2002.

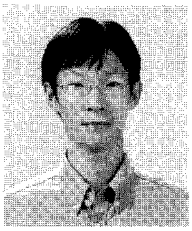
[9] C. denBesten, R. E. G. van Hal, J. Munoz, and P. Bergveld, "Polymer bonding of micro-machined silicon structures, micro electro mechanical systems", *IEEE MEMS*, vol. 92, pp. 104-107, 1992.

[10] J. E.-Ali, I. R. P.-Nielsen, C. R. Poulsen, D. D. Bang, P. Telleman, and A. Wolff, "Simulation and experimental validation of a SU-8 based PCR thermocycler chip with integrated heaters and temperature sensor", *Sensors and Actuators A*, vol. 110, pp. 3-10, 2004.

[11] E. Kukharenka, M. M. Farooqui, L. Grigore, M. Kraft, and N. Hollinshead, "Electroplating moulds using dry film thick negative photoresist", *J. Micromechanics and Microengineering*, vol. 13, pp. S69-S74, 2003.

[12] K.-D. Song, Y.-I. Bang, S.-M. Lee, Y.-S. Lee, N.-J. Choi, B.-S. Joo, M.-G. Seo, J.-S. Huh, and D.-D. Lee, "Characteristics and fabrication of micro gas sensor with single electrode", *J. Sensors Soc.*, vol.

11, no. 6, pp. 350-357, 2002.
[13] A. F. R. Huhmer and J. P. Landers, "Noncontact infrared-mediated thermocycling for effective polymerase chain reaction amplification of DNA in nanoliter volumes", *Anal. Chem.*, vol. 72, pp. 5507-5512, 2000.
[14] D. K. Schroder, R. NoelThomas, and J. C. Schwartz, "Free carrier absorption in silicon", *IEEE J. Solid State Circuits*, vol. SC-13, pp. 180-187, 1978.



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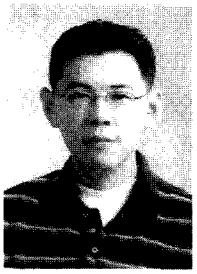

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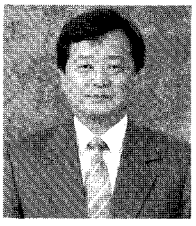

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