A PDMS BASED INTEGRATED PCR MICROCHIP FOR GENETIC ANALYSIS

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Abstract: An integrated continuous-flow microfluidic chip was fabricated on glass substrate with PDMS based microchannels, cell lysis and PCR modules. Gold-microelectrodes were used to produce electrochemical cell lysis, while, indium-tin-oxide (ITO) microheater was used for thermal cycling of PCR reaction. The fabricated device was used for 20 cycles of PCR amplification of pancreatic cancer DNA marker (*SMAD4*) from non-tumorigenic MCF10a human cell lines. The 193 bp PCR amplicon obtained through on-chip PCR was confirmed in case of MCF 10a cells through agarose gel electrophoresis, whereas no product was detected in case of tumorigenic MCF7 cells. The total time required for entire reaction was less than 90 min. Therefore, we propose that such microchip can be helpful in predicting the risk of cancer by analysis of genetic tumor markers from human samples and can also be used for other genetic analysis involving PCR reaction.

1 INTRODUCTION

Early detection of metastasis has always remained as elusive as necessity. Such detection often involves invasive tissue biopsy or expensive and unreliable tumor marker antigen study. However, the probable occurrence of a certain forms of cancer can be predicted as early as gastrulation using genetic markers. *SMAD4* is one such gene, which is either deleted or mutated in more than one third of pancreatic cancer patients (Dixit and Juliano, 2008). These genetic markers can be identified by using Polymerase chain reaction (PCR), which is a widely used molecular biology technique for amplifying specific regions of DNA using DNA polymerase enzyme. The PCR technique is also applicable in cloning, genotyping, drugs discovery, forensic, environmental and ever growing application areas. A majority of PCR applications involves analysis with whole cells and requires the extraction of template DNA prior to PCR. These steps are time consuming and labor extensive and require expensive chemicals and instrumentation.

Since, further miniaturization of this technique is possible, we propose in this regard, a continuous-flow PCR chip on glass substrate using indium-tin-oxide (ITO) heater and microchannels laid in polydimethylsiloxane (PDMS). As, cell lysis is an important step for extraction of DNA prior to PCR analysis, for this reason, we also integrated a cell lysis device on the same chip. While, PDMS was chosen for microfluidic devices fabrication as it can easily and repeatedly be fabricated by the molding method and requires no channel pretreatment as in case of materials such as Poly(methyl methacrylate) (PMMA); ITO heater electrodes were the choice for thermal cycling due to ease of its fabrication and linear variation of its temperature by application of DC power.

2 EXPERIMENTAL

The Micro chip consisted of two parts (Fig. 1). The

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PDMS microchannel was fabricated using negative molding method. Negative photoresist (SU-8 2075, Micro Chem) was spin-coated onto a silicon wafer. SU-8 was patterned to make a microchannel using photolithography technique (Fig. 2). The PDMS (DC-184, Dow Corning) mixture was poured on the SU-8 patterned wafer and cured for 4 h at 72°C. The PDMS was then peeled off and manual drilling was performed to produce access holes. The width and depth of the microchannel were 250 and 200 µm respectively, and total length was 1100 mm for 20 PCR cycles. The PCR channel was divided into 2:2:3 length ratio for three different temperature zones namely denaturation, annealing, and extension. ITO heater/electrode for PCR was fabricated using conventional photolithography and wet etch process. Positive photoresist (AZ-1512, Chariant) was spin-coated on ITO film deposited glass. AZ-1512 was patterned to make electrode using photolithography. ITO film was etched using $FeCl_3$/HCl solution for 1 h and photoresist was removed. Gold electrodes for cell lysis were fabricated using photolithography and evaporation method. AZ-1512 was spin-coated on glass and patterned using photolithography. After photolithography process, Gold electrode was deposited using evaporator. For electrical isolation of electrodes from test fluid, PDMS was spin coated onto the ITO patterned glass and baked at 95°C for 30 min. Fabricated PDMS microchannel and ITO/Gold electrode chip were bonded with each other after UV-ozone treatment for 40 min. ITO heaters were calibrated for liquid temperature control by incorporating thermocouple (CHAL-0001, Omega) into the microchannel during UV-ozone bonding of PDMS to the glass substrate.

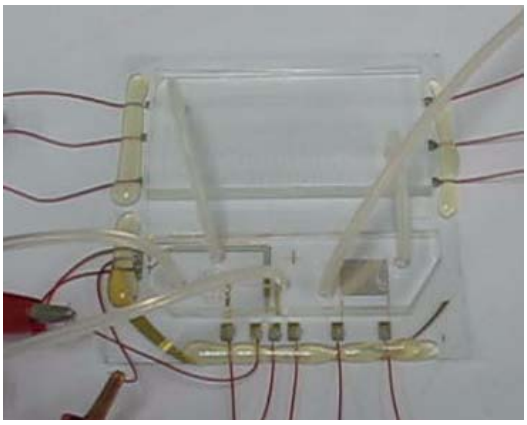

Figure 1: The PCR microdevice containing microchannels for cell lysis and PCR modules, ITO microheater and the gold microelectrode for electrochemical cell lysis.

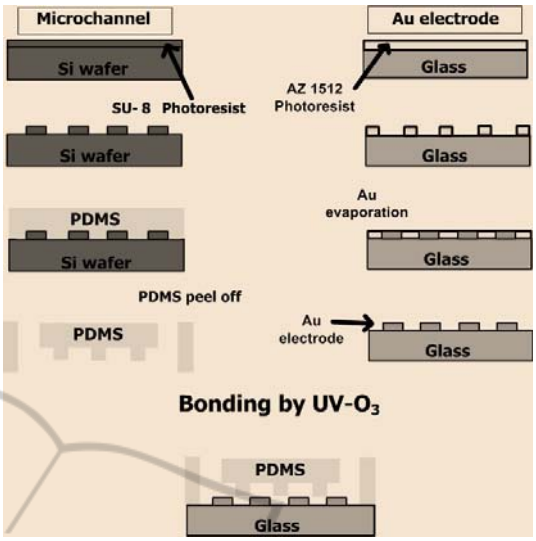

Figure 2: Fabrication process for PDMS based microchannel and Au microelectrodes.

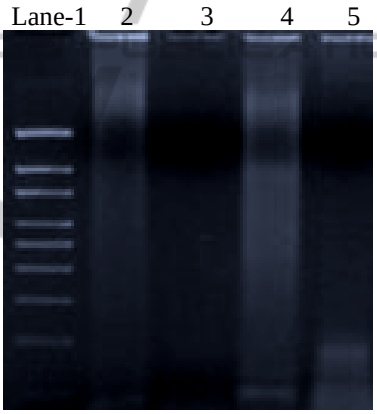

Figure 3: Gel-doc from agarose gel electrophoresis of PCR amplicon of MCF 7 and MCF 10a cells (SMAD4 gene. Condition: injecting sample with flow of air at 5 µl/min rate. Lane: 1= 1.5 kb marker; 2 = Pre PCR lysate for MCF7; 3 = PCR of MCF7; 4 = Pre PCR lysate for MCF10a (Non-cancerous); 5 = PCR of MCF10a. The 193 base pair band on lane 5 is the desired PCR product after 20 cycles.

3 RESULTS

The fabricated device was used for lysis and PCR amplification of genomic DNA of non-tumorigenic MCF10a and tumorigenic MCF7 human cell lines. Approximately 10^6 cells were suspended in 50 µl PBS and 50 µl 2X PCR mix containing Taq-DNA polymerase and 2 µl each of the primers against *SMAD4* gene were added. The mixture was injected into silicone tube carrying air to microchannel with

the help of precision syringe pump. The PCR product (193 bp) was verified by agarose gel electrophoresis (Fig. 3) as well as spectroscopic method and a yield of ~ 250 ng DNA/10^6 MCF 10a cells was recorded, while no product was detected in case of MCF7. These results suggested the success of miniaturized PCR device for rapid PCR amplification of *SMAD4* gene in tumorigenic human cells and therefore, early prediction of occurrence of pancreatic cancer. In conclusion, the developed device can also be used in almost any other genetic analysis involving DNA extraction and PCR amplification. Based on these preliminary results, we propose that the integrated device will be helpful in reducing the reaction time for DNA extraction as well as PCR amplification of DNA in a variety of samples. Further work is under progress to use this integrated chip in genomic analysis from a variety of samples.

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