PROTOTYPE OF A MULTIPLE REACTIONS DNA ANALYSIS DEVICE WITH A VERSATILE MICROFLUIDIC SAMPLE MANIPULATOR

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Abstract
An integrated DNA analysis device that can perform multiple reactions and electrophoretic separation of DNA samples has been designed, fabricated and tested. A microfluidic sample manipulator has been developed to precisely control the sample movement on this device. By controlling the external air pressure and vacuum applied to the device through the manipulator, fine movement of fluid motion inside microchannels was achieved. Two sequential restriction digest reactions using a mouse DNA template, followed by an electrophoretic separation were successfully performed on the device. This microfabricated device, integrating precise fluidic manipulation, multiple thermal reactions and on-chip electrophoretic separation, has potential applications for fast, highly integrated chemical and biological analysis.

Keywords: Microfluidic, sample manipulator, DNA analysis

Introduction
Recently there has been a worldwide trend of miniaturization of analytical devices in many areas such as biology, biochemistry, chemical engineering and biomedical engineering. Although so far most reported work focuses on single functional components such as fluidic handling [1,2], reactions [3,4], and separations [5,6], it is the integration of these functional components into a single analysis device that makes miniaturized analytical devices truly advantageous over the conventional analytical instruments.

Integrated devices can extract useful information from the raw samples by a series of automated operations. Highly integrated systems offer the advantage of high throughput, short analysis time and low contamination. Much progress has been made on device integration, and several integrated DNA analysis devices have been reported [7-9]. We designed and fabricated an integrated microfluidic DNA analysis device that can perform two sequential biochemical reactions, followed by an electrophoretic separation with minimal sample volume (~200 nanoliters). A removable microfluidic sample manipulator has also been designed and fabricated.

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for precise liquid sample movement on the device. This sample manipulator uses a vacuum chamber to attach to and detach from the device, and can be applied to a variety of microfluidic chemical and biological analysis devices.

Fabrication

The device structure is comprised of two sides: a glass substrate with microfluidic channels and a silicon substrate with heaters, temperature sensors and electrodes (Figure 1 & 2). The microchannels are etched in the glass substrate using standard photolithography and wet etching techniques. The metal heaters and temperature sensors for temperature control are evaporated on top of a silicon dioxide layer thermally grown on the silicon substrate. A Low Temperature Oxide (LTO) layer is deposited over the heaters and temperature sensors as a passivation layer. This passivation layer works as a barrier layer to insulate the electronic elements from the liquid solution in the microchannels. Metal electrodes (Ti 300Å/Pt 1000Å) are fabricated on top of the LTO layer at defined locations for use in electrophoretic separation. The two sides are then bonded together to yield the final device.

![Device photograph.](image1)

![Device structure.](image2)

The manifold was fabricated by connecting transparent plastic block (Lucite®, Huntersville, NC) and Teflon tubes (Small Parts Inc., Miami Lakes, FL). First a plastic block was measured and cut to fit on top of the fabricated device. Then the piece and the device were aligned together and the locations of the holes were marked on the piece. Next the holes were drilled using a microdrill bit (0.86 mm in diameter) and connections for Teflon tubes were drilled horizontally. Once the plastic part was completed, the Teflon tubes were inserted into those holes. They were glued together by using an epoxy resin; this resin was used for sealing the gaps between drilled holes and inserted Teflon tubes.

The fluidic manifold can be easily and reversibly attached to the chip. A rectangular shape is cut as a vacuum chamber and a vacuum line is connected to this chamber. Because of this vacuum chamber the manifold will adhere to the surface of glass substrate and can be easily removed from the fabricated device when vacuum is shut off. Two way and three way solenoid valves were used to control the movement of fluids in a microchannel. Valve controller was made with an electric circuit board. Figures 3 and figure 4 show the pictures of the fabricated manifold and the sample manipulator, with valve system and sample movement controller.
Experiments and Results

We tested fluidic motion control and the mixing effect using the sample manipulator. Three different colored samples were preloaded into the device to visualize mixing. The first two samples were mixed together and delivered to the third sample channel. This mixed product and the third sample were then mixed again and the final product was loaded into the gel electrophoresis channel. Figure 5 shows sequentially preloaded samples, sample movement and mixing.

Figure 5. (A) Two samples (blue in right and yellow in left) are injected through sample inlet holes and stopped at the hydrophobic patches patterned for sample volume metering. (B) The sample manifold was placed on top of glass substrate and sample fluids are moving forward by applied air pressure. (C) Mixed sample (light blue) passes through the first U-shaped reaction channel for the second reaction. Another sample (green) is loaded and the sample mixture of first two samples is stopped at the hydrophobic patches again for sample metering. (D) Second mixing happens when the two fluids move forward by air pressure. (E) Mixed sample passes the second U-shaped reaction channel. (F) Final product is loaded into a separation channel for gel electrophoresis.
Enzymatic reactions have been performed in the thermally controlled reaction chambers. Thin film metal (Ti 300Å/Pt 1000Å) resistive heaters and temperature sensors deposited on the silicon substrate are used for temperature control. Temperature sensors are calibrated based on the fact that the resistance varies linearly with temperature. A LabVIEW (National instrument, Austin, TX) program is used to adjust the power output to the heaters based on the measured and set point temperatures.

We performed two consecutive restriction digest reactions on this device. A mouse DNA PCR product (996bp) is used as the template, and is digested consecutively by two different restriction enzymes. Each enzyme cuts the template once, and consequently generates three DNA fragments (149bp, 276bp and 571bp) after the two reactions. Both reactions are performed at 37°C for 10 minutes, and the temperature profile is shown on Figure 6. No significant evaporation was observed during the reaction because of the relatively low reaction temperature.

A UV-polymerized gel was cast using a previously described masking procedure. This technique allows a well-defined flat gel interface to be precisely positioned inside the electrophoresis channel. The sample is then loaded and compacted at the gel interface by applying an electric field of about 25V/cm and any excess sample is replaced with running buffer. Fluorescence from the migrating DNA bands was detected using an Olympus fluorescence stereoscope with a mercury arc illumination source and imaged using a Hamamatsu digital camera (Hamamatsu Corporation, Bridgewater, NJ). The camera output was recorded, digitized and intensity profiles were extracted using Transform 2D image analysis software. Separation of reaction products is achieved in less than 6 minutes in a distance of 1mm (Figure 7).

![Figure 6. Temperature profile of the reaction chamber.](image1)

![Figure 7. Two serial restriction digest reactions with electrophoretic separation in integrated devices.](image2)

**Conclusions**

The prototype of device for DNA analysis presented in this paper offers an integrated mechanism for fluidic transport, reaction and separation in a miniaturized format. The pneumatic sample manipulator makes parallel manipulation of nanoliter volume liquids practical and reliable in μTAS devices. Integration of multiple reactions with separation makes this device suitable for a variety of chemical and biological assays involving different steps, and therefore makes this...
prototype device particularly attractive for single-use and disposable microfluidic devices for chemical and biological applications.

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References