Genetic Testing on Electrowetting-on-dielectric Chips for Magnetic Bead-based DNA Extraction

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Abstract: To develop and realize the personalized medicine and point-of-care in genetic testing, the sequences of DNA extraction process should be integrated. This thesis introduces the implementation of magnetic beads (MB) based DNA extractions on electrowetting on electrowetting-based digital microfluidics (DMF). The reagents are DNA extraction kit. They are characterized as a droplet on DMF. These droplets can be precisely manipulated by electric signals, which simplifies the whole genetic testing. The result from the on-chip DNA of extraction are validated by SYBR® GREEN I. DNA is successfully extracted from 90nl whole blood. Finally, our EWOD chip has been optimized in the following three aspects: (1) it has independent paths of the electrodes for different reagent to avoid the cross-contamination problem. (2) It utilizes MBs to replace the complex centrifugation in tradition DNA extraction procedures. (3) Ratio separation electrodes are designed to re-suspend the MBs and to improve the efficiency of the wasting process. Therefore, our DMF chip not only can successfully extract the DNA from whole blood, but also demonstrate the possibility to use less sample/reagent and shorter process time to purify DNA on chip for point –of-care genetic testing.

Keywords: DNA extraction, electrowetting, Magnetic beads, Genetic testing

I. Introduction

Deoxyribonucleic acid (DNA) is a biological macromolecule, which contains genetic instruction to guide biological development and vital functions. DNA exhibits in all living species and shows variability among individuals. Nucleic acid sequence is a series of letters that indicate the order of nucleotides within a DNA. Through realizing the DNA sequence of organisms or human, geneticists can understand their evolutionary history. Doctors can make proper diagnosis on the patients, who have genetic diseases. Therefore, in order to prevent these illnesses from human, scientists have devoted their effort to understand the complex relationship between gene and these illnesses through genetic testing.

There are three steps before the genetic testing: (1) DNA extraction from biological sample, (2) DNA amplification by the PCR and (3) the testing of DNA separation and selection.

DNA extraction is often used in many diagnostic processes used to detect viruses and bacteria in the environment as well as diagnosing disease and genetic disorder. The results of DNA extraction also require high quality and free-of-contaminants for the following step, sequence amplification [1].

In the 1980s, Micro-total-analysis-system (μ-TAS) which is so-called Lab-on-a-chip (LOC) was started to develop due to the inkjet printing technology. The concept of LOC emphasizes the miniaturization and integration of different sample preparation and biochemical processes into a single chip. It has many advantages which compare to the traditional bulky-liquid-handling system. It reduces sample/reagent consumption, thereby making the automation of complicated procedures possible and speeding up analysis time.

There are two approaches to manipulate reagent and sample solution in the form of continuously flow in microchannel (Continuous-flow microfluidic), or discrete droplets on the chip (Digital microfluidic). Continuous-flow microfluidic devices are usually made by using soft lithography, and they can be easily made. However, Continuous-flow microfluidic devices still has two main disadvantages of manipulate: (1) The devices have to be reprogrammed when they are integrated with additional biochemical procedure. (2)The dead volume inside the microchannel will cause excessive waste in reagents and samples.

Until 2003, C-J Kim et al. manipulate droplets to creating, transporting, cutting, and merging by Electrowetting-Based actuation for Digital Microfluidic Circuits [2]. Digital microfluidic (DMF) is growing in popularity in recent years. DMF controlled the fluids as discrete droplets on an array of independent electrodes. The droplets can be created from reservoirs, mixed with other and split into multiple droplets. DMF can overcome disadvantages of continuous-flow microfluidic devices [3].

The re-programmability and easy controllability of DMF provide high flexibility which is suitable for applications that involve complex, multistep protocols. Due to the advantages of DMF, we aim to do DNA extraction and PCR on EWOD chip.

II. Principle

A. EWOD

In this research, we apply Electrowetting-on-dielectric (EWOD) to manipulate reagent /biological samples. EWOD is an electric means to change the wettability of a dielectric solid surface by applying a voltage across the dielectric layer. In 1805[4], Young found that the contact angle of a sessile drop placed on a solid surface is determined by the interfacial tensions between liquid and solid, liquid
and gas, and solid and gas. After 70 years, Lippmann studied electro-capillary and found that the capillary force at the interface between liquid metal and electrolyte is changed by externally applied electric charges [5]. Then, we combine Young’s result and Lippmann’s result to gain Young-Lippmann equation, which is described as:

$$\cos \theta (V) = \cos \theta_0 + \frac{\varepsilon_0 \varepsilon_d}{\gamma_{lg}} t V^2$$  \hspace{1cm} (1)$$

Where \(\varepsilon_0\) is the permittivity of vacuum, \(\varepsilon_d\) and \(t\) are the relative permittivity and thickness of the dielectric layer, respectively, and \(\gamma_{lg}\) is the liquid–gas interfacial tension. When voltage \(V\) is applied across the dielectric layer, the contact angle changes from \(\theta_0\) to \(\theta (V)\).

We design a device which consists of a top plate, liquid droplet and a bottom plate, denoted as A, B, and C in Fig.1[6]. The top plate contains a blank electrode covered by a hydrophobic Teflon layer. We select ITO glass which is substrate for observation purposes. An array of driving electrodes is patterned on the bottom plate and coated by a dielectric and a hydrophobic layer. The droplet is placed between top and bottom plates. We use the thickness of double faced adhesive tape to determine the height of the droplet. Then, we apply a voltage between the top and bottom plate. The surface above the energized driving electrode is changed from hydrophobic to hydrophilic. Therefore, the droplet moves to the right as illustrated in Fig.1.

Owing to Young-Lippmann equation and our EWOD-device, we can manipulate reagent and biological sample to translate, merge and be cut each other.

![Fig.1 Configuration of the device: A: top plate, B: liquid droplet, and C: bottom plate.](image1)

**III. Experimental**

A. Material

The MBs-based extraction kit is Agencourt Genfind V2(Beckman Coulter, Brea, California, USA). The kit includes Proteinase K, Lysis Buffer, Binding Buffer which include magnet beads, Wash Buffer 1, Wash Buffer 2 and Elution Buffer. Whole blood is provided by Industrial Technology Research Institute (ITRI, Taiwan). The function /manipulating volume ratio to blood of extraction kit is depicted as shown in Fig 3.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Function</th>
<th>Volume ratio to blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood(9nl)</td>
<td>Sample</td>
<td>1X</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>Hydrolyse protein</td>
<td>0.045X</td>
</tr>
<tr>
<td>Lysis Buffer</td>
<td>Blood cells lysis</td>
<td>2X</td>
</tr>
<tr>
<td>Binding Buffer</td>
<td>Absorb DNA by magnet beads</td>
<td>1.5X</td>
</tr>
<tr>
<td>Wash Buffer 1</td>
<td>Remove proteins and salts</td>
<td>4X</td>
</tr>
<tr>
<td>Wash Buffer 2</td>
<td>Remove proteins and salts</td>
<td>2.5X</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>elute DNA from magnet beads</td>
<td>1X</td>
</tr>
</tbody>
</table>

B. EWOD Chip Design

The design of our DMF chip with all electrodes was depicted as shown in Fig. 4. These electrodes could be divided into four categories according to their function: (1) reservoir, (2) droplet generation, (3) droplet transportation and (4) ratio separation electrodes. Reservoir electrodes were responsible to provide or store the liquid. There were eight kinds of reagents in our protocol of MBs-based DNA extraction and PCR. Reagent solutions would be drawn from the reservoir electrodes, and separated through the droplet generation electrodes to form droplets. The droplet was transported to the designated position by using the droplet transportation electrode. The droplet transportation electrode worked as droplet dispensation, droplet transportation and reaction areas. The design of the lay out of electrodes was purpose to avoid the cross-contaminations of reagents.

![Fig.2 Traditional protocol employs MBs to isolate DNA from whole blood in an Eppendorf tube](image2)

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**Fig. 3 The function /manipulating volume ratio to blood of extraction kit.**
C. EWOD Chip fabrication.

EWOD device fabrication started with lithography technique. These electrodes were patterned on ITO (indium tin oxide) glass (7 Ω/sq, Ruilong Inc. Taipei, Taiwan). The ITO glass was sequentially cleaned in acetone and methanol with an ultrasonic cleaner for 10 minutes. The ITO glass was baked at 95°C for 5 minutes for dehydration. Positive photoresist of FH-6400 (MicroChem Corp. Westborough, MA, U.S.A) was dispensed and spin at 3000 rpm for 30 seconds. The thickness of FH-6400 was 1.5μm. The ITO glass was soft baked at 95 °C for 5 minutes. It was then exposed for 3.5 seconds to achieve a total dose of 125 mJ/cm² with a UV-light of 365 nm wavelength. The ITO glass was immersed in TMAH developer (MicroChem Corp. Westborough, MA, U.S.A) for 20 seconds and rinsed with DI water. Hard bake was completed at 150 °C for 10 minutes. The sample was immersed in aqua regia for 300 seconds for 44°C to pattern the ITO. After etching process, the sample was immersed in photoresist stripper (ALEG-310, Avantor Performance Materials Co., Pennsylvania U.S.A) for 20 seconds and rinsed with DI water. Hard bake was completed at 150 °C for 10 minutes. The sample was immersed in aqua regia for 300 seconds for 44°C to pattern the ITO. After etching process, the sample was immersed in photoresist stripper (ALEG-310, Avantor Performance Materials Co., Pennsylvania U.S.A) for 20 seconds and rinsed with DI water. Hard bake was completed at 150 °C for 10 minutes. The sample was immersed in aqua regia for 300 seconds for 44°C to pattern the ITO.

The top plate was also an ITO glass, whose ITO layer worked as a common ground electrode. The resistance of top plate ITO glass was 450Ω/sq. A 55 nm-thick Teflon® layer was coated. The top plate was obtained.

D. Experiment of the contact angle of reagent.

There are a important things about contact angle. We can decide the approaches, which is dispensing liquid before EWOD device assembly and injecting liquid from side after device assembly, to load reagent onto EWOD chip due to the contact angle of reagent. In the first approach which applied to all kinds of reagents, the reagent was loaded onto chip with pipettes before covering the top plate. There is a drawback for the first approach. When you cover the top plate onto the EWOD chips, all liquids were pressed, flowing into each other, and mixed. The second approach can prevent this problem. The liquid was loaded with a pipette along the edge of the top plate of the EWOD device, or in between the top and bottom plates to dispense the liquid to reservoir. We load every kinds of reagents on the bottom plate without top plate, and observe the contact angle of reagents on side view by zoom camera shot (EIA G20E20, CIS corp. Japan ). Then, we measured the contact angle of reagent, which were depicted as shown in Fig. 5, with the software(Fta32 Video) on computer. The liquid which has smaller contact angle (<90°) means that this liquid had low interface between the liquid and the substrate, or was hydrophilic; It could easily flood to the gap in between two plates. In Fig. 6, we can understand that lysis Buffer, binding buffer, Wash buffer1 and wash buffer 2 were able to flood to the gap in between two plates easily. In fig. 7, it is process of liquid, which has a contact angle smaller than 90°, loading by injecting liquid from side after device assembly.

![Fig. 6 The contact angle of blood and reagents of Agencourt Genfind V2.](image)

![Fig. 7 (a)–(c) The process of liquid, which has a contact angle smaller than 90°, is loaded by injecting liquid from side after device assembly. (a) Top view of the gap between top plate and bottom plate. (b)–(c) Injecting liquid from side by pipette. When injecting liquid from side slowly, we would open the electrode of reservoir to fit the reservoir by liquid.](image)
E. MBs DNA extraction on EWOD chip.
The MBs extraction on the EWOD chip was implemented. The first few steps of DNA extraction on the chip were as shown in Fig.8. Before the DNA extraction, we would inject the silicone oil between top and bottom plate as the surrounding.
Proteinase K and Lysis Buffer mixed with blood sequentially. Proteinase K, which digested proteins and degrade was added into the whole blood (Fig. 8 (b)) and mixed in a loop motion until the color of the droplet became uniform (Fig. 8 (c)). Lysis Buffer, which broke the cell membrane to release DNA, was sequentially added (Fig. 8 (e)). The lysate was mixed by moving in a loop motion (Fig. 8 (f)).

![Fig.8 Proteinase K and Lysis Buffer were sequentially add into whole blood and mixed in a loop motion. (a)–(c), Proteinase K was added to degrade nucleases. (d)–(f), Lysis Buffer was add to break cell membrane.](image)

After mixing with lysis buffer, the lysate was mixed with binding buffer which has magnetic beads at 2:1 volume ratio onto the magnetic beads (Fig.9 (a)–(b)). The binding buffer serve a surrounding which make MBs bound with DNA. The MBs bound with DNA were collected and separated from the mixture. The MBs were collected by using the meniscus-aid MBs collection technique (Fig.9 (c)–(d)). We moved the droplet to the north direction in Fig. 9 (d) by EWOD, and attracted the MBs to south direction by magnet. The droplet would proportionally into two droplets, which are

![Fig.9 Binding buffer was added into lysate and mixed in a loop motion. (a)–(b), binding buffer was used to bound DNA onto the MBs. (c)–(d), the method of splitting two droplets, which is called the meniscus-aid MBs collection technique.](image)

Wash buffer 1 was added to the droplet, which has magnet beads, to remove the salts and proteinase in the mixture. After washing protocol, we use the meniscus-aid MBs collection technique to get higher concentration of DNA. Wash buffer 2 was added to the droplet sequentially to do wash protocol. After washing protocol, we also use the meniscus-aid MBs collection technique to get higher concentration from residual droplet. Finally, elution buffer was added to the residual droplet. Adding elute buffer is used to elute DNA from MBs to the droplets. Then, we got elution buffer with DNA by meniscus-aid MBs collection technique.
Fig. 9 Washing buffer 1, washing buffer 2 and elution buffer were sequentially added into residual droplet. (a)–(b), washing buffer 1 was used to remove salts and proteinase K. (c)–(e), washing buffer 2 was added to wash again. (f)–(j) elution buffer was added to rinse the DNA from MBs.

Then, we pulled up top plate carefully and slowly to prevent tiny droplet of elution buffer with DNA evaporate and mix other reagent. After pulling up the top plate, we used the outside wall of tip to adhesive the tiny droplet with DNA and take it to Ependorf with 50 μl deionized water.

Fig. 10 The approach to get DNA from chip is accomplished by pipette. (a) Top view of elution buffer with DNA onto the bottom plate. (b) Top view of tip, whose wall has a tiny droplet with DNA.

F. DNA validation.

Once we extract the DNA from EWOD chip, we had to prove that we surely extract DNA successfully. There is a dye, which is called SYBR® GREEN 1, that can intercalate to the DNA. The resulting DNA-dye complex absorb blue light (λmax=497 nm) and emits green light (λmax = 520 nm).

SYBR® GREEN 1(2X) was added to the DNA-droplet and mixed. SYBR® GREEN 1 intercalated with double-stranded DNA (ds-DNA) and showed fluorescent signals under fluorescent microscope.

III. Conclusions

We have developed an on-chip DNA extraction protocol with MBs on a EWOD chip. Our EWOD system extracts the DNA from whole blood successfully. There was variety of sample and reagent liquids that were successfully driven on a EWOD chip. We also developed two approaches that droplet put in the chip by testing reagent’s contact angle. After on-chip DNA extraction, The SYBR® GREEN 1 stained DNA could be easily observed under a fluorescent microscope. The low consumed reagents, low required whole blood volume and faster on-chip DNA extraction was successfully developed.