A Droplet-based Device for Triglycerides Colorimetric Measurement

Chih-Sheng Yu*, Ming-Yu Lin, Heng-Tsang Hu, Yi-Chiuen Hu and Haiao-Yu Chou

Instrument Technology Research Center, National Applied Research Laboratories
Hsinchu, Taiwan 300, R.O.C.

Abstract

We characterize a droplet-based biochemical reaction device with the features of droplet self-positioning, self-mixing and self-alignment optics on a micro-patterning PDMS chip. The phenomenon of droplet self-positioning is explained as follows: the different texture zones generate gradient of surface tension force to manipulate droplet. Droplet-based reagents can be transported, precisely positioning, and mixed on the detection zone without any power source. The study was carried out to experimentally determined triglycerides (TG) using this droplet manipulation device with correlation coefficient 0.922 from 19 mg/dL to 480 mg/dL.

Key Words: Droplet Manipulation, Droplet Self-mixing, Triglycerides Assay

1. Introduction

Triglycerides are the chemical forms which most fat exits in the food, and in the body. Excess triglycerides in plasma are so called hypertriglyceridemia, a disease closely related with cerebrovascular diseases and cardiac vascular diseases, including coronary artery disease. Blood sugar is out of control caused by diabetes mellitus (DM), which may also cause high concentration of triglycerides in plasma. Daily dietary plays a key role in controlling blood sugar and triglycerides [1]. Therefore, it raises increasing demands for a point-of-care device to real-time monitor the blood level of triglyceride in medical rapid diagnostics.

Recently, the droplets-based manipulation is receiving increasing amount of attention in the microfluidic biosensors application. Owing to the advantages of the low sample and reagent volume for reacting, rapid mixing [11–13], the short reaction time and the property of non-contaminating on the surface of a low surface energy. Therefore, many efforts are complete for studying the droplet behavior in the recent years, and different type of droplet-based control devices are developed accordingly [2–4].

Surface tension is domain force in the micro fluidics devices and the roughness can be enhancing the property of the surface energy [5]. He and Lee [6] proposed a droplet motion by switched the (polydimethylsiloxane) PDMS member devices, which the wettability is dynamically form superhydrophobic state to hydrophobic state.

In this paper, we characterize a gradient of surface tension force to manipulation droplet and a droplet-based

Figure 1. Droplet self-motion by gradient of surface tension.
reagent can be self-motion precisely positioning without any power source, as show in Figure 1.

2. Theory

The contact angle of the droplet on a composite surface could be quantitatively calculated by a general equation [7]:

$$\cos \theta_a = f_1 \cos \theta_1 + f_2 \cos \theta_2$$

(1)

Where $\theta_a$ is the contact angle of the droplet on the composite surface, $f_1$ is a contact ratio of the solid-liquid, $\theta_1$ is the contact angle of the droplet on the solid-liquid, $f_2$ is a contact ratio of the air-liquid and $\theta_2$ is the contact angle of the droplet on the air-liquid.

The conditions of the contact interface between the droplet and the ambient air should comply with the Laplace-Young equation [8]:

$$\Delta P = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$

(2)

Where $\Delta P$ is a difference between the internal pressure and the external pressure of the spherical surface of the droplet, $\gamma$ is the surface tension of the droplets; $R_1$ and $R_2$ are the radius of curvature of the surface, respectively.

The hysteresis effect is importance factor and to cause a droplet moving on solid surface [9]:

$$F = \gamma \left( \cos \theta_a - \cos \theta_s \right)$$

(3)

Where $\gamma$ is the surface tension, $\theta_a$ and $\theta_s$ are the advancing and receding contact angles, respectively.

3. Design and Fabrication

3.1 Basic Concept

The basics design is show in Figure 2. The texture pattern was designed to have several radiate-texture, which had gradient of surface tension. The wettability was gradually decreased from the outer zone (Super-hydrophobic) to the center zone (Hydrophobic). When the droplet was dripped on this surface Figure 3. (a), they could be self-transportation and self-position to the center zone (b) is due to hysteresis forces form equation (3). In this device, the hysteresis forces will drive the droplet to move toward the surface having a smaller contact angle. That is to say, the droplet will be moved from the super-hydrophobic surface to the hydrophobic surface.
3.2 Detection System Setup

Figure 4 shows a concept diagram of the integrated biochemical detection system, which is based on colorimetric absorbance and consists of a small size droplet, low cost LED and photodiode for triglyceride quantitation. Droplets of the triglyceride and the reagent are dripped using pipette. They are self-motion by gradient of surface tension force, which can be guided the droplet with direction of similarity to center zone without any accuracy system and power source. When the droplet mixing is occurs, the absorbance is measured using photodiode.

3.3 Chip Fabrication

The droplet-based detection device was fabricated by molding process. Major fabrication step are illustrated in Figure 3.

The silicon master was fabricated using standard photolithography and Bosch etching (STS, Multiplex ICP) process (a) (b) and picture of silicon master show in Figure 6. After etching of 15 μm, the photoresist was stripped by acetone. In order to replicate the biochip by molding process, the PPFC (plasma polymerization fluorocarbon) film was deposition on the silicon master by passivation process (c) to provide anti-adhesion property. The PDMS (polydimethylsiloxane) is an elastomer material, which has several advantages in this system, such as high transparent, low surface energy, biocompatibility and low cost fabrication. The two PDMS components, part A and part B (Dow Corning, Sylgard 184) are mixed in a 10:1 ratio, and mixture degassed under vacuum with 30 min to remove bubbles.
And then poured over between silicon master and bottom, loading 12 kPa and heated 90 °C for 1 hr to cure the PDMS (d). Subsequently, the chamber is cooled down, and replicated chip is demolding form the molding system (e) and PDMS devices show in Figure 7.

3.4 Triglycerides Assay

Triglycerides, esters of fatty acids and glycerol, are bound to proteins as lipoproteins circulating in plasma. The procedure involves enzymatic hydrolysis by lipase of the TG to glycerol and free fatty acids. The formed glycerol is further reacted with ATP, Q2, 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl)m-nisidine (ESPA) in a coupled enzymatic (Glycerol kinase, GK glycerol phosphate oxidase, GPO, peroxidase, POD) methods. (Sigma Chemicals, St. Louis, Missouri, USA).

\[
\begin{align*}
\text{Triglycerides} & \rightarrow \text{Lipoprotein Lipase} \rightarrow \text{Glycerol + Fatty Acids} \\
\text{Glycerol + ATP} & \rightarrow \text{GK} \rightarrow \text{G-1-P + ADP} \\
\text{G-1-P + ADP} & \rightarrow \text{GPO} \rightarrow \text{DAP + H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 4\text{-AAP + ESPA} & \rightarrow \text{POD} \rightarrow \text{Quinoneimine Dye + H}_2\text{O}
\end{align*}
\]

4. Result

4.1 Droplet Manipulation

Figure 4 shows sequence of droplet merging by high-speed camera system (X-Stream VISION, IDT, Inc.) a frame rate of 100 frames/sec. The droplets volumes are 4 μl and 7 μl, respectively. The 4 μl droplet was precisely positioning on the center zone. The 7 μl droplet drove automatically toward the center and simultaneously triggered rapidly merging. The varied texture is support the droplet to generate a velocity (about 20 mm per sec) and restrict within center zone, then coalescence occurs. When the gap between the

![Figure 7. SEM photograph of PDMS.](image_url)

![Figure 8. Droplet self-motion process.](image_url)

![Figure 9. The mixing phenomena of triglycerides reaction.](image_url)

![Figure 10. Triglyceride quantification measurement.](image_url)
two droplets is very closely, the van der Waals’ force is dominant factor to cause the droplet merging [10].

Theses images showing how a droplet self-motion on gradient of surface tension. For this recording, which took a few second. (a)–(c) is the droplet self-moving, self-merge(d), and self-vibration (f)–(h). Finally stop in center zone (i). Use this method has several advantage: a) the droplet manipulate without any power source, b) the droplet can be precisely position without accuracy machine, c) reaction in short time, and low volume re-agent for mixing process, d) real-time detection. Moreover, this momentum assisted in molecule diffusion and it’s effective improved the mixing process.

4.2 Triglycerides Assay

We use 1 μl TG droplet self-positioning on the reaction zone and 9 μl reagent droplet mixing for the colorimetric assay. We perform a TG measurement on this self-alignment optical detection system.

Figure 9 as show the mixing process; we simultaneously detected the optical density of absorbance with the time course study in our detection system. The optical density of the transmission decreased as time goes and the whole biochemical reaction was finished within 30 seconds.

Figure 10 show as the concentration of triglyceride is proportional to absorbance with correlation coefficient 0.922 from 19 mg/dl to 180 mg/dl.

5. Conclusions

A novel biochemical reaction device for real-time measuring triglycerides based on the droplets manipulation was proposed. Using this system, we successfully performed the triglycerides quantification assay with a non-power source for droplet self-positioning, self-mixing and self-alignment on the PDMS hydrophobic surface. The whole system is able to detect the physical concentration of triglycerides from 19 mg/dl to 180 mg/dl, which also meets the requirement for the commercial triglycerides meter. This droplet-based manipulation system with advantages of self-positioning, self-mixing, and self-alignment can be further developed for the prospects of developing multi-functional biochemical assays array.

References

[12] Paik, P., Pamula, V. K., Pollack, M. G. and Fair, R. B.,


*Manuscript Received: Jan. 15, 2005*

*Accepted: Jun. 2, 2005*