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Droplet device for immunoassay detection

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1. Introduction

Droplets form in nanoliter volumes are expected to be used in microfludic environment as fluid carriers and reactors to allow us to accelerate and confine chemical reaction effectively inside droplets [1]. Taking this advantage, we have developed a microfludic device for droplet immunoassay detection which leads to development of multiple analyses detection using droplets including a lot of analysts with low concentration.

Interferons (IFN) are best known for their critical functions in host defense mechanisms. The type I interferons include IFN-alpha family, IFN-beta, -omega, -kappa and Limitin and are important for marshalling the immune response to viral infection. The type II interferon group includes only IFN-gamma. It is a multifunctional cytokine that exerts aniti-proliferative, immunoregulatory, and pro-inflammatory effects, which are important for numerous aspects of the immune response. In this study we focus on miniaturization of PDMS chips used for interferons immunoassays, especially in detection of IFN-γ. It is a power method for cancer diagnosis in a microchip, as well as to measure anticancer activity in human body.

2. Experiments

The PDMS based chip (Fig. 1) was made using hard-embossing procedures, in which the embossing mold was Si fabricated using ICP dry etching and the method can also be used to deposit a hydrophobic surface with Teflon on microfluidic channel to prevent non-specific binding inside the channels. In this study, syringe pumps were used to provide transportation for both carrier fluid (oil) and analytes (antibody and antigen based solution) at a speed ranging from 0.1 μL/min to 10 μL/min. The designation (Fig. 2) and functions of the PDMS chip can be explained as follows; firstly, the carrier oil (Fomblin Y L VAC 16/6) is introduced from oil inlet to provide overall transportation power inside the channel; secondly, microbeads (10 μm) immobilized with protein G are added from reagent inlet 1 and can be blocked in the PDMS pillar regions (Fig. 3) due to the gap between PDMS pillars is fabricated in less than 10 μm; thirdly, anti-antibody IFN-γ is introduced from reagent inlet 1 to bind protein G which is specific for IFN-γ antigen recognition; fourthly, the second antibody of biotinylated IFN-γ antibody reagent is used to provide binding site for streptavidin-HRP, which is introduced from reagent inlet 2 followed by using TMB substrate reagent (3, 3', 5, 5'-tetramethylbenzidine). The TMB is injected from reagent inlet 3 to fuse droplets of IFN-γ protein complexes; therefore the concentration of droplets can be determined in optical density at 450nm.

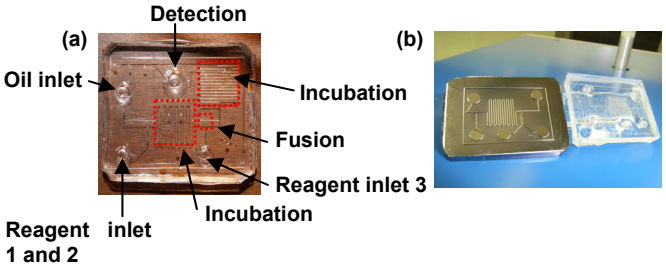

Fig. 1 (a) PDMS chip and (b) Si molding plate

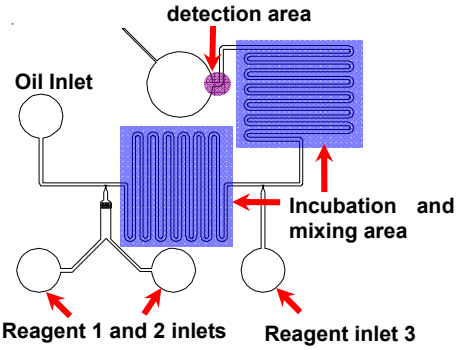

Fig. 2 PDMS chip designation

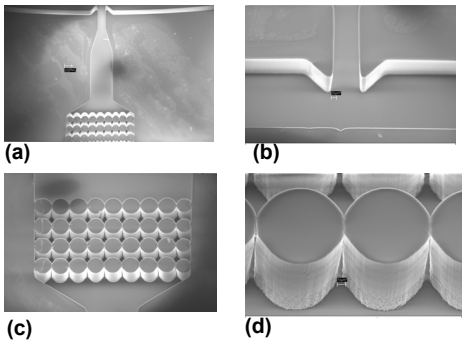

Fig. 3 SEM images of PDMS chip, (a) overall image of reagent outlet 1 and 2 (b) enlarged area of reagent outlet 1, 2 and 3 in jet-shape, (c) PDMS pillars region (800μm x 500μm), (d) magnified PDMS pillars (pillar size of 100μm height x 100μm in diameter with 5μm gap between).

3. Results and Discussions

In this immunoassay study, one of the key objects is to control droplet size. During the experiments, droplets in nano-liter containing IFN-γ protein complexes are generated from jet-shape channel and the droplet size can be differentiated well with good control of oil and reagents flow rate. From Figs 4 and 5, it is clear to see the droplet size can be produced in different mixture of flow rate. In some cases, if the flow rate ratio between oil and reagents is over 30 times higher or either way around, due to the suppressing pressure is too high, no droplet can be formed in this critical condition.

A measurement setup can be seen in Fig. 7 (a). Fig. 7 (b)

shows background signal due to the PDMS material itself. No absorption in spectrum region (260nm to 800nm) was observed. In Fig. 7 (c), it indicates that the detector moves toward microfluidic channel and it shows absorption band started from 300nm owning to the Fomblin oil is used as a medium inside the channel. The intensity of absorption increases as a function of time, Fig. 7 (d). In Fig. 7 (e), it gives intensity of droplet OD (optical density) at 405 nm, each peak represents one droplet and the signal can be plotted as a function of time in seconds. The difference in intensity is explained that various concentration of IFN-γ in droplet is differentiated from each other and this differentiation technique has potential to be used for multiple analytes in the future.

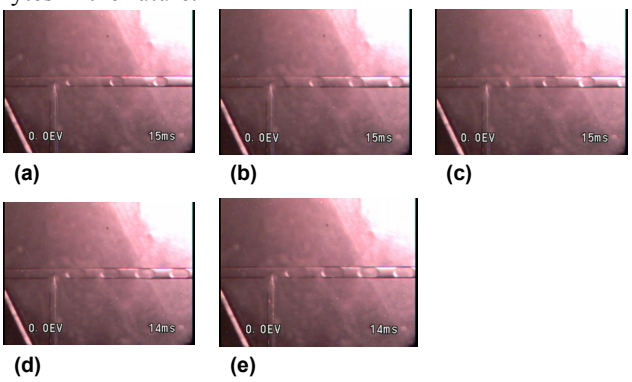

Fig. 4 Droplet size variation, fixed oil flow rate 2μL/min, (a) reagent flow 0.5μL/min, (b) reagent flow 0.8μL/min, (c) reagent flow 1μL/min (d) reagent flow 1.5μL/min, (e) reagent flow 2μL/min.

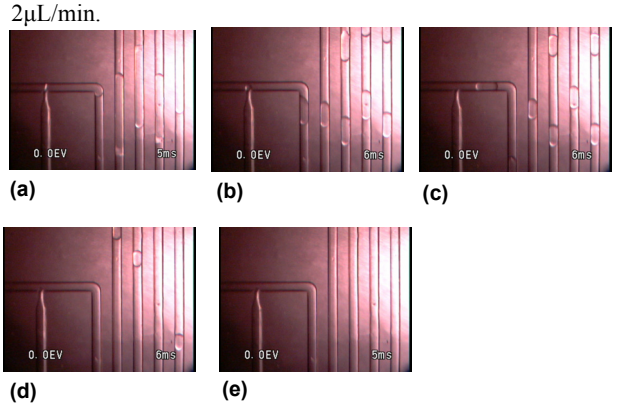

Fig. 5 Droplet size variation, fixed reagent flow rate 0.1μL/min, (a) reagent flow 0.1μL/min, (b) reagent flow 0.5μL/min, (c) reagent flow 1μL/min (d) reagent flow 2μL/min, (e) reagent flow 3μL/min.

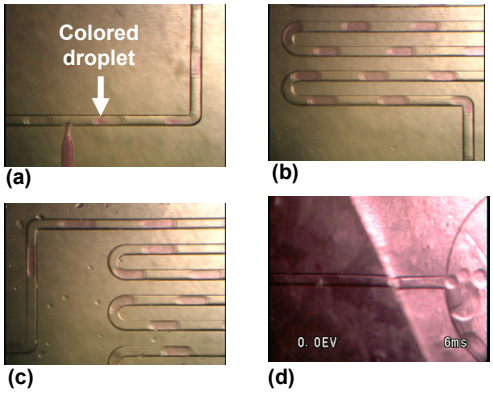

Fig. 6 Operation of PDMS chip, (a) Fusion of droplets, (b) incubation, (c) complete mixing, (d) droplet transportation into detection area.

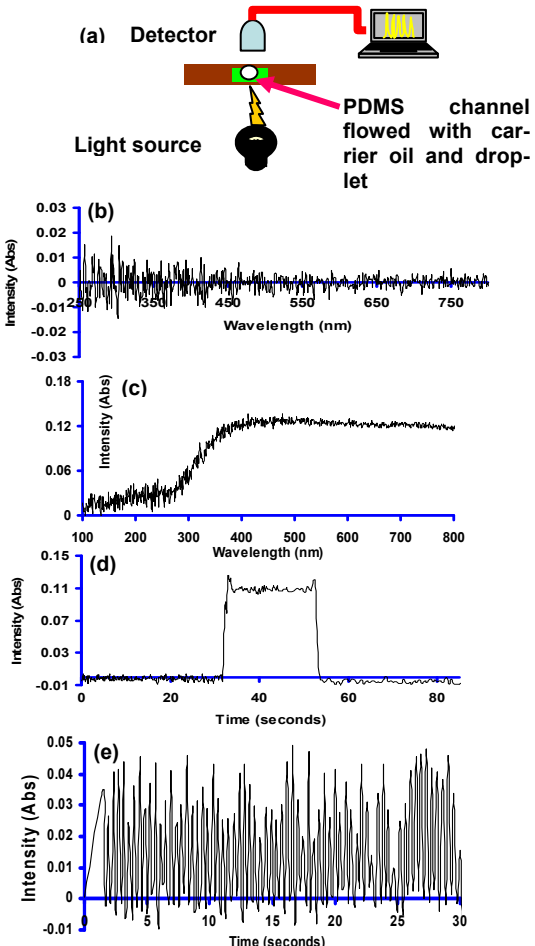

Fig. 7 (a) measurement setup, (b) measurement with PDMS only, (c) channel measured as intensity increased, (d) channel detected as a function of time, (e) droplet detection signals.

3. Conclusions

We developed a diagnostic chip for IFN-γ immunoassay employing nano-liter droplets in a PDMS chip and based on this study, we will be able to establish this technique for multiple analysts detection (up to a few dozen samples).

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References

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Appendix

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