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Parallelized High Throughput Emulsification and Emulsion PCR for Clinical Use of BEAMing Technology

Cai Kunpeng[1], Yamawaki Koya[1], Kawamoto Yasuko[1], Nakanishi Katsumi[1], Tagawa Ayato[1]

[1] Central Research Laboratories, Sysmex Corporation.
4-4-4 Takatsukadai, Nishi-ku, Kobe 651-2271, Japan
Phone: +81-78-991-1911 E-mail: Tagawa.Ayato@sysmex.co.jp

Abstract

Circulating tumor DNA (ctDNA) in plasma fractions from blood is believed to be a useful diagnostic and prognostic marker in comparison to stand methods. BEAMing is a highly sensitive dPCR technology, which is capable of ctDNA enumeration at extremely low ratio presentation. A microfluidic BEAMing system has been proposed by our team, while this work focuses on achieving high-throughput, high-efficiency and high detection sensitivity BEAMing procedures for clinical application.

1. Introduction

BEAMing (Beads, Emulsion, Amplification, and Magnetics) is reported as a highly sensitive digital PCR technology, with the capability of detecting ctDNA at extremely low concentration [1]. Microfluidics based solutions have been proposed by our team to replace complicated manual assays [2], including Pre-amplification / Dilution / Emulsification / Emulsion PCR (EmPCR) / Breaking and Hybridization / Flow cytometry sequentially [3]. High throughput, high efficiency, high sensitivity and automation of continuous microfluidic BEAMing procedures are further achieved in this work for large scale clinical applications.

2. Experiment

Working Principle and Chip Design

To reduce time consumption of sample transferring and eliminate contamination between Emulsification and EmPCR procedures, an integrated bio-chip enabling continuous emulsification and EmPCR was designed. As shown in Fig. 1, single DNA molecule and a magnetic bead coated with primers are encapsulated into a same droplet with deoxynucleotides (dNTPs) and polymerase, by passing through a junction (20 μm × 20 μm) inside the bio-chip. The as-generated emulsion will be continuously flowed into an S-shaped chamber for EmPCR.

The developed disposable bio-chip (122×144 mm) is shown in Fig. 2a, where polydimethylsiloxane (PDMS) reaction chambers were fabricated by photolithography separately for emulsification and EmPCR. For generation of small BEAMing emulsion (D ~ 8 μm) at high throughput (~ 45,000/s), a depth of only 20 μm is demanded for emulsification chamber. Yet, to accommodate sufficient volume of samples for EmPCR and FCM detection, depth of 520 μm is needed for EmPCR chamber. A jumper channel (depth of 20 μm) was then introduced to connect these two micro chambers on a glass substrate, as shown in Fig. 2b. 12 channels were integrated on one bio-chip to enable parallelized operation. Polycarbonate reservoirs for sample input and collection were laid out separately on two sides of the bio-chip at 9 mm pitch for convenient handling by conventional pipetting methods.

Continuous Emulsification and EmPCR

A bio-chip loaded with DNA samples (30 μL) was set onto an automated controlling device for emulsification and emulsion PCR. A contamination free method, shown in Fig. 3, was innovated for DNA sample injection at high precision, which is necessary for generating small droplets for clinical application. For emulsification, flow rate of oil phase was set to be 3.25 μL/min, while 1.3 μL/min was set as flow rate of DNA samples. A Peltier heater unit was included for PCR temperature cycles.

Observation and Evaluation

Emulsification processes were real-time monitored using digital microscope (AM4113ZT Pro Digital Microscope, Dino-Lite). The generated emulsion were filled into glass slice plates (UR157S, Sekisui), observed under a high resolution microscope (BZ-X700, KEYENCE) and analyzed using imaging software (Image-pro plus, Media Cybernetics). Breaking and hybridization were manually conducted for fluorescent detection, using FCM scatters obtained on a flow cytometer (Accuri C6 BD), where 120 μL of sample solution or 1 million counts of beads was measured for each sample. The obtained FCM scatters were then analyzed using FlowJo software (FLOWJO LCC). Details of detections and evaluations could also be found in our previous work [2].

3. Results and Discussion

Bio-Chip Evaluation

High-throughput continuous emulsification and EmPCR procedures were accomplished using the as-developed 12-channel integrated bio-chip. Parallelized emulsification in 12 channels was confirmed by real-time observation. Emulsion with homogeneous diameter dispersity have been successfully generated, as shown in Fig. 4. Diameter of generated emulsion were tuned from 8.8 μm to 90 μm by adjusting flow rate of oil phase and DNA samples. The applied flow rate ranges from 0.1 μL/min to 6.5 μL/min in our experiments.

Sensitivity

Fig. 5 displays typical FCM scatters (Fig. 5a and Fig. 5b) and summary of FCM scatter analysis results (Fig. 5c) from a collection of samples. It is shown that DNA

samples with mutation rate of as low as 0.02 % were successfully differentiated from normal wild type DNA sample, which indicates very high detection sensitivity and high clinical application potential.

4. Conclusion

High throughput, high efficiency and high sensitivity of BEAMing procedures were achieved by parallelized emulsification and EmPCR on an integrated bio-chip. Further results of evaluation on the bio-chip and automatic controlling device for clinical use will be reported at the conference.

References

[1] E. Heitzer *et al*., *Clinical Chemistry*, vol. 61(2015), 112.

[2] A. Tagawa *et al*., *International Conference on Solid State Device and Materials*, 2016, H-5-03.

[3] Vogelstein *et al*., *Proc. Natl. Acad. Sci*. 96(1999), 9236.

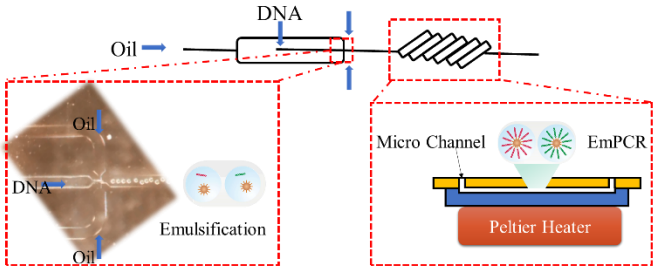

Fig. 1 Principle of continuous emulsification and EmPCR.

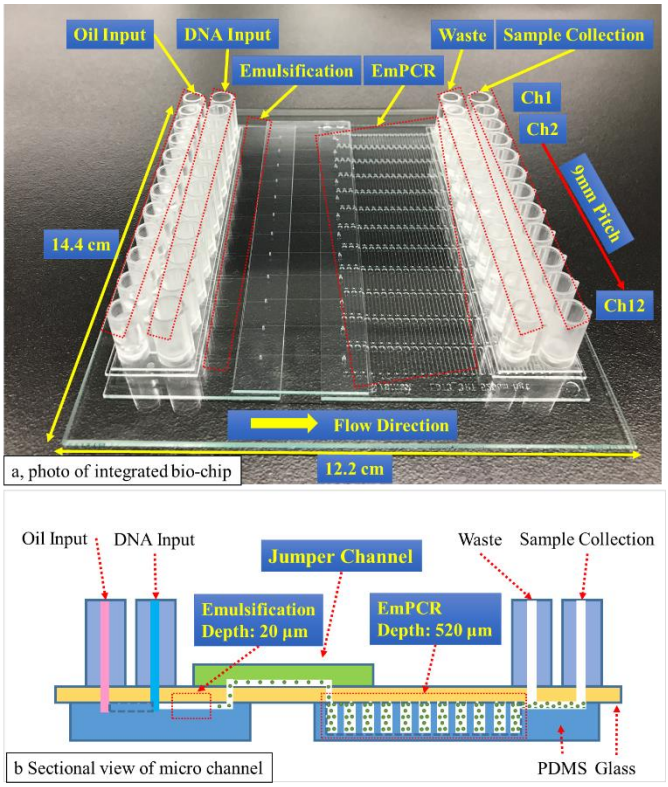

Fig. 2 Structure of the 12-channel integrated bio-chip.

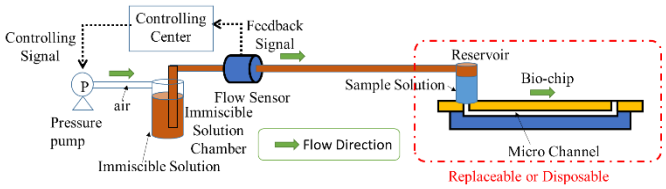

Fig. 3. A contamination free method for injecting DNA samples.

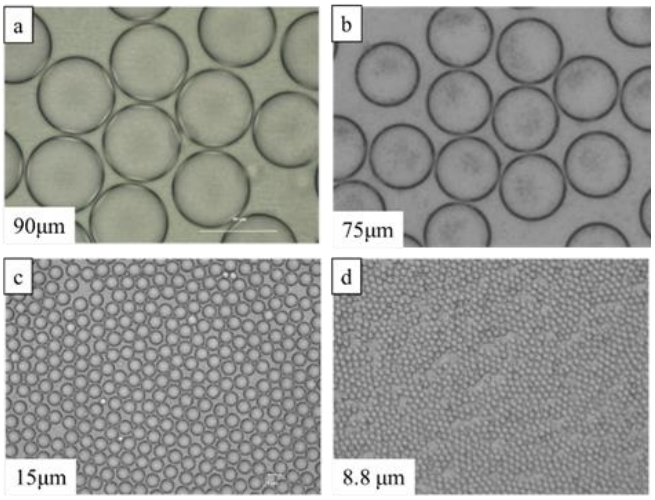

Fig. 4 Photos of generated emulsion

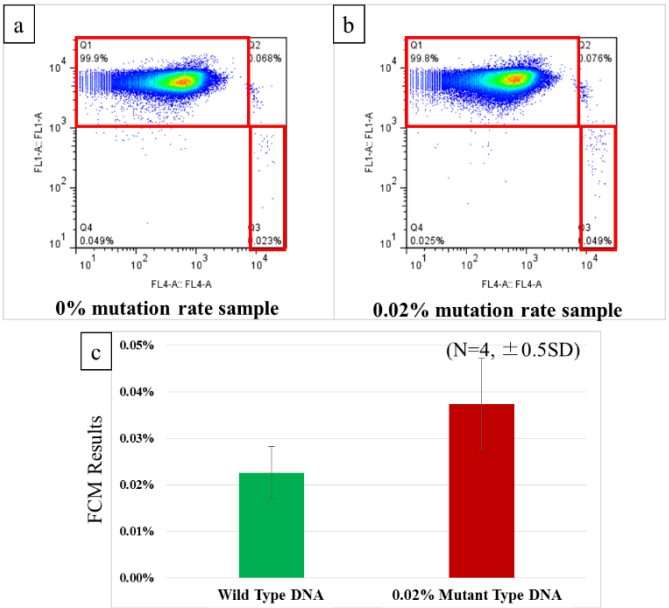

Fig. 5 Differentiation of 0.02% mutant DNA samples from wild DNA samples with no mutation.