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BEAMing (Beads, Emulsion, Amplification, Magnetics) on a Chip for Liquid Biopsy in Clinical Diagnostics

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Abstract

ctDNA (circulating tumor DNA) is expected to be a new generation of cancer biomarker and BEAMing digital PCR is one of the ultra-sensitive ctDNA detection technologies. We are developing automated devices of BEAMing for large-scale clinical trials to realize liquid biopsy. In this presentation, I will show the results of fundamental evaluation using microfluidic system.

1. Introduction

The importance of CDx (Companion Diagnostics), in which diagnosis and therapy are conjoined, is growing in concert with the progress of recent years in molecularly targeted antitumor agents. The range of cancer types indicated for testing by these agents is expected to grow and ultimately include all types. At present the approved CDx test targets are tissue specimens derived from the primary lesion obtained by surgery or tissue biopsy. However, repeated highly invasive tissue biopsies are difficult to perform and place a heavy burden on the patient. A method of detecting cancer-originated DNA in the blood is therefore highly desirable. BEAMing (beads, emulsion, amplification, magnetics) has been proposed for detection of minute quantities of ctDNA (cell-free circulating tumor DNA) in the blood [1][2]. It is one of the digital polymerase chain reaction (dPCR) techniques [3] comprising a sequence of manual processes performed in the order DNA extraction, pre-amplification, beads emulsification, emulsion PCR, emulsion break, hybridization and flow cytometry. We plan to develop an automated BEAMing system including a microfluidic system as shown in Fig. 1 to react pre-amplification to hybridization that will enable its application to large-scale clinical trials.

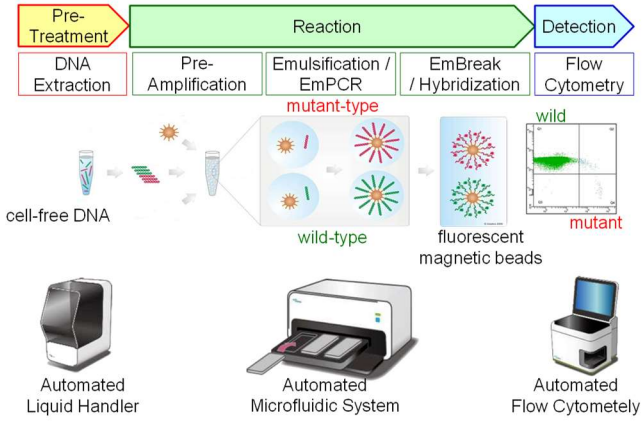


Fig. 1 Automated BEAMing system for clinical use

2. BEAMing with microfluidic system

The concept of BEAMing with microfluidics [4-6] is shown in Fig. 2. Template DNA with master mix flowed through 3 different temperature hotplates as pre-PCR as shown in Fig. 2(a). The amplified DNA was diluted in the micro-chamber heated half dimention to generate convection flow in Fig. 2(b) to realize limiting dilution to encapsulate single DNA and single bead in one droplet.

Using a flow focusing chip in Fig.2(c), the normalized DNA with 1 µm primer-coated magnetic beads and master mix were encapsulated in 7-8 µm diameter micro-droplets as shown in Fig. 3. Approximately 92.4 million droplets were generated and 6.7 million beads were encapsulated within ten minutes. The bead-droplets continuously flowed over 2 different temperature hotplates in a serpentine channel as shown in Fig.2(d). The bead-droplets were maintained after the emulsion PCR.

The droplets then flowed through the channel with alcohol in the device with a heater and a magnetic bar as shown in Fig. 2(e), where the droplets are broken by the alcohol. Then, the breaking solution flows through a micro-chamber over a magnetic bar, to expel the alcohol and oil via an output port, thus leaving only the magnetic beads in the micro-chamber. After several washings, the double-stranded DNA bonded to each bead was denatured by filling with an alkaline solution, and then were hybridized by filling with fluorescent probes. By moving the magnet away from the micro-chamber, the magnetic beads can be collected via the output port.

The collected fluorescent beads related to the amplified wild and mutant-DNA were analyzed with flow cytometry (FCM) and identify the mutant and wild type DNA signals as shown in Fig. 4(a). The signals of mutation increased as the concentration of input mutant-DNA increased as shown in Fig. 4(b) with 0.02 % LOD.

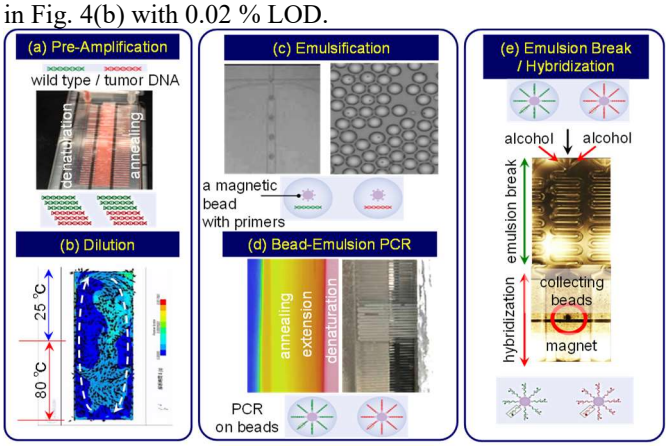


Fig. 2 BEAMing with microfluidics

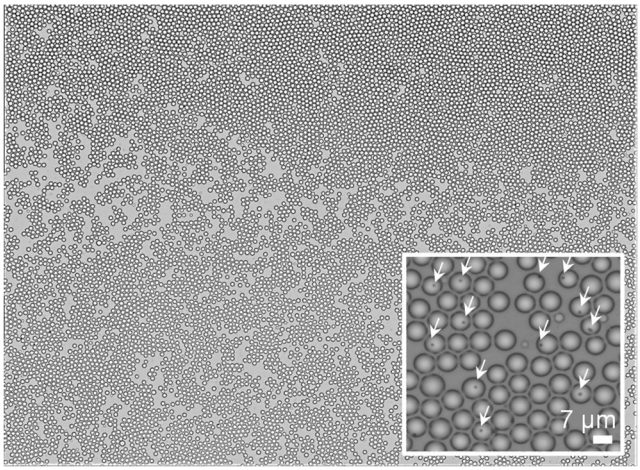
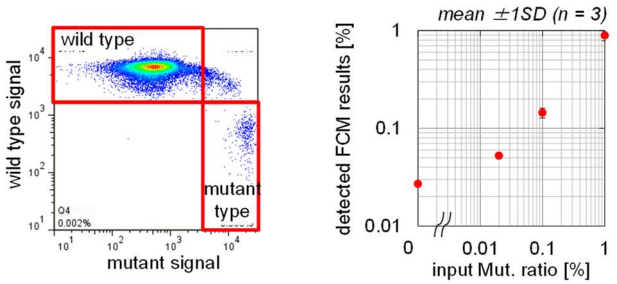


Fig. 3 Femtoliter bead-in-droplet



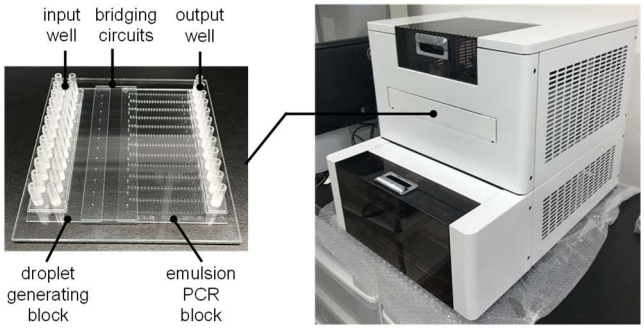
(a) Scattergram (b) Calibration curve

Fig. 4 Detected 0.02 % mutant-DNA with microfluidics

3. Automated system

Fig. 5 shows the fully automated emulsion PCR device. The cartridge shown in Fig. 5(a) carries out bead-droplet generation and emulsion PCR. The cartridge is composed of three different fluidic circuit blocks of a 12-channel droplet generation fluidic block and a 12-channel emulsion PCR fluidic block with a bridging block to connect droplet generation and emulsion PCR. We can separately evaluate the functions of droplet uniformity and reaction of DNA in droplets for quality control. To optimize the cartridge for clinical lab use, 12 flow paths are located at the 9-mm pitch same as standard lab pipettes.

DNA samples, primer-coated beads and master mix are mixed and input in the 9 mm-pitch wells of the cartridge with lab pipette and set in the automated device as shown in Fig. 5(b). After starting with the software, the sample is loaded into microfluidic circuits and start reaction automatically. After finishing the process, the bead-droplets after PCR are collected in output wells. The most important factor for high-sensitive DNA testing is how to prevent contamination. We specially designed the system to block aerosol injecting into the instrument.



(a) Cartridge (b) Automated System

Fig. 5 Automated emulsion PCR system

4. Conclusion

The results of this study show that BEAMing reaction functions effectively in a micro-channel. High-sensitive molecule detecting technologies using droplets have been the subjects of widespread studies for many years. The targets have now extended from DNA to cells and proteins [7-9]. Progress has been rapid with regard to development of instruments for research. In the near future, diagnostic instrument development lies ahead and our goal is to produce world-leading systems with BEAMing technology.

References

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