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An Integrated One-chip-sensor System for miRNA Quantitative Analysis Based on Digital Droplet PCR

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Abstract

A one-chip-sensor for miRNA quantitative analysis was developed, based on digital droplet PCR (ddPCR). Reverse transcription and PCR were performed sequentially on chip, and fluorescence from droplet was detected by newly developed off chip miniaturized detector. Quantitative results, obtained by analyzing samples containing known concentration of miRNA, prove the functionality of the sensor.

1. Introduction

miRNAs are endogenous short non-coding RNAs. miRNAs play a critical role in various cellular, developmental and physiological processes by means of post-transcriptional regulation of gene expression. Correlation between changes in the expression patterns of miRNAs and different diseases has been reported [1]. As more and more diseases and disorders are being linked to miRNA [2], a simple fast and quantitative analysis of miRNA in the blood becomes of greater need. At present, miRNAs analysis is performed using conventional laboratory techniques. It is time consuming and requires skilled personnel. In order to decrease costs and time to result, it is important to develop simple, small benchtop tools for miRNAs analysis. These tools could be used both at the hospital and at the doctor office and would require only a drop of blood as initial sample.

We have recently proposed a “one-chip-sensor” to realize fast and automatic SNP (Single Nucleotide Polymorphism) analysis from a small volume of blood [3]. An automated miRNA analysis system can be realized by implementing quantitative analysis techniques based on digital droplet PCR (ddPCR) in this one-chip-sensor. In addition, a miniaturized fluorescence detector is also developed to further reduce the form factor of the bench top size system.

In this paper, we have successfully demonstrated on chip miRNA quantitative analysis by using ddPCR technology with a miniaturized fluorescence detector.

2. The basic principle and the system design

Digital droplet PCR techniques (ddPCR)

Digital droplet PCR (ddPCR) is an emerging technology enabling a direct quantification of nucleic acids. It is based on partitioning the sample into small droplets, such that at least some contain no template. The average template number per droplet before PCR is determined from the ratio of positive droplets after PCR to the total droplets, making use of the Poisson distribution. Figure 1 (a) shows concept image of one-chip-sensor for quantitative analysis of miRNAs. The chip integrates reverse transcription (RT) and ddPCR modules. The droplets are counted by using a miniaturized fluorescence detector based on optical pickup head (OPU). Figure 1 (b) shows optical microscope image of generated droplets in the reactor.

One-chip-sensor design of miRNA analysis

Figure 2 shows the structure of the one-chip-sensor. Microfluidic structures are etched in a Si wafer and sealed with Pyrex glass by anodic bonding, fluidic connections are opened by a backside etch step [4]. It consists of five reactors, each with a 0.2 μL volume. They are thermally insulated from each other and from the remaining chip. Two reactors are allocated for RT, and the remaining three for ddPCR [5]. A thermoelectric Peltier element is used to accurately control the temperature of these reactors. A mixer and a T-junction channel are positioned between the reactors for RT and those for ddPCR. The template cDNA produced during RT is mixed with PCR reagents in the mixer. The droplets are generated at the T-junction channel by mixing the RT solution with oil. After PCR, the droplets are fed to the optical detection channel at a constant speed, and the optical detector counts both the number of fluorescent and non-fluorescent droplets.

A miniaturized fluorescence detector

We developed a miniaturized fluorescence detector by modifying a DVD’s OPU. Figure 3 shows the photograph of newly developed detector. This unit consists of 661 nm laser diode (LD), and an objective lens with numerical aperture 0.65. The focused laser beam is irradiated to the optical detection channel in a chip. The fluorescence light passes through the same objective lens, some optical filters and a pinhole and is detected by a photomultiplier (PMT) which is set on the OPU unit. The pinhole is placed in the position conjugated with the focused beam on the chip, and shields the scattered and reflected beams at the emission wavelength.

3. Results

Droplet counting by a miniaturized fluorescence detector

The performance of the fluorescent detector based on DVD OPU was evaluated using PCR solutions prepared off

chip. The solution for positive droplets was obtained by amplifying in a conventional PCR tools a mix of Biorad supermix, ACTB Cy5 primer, and 2.5ng/μL human genomic DNA. The solution for the negative control was prepared likewise, but the human genomic DNA was omitted. Positive and negative droplets, with a volume of approximately 300 pL were generated at the T-junction channel, and were counted directly by the fluorescent detector. 2.2 mW laser beam was focused at the center (both in depth and width) of the fluidic channel in the chip. Figure 4 shows the detection results. The positive and negative droplets could be easily distinguished with good signal ratio. We achieved a very high speed counting, exceeding 1700 droplets per second. It is realized to analyze all droplets filled in 3 PCR reactors within approximately 10 sec.

miRNAs quantitative analysis on the chip

The miRNAs analysis was performed on the sensor chip. Synthetic miRNA samples with concentration of 3000, 1500, 900, and 300 templates per μL were prepared off-chip. The miRNA was mixed in a 1:2 ratio with RT buffer (Taqman miRNA reverse transcription kit, Applied Biosystems) and stem-loop primers (has-Let-7a, Applied Biosystems). This mixed solution was fed to the 2 reactors for RT to generate cDNA by varying the temperature with 3 steps (30 min. at 16 °C, 30 min. at 42 °C and 5 min. at 85 °C). In the next step, the cDNA was mixed on chip in a 1:3 ratio with the PCR reagents. This solution was mixed with fluorinated oil with appropriate surfactants (Biorad 186-3005) to generate the droplets. The flow rate of the solution and the oil streams were adjusted to 0.4 and 0.8 μL/min, respectively. The droplets were subjected to a heat-activation step for the polymerase (5 min. at 95 °C) and to at least 50 PCR cycles (15 sec. at 95 °C, 45 sec. at 60 °C). Figure 5 shows the comparison of expected concentration and measured concentration of template. The both concentrations have a clear correlation, demonstrating the capability to directly quantify miRNAs using our miniaturized system.

4. Conclusions

A miRNA quantitative analysis system was fabricated and evaluated. The RT and ddPCR were performed sequentially on the same chip, and fluorescence was detected by a miniaturized optical system. This technology is very promising for the realization of compact and automatic miRNA analysis system on site such as the doctor office.

References

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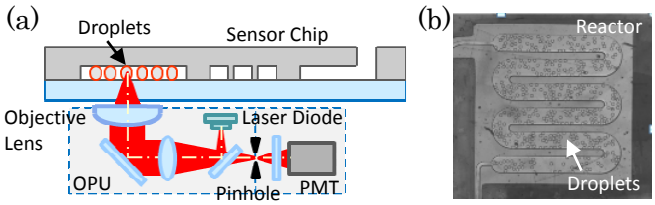

Fig. 1 Schematic of one-chip-sensor (a) concept image (b) droplets image generated in a reactor in the chip

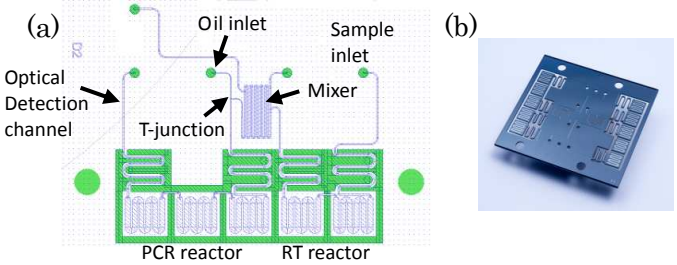

Fig. 2 (a) one-chip-sensor design for miRNA quantification, (b) Photographs of fabricated chip.

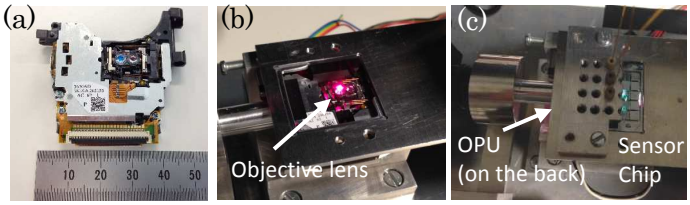

Fig. 3 Photographs of a miniaturized fluorescence detector based on DVDs optical pick-up. (a) Optical pick-up unit (b) Laser emission image (c) Assembled image

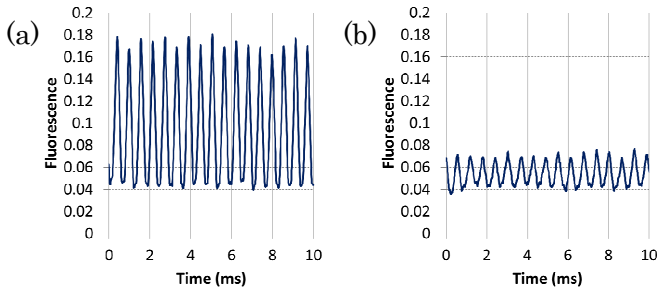

Fig. 4 Droplet detection results using a miniaturized fluorescence detector. (a) Positive droplets (b) Negative droplets

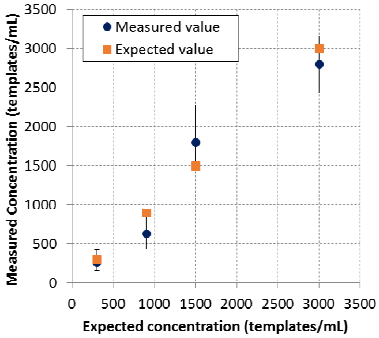

Fig. 5 Quantitative analysis of miRNA concentration. Comparison of measured and expected concentration. Error bar shows 95% confidence interval.