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REVIEW

Recent Development of Droplet Microfluidics in Digital Polymerase Chain Reaction

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Abstract: Digital polymerase chain reaction (PCR) has been experiencing a rapid development during the past few years. Compared with the traditional qPCR, the accuracy of digital PCR for the absolute quantification of target gene has been significantly improved with the same primer and probe as qPCR. The development of digital PCR is directly related to the development of microfluidics. The integrated fluid circuit, which has a complicated fabrication process with high cost, is an early integration of the microfluidics and digital PCR. Recently, researchers are trying to apply the droplet microfluidics in digital PCR. A droplet microfluidic chip is able to generate millions of droplets within a short time. Each of these droplets containing no more than one target gene is a reaction chamber during the amplification process. After amplification, each droplet is tested to obtain the absolute quantification of the target gene. This paper reviews the recent progress of droplet digital PCR and the application of droplet digital PCR in biological, medical and environmental area.

Key Words: Digital polymerase chain reaction; Droplet microfluidics; Genetic testing; Gene sequencing; Review

1 Introduction

The concept of digital PCR was first proposed in 1992 with the prediction of its significant advantages in quantitative analysis of the target DNA[1]. The initial development of digital PCR was quite slow due to the huge consumption of the sample and reagent using the 96 or 384 well plate[2]. The experimental processes of digital PCR analysis using well plates were usually complicated and required to be conducted simultaneously in several well plates, which was a huge/main barrier to conduct the research in digital PCR. In addition, with the rapid development of real-time quantitative PCR (RT-qPCR) in the past few years[3,4], researchers tend to use qPCR instead of digital PCR in the field of quantitative analysis of target gene.

Digital PCR got a revival recently by the combination with microfluidics technology[5]. With the boost in sensitivity and accuracy, the microfluidics-based digital PCR has gain more and more applications in single cell study[6,7], gene sequencing[8], medical diagnosis[9–11], environmental monitoring[12,13], and food safety[14–16]. In recent years, the commercial instruments of the digital PCR were also invented. In this study, we briefly introduced the concept of digital PCR and droplet microfluidics and discussed the wide applications of the droplet microfluidics-based digital PCR in biological, medical and environmental area.

2 Digital PCR

In comparison with the traditional qPCR, the digital PCR can obtain the quantitative information of the target gene in digital form instead of exponential form. The working principle of the digital PCR is shown in Fig.1[17]. The solution, which contained target gene, primer, DNA polymerase, etc., was diluted into hundreds to millions of units, each of which contained no more than one target gene. Then the solutions would go through the traditional thermal cycling amplification process. After amplification, each unit was checked if it

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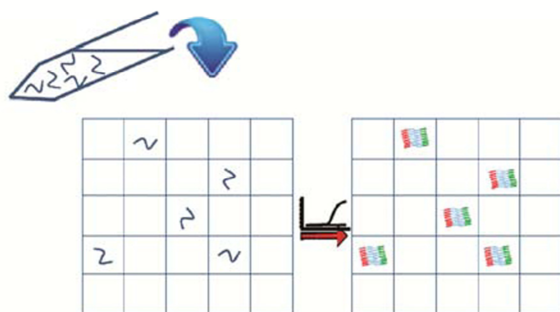

Fig.1 Schematic diagram of digital polymerase chain reaction (PCR) process[17]

contained the target gene. The positive unit was marked as “1” while the negative unite was marked as “0”. These “1” and “0”were similar to open and close in digital circuits, which was the reason that the process was named “digital PCR”. The exact quantitative information of the target gene could be obtained by counting the positive unit after PCR. Considering the fact that there might be more than one target gene in the original reaction unit, the Poisson correction could be used for the more precise calculation.

Compared with the conventional PCR progress, since the thermal cycling and fluorescent probes techniques are relatively mature at current stage, the major technological difficulty is to massively divide the reaction solutions. To divide the solutions, 96-well or 384-well plate was used initially; however, the major drawbacks of this technique were the huge consumption of the reaction reagent (5 μL for each well) and limited reaction units. Even though some progresses are made for this well plate-based method, for instance, Shen *et al*[18] fabricated 1280 wells on glass substrates, but the two major issues on reagent consumption and reaction units are still not resolved. The combination of microfluidics and digital PCR significantly boosts the number of reaction units, which will be introduced in details in later sections in this review.

The example of fetal medical diagnosis with digital PCR (Fig.2) further explains the working principle of digital PCR. Figure 2a shows one of the twelve well plates, which contains 765 reaction units with a volume of 6 nL level for each unit. The solution containing target gene, primer, fluorescent probe, etc. were distribute to the wells evenly using microfluidics technology. After thermal cycling, the units marked with TaqMan fluorescent probes were easily to be detected through fluorescence microscope. After data processing, the heat map (shown in Fig.2b) could be obtained, in which each red dot represented a unit with target gene. The heat map revealed the precise quantitative information of the target gene.

Figure 2c shows the amplification curves during the digital PCR process. It has to be pointed out that the amplification curve is not necessary to obtain the quantitative information of the target gene using digital PCR. In contrast, the qPCR analysis relies on the comparison between amplification curve and standard curve. Since the amplification efficiency and some other variables may unequal for amplification curve and standard curve, the measurement accuracy of the qPCR is relatively low. Hindson *et al*[19] and Whale *et al*[20] compared the qPCR and digital PCR process and found that digital PCR had significant advantages in sensitivity, accuracy and measurement time over the qPCR. For measurement accuracy, the coefficient of variation was reduced by 37%–86%[19]. As for time consuming, the day-to-day reproducibility was improved by a factor of seven[19].

As the early integration of microfluidics and digital PCR, the integrated fluid circuit (IFC)[21]-based digital PCR chip had every complicated microchannels with pumps and valves fabricated by multi-layer photolithography. The reaction solution was pumped into the reaction chamber arrays with the help of these pumps and valves. The sophisticated structures on IFC chips significantly raised the fabrication cost. Restricted by the fabrication cost and fabrication technology, the number of reaction chamber was usually less than ten thousand[22].

Nowadays, researchers in digital PCR start looking for options from droplet microfluidics technique that has been developing rapidly over the past few years. Using droplet microfluidics technique, highly diluted reaction solution that contained target DNA and reagent was divided into hundreds to millions of droplets[23] in nano-liters[24] or pico-liters[25]. Each of these droplets was a reaction chamber containing no more than one target gene. After amplification, the quantity of the target gene could be obtained from the number of droplets which had positive reaction.

Compared with the IFC-based digital PCR method, the advantages of droplet digital PCR (ddPCR) do not only lie in the dramatic increase of the number of reaction units, but also in the cost. The fabrication process of IFC usually involves multi-layer fabrication in nano-scale, which largely increases

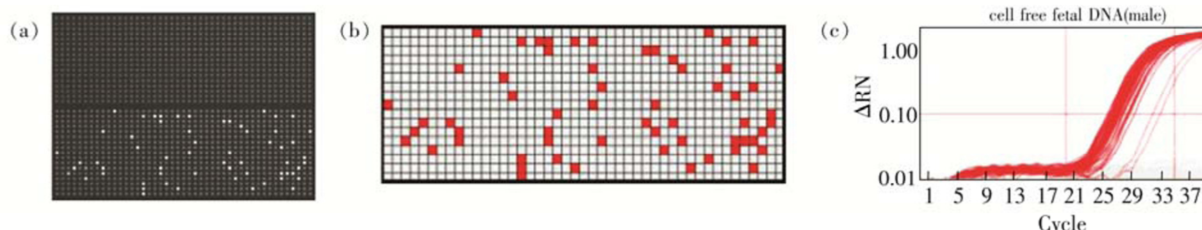

Fig.2 Digital PCR amplification of fetal DNA from maternal plasma analyzed in a digital array microfluidics chip[10]
(a) Same chip before and after PCR amplification process; (b) Heat map for positive reactions; (c) Exponential plot for amplification process

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