Poster

DIGITAL PCR ON A COMPACT STEP EMULSIFICATION DEVICE AND ITS APPLICATION IN DETECTION OF COPY NUMBER VARIATIONS OF CANCER

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Droplet digital PCR (ddPCR) is a revolutionary alternative to conventional real-time PCR for measuring nucleic acids with high precision and specificity, and is playing a more and more important role in biomedical research and clinical diagnostics. However, current commercial-available ddPCR systems requires different instruments to perform droplet generation and droplet reading, which makes the operation complicated and limited the wide application of ddPCR. To simplify the process, novel microfluidic devices have been reported, such as Abraham Lee's 1−million droplet array device[1], centrifugal step emulsification chip[2], and SlipChip[3]. However, it's still challenging to further simplify and reduce the cost of ddPCR for point-of-care applications.

In this work, we present a simple step emulsification ddPCR (SE-ddPCR) device which integrates sample loading, droplet generation, thermal cycling, and on-chip fluorescence imaging. Compared with previous methods, our compact device（1 inch × 3 inch in size）can simultaneously analyze 8 samples, generating around 10,000 droplets for each sample. The device with droplet array generated is shown in Fig 1. We designed a sample loading channel in the middle, and droplets were generated through nozzles based on step emulsification. The droplets arranged into monolayer array in the chamber automatically. This ddPCR device use a single pressure pump to drive droplet generation of 8 samples in parallel. Importantly, we used an optimized mineral oil instead of fluorinated oil, which further reduced the cost and avoided bubbling during thermal cycling[2].

We evaluated the performance of SE-ddPCR by measuring the concentration of a constructed plasmid containing *HER*2, a gene related to breast cancer. As shown in Fig 2, a linear regression was obtained in the range of 10 to 2000 DNA copies/μl, demonstrating high robustness of SE-ddPCR. Next, we measured copy number variation of *HER*2 in 16 clinical samples from patients with breast cancer, by simultaneous counting the *HER*2 gene and a reference gene (RNase P). The results of SE-ddPCR was compared with that using commercial QuantStudio 3D digital PCR system. Our SE-ddPCR system successfully detected the overexpression of *HER*2 in 10 samples, and the other 6 were identified as normal. The results were comparable with those obtained from the commercial systems. Current work is directed toward development of a fully automated SE-ddPCR system, which integrates liquid handling, SE-ddPCR and fluorescence imaging and analysis.

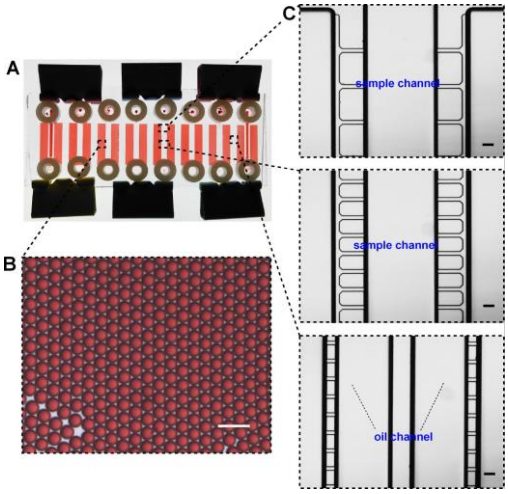

Fig 1. Illustration of the step emulsification ddPCR device. (A) Photo of the microfluidic chip with droplet arrays generated using red dye solution; (B) Zoom-in view of the droplet array. (C) Geometry of the step emulsification nozzles and oil draining channels surrounding the chamber. Scale bar = 200 μm.

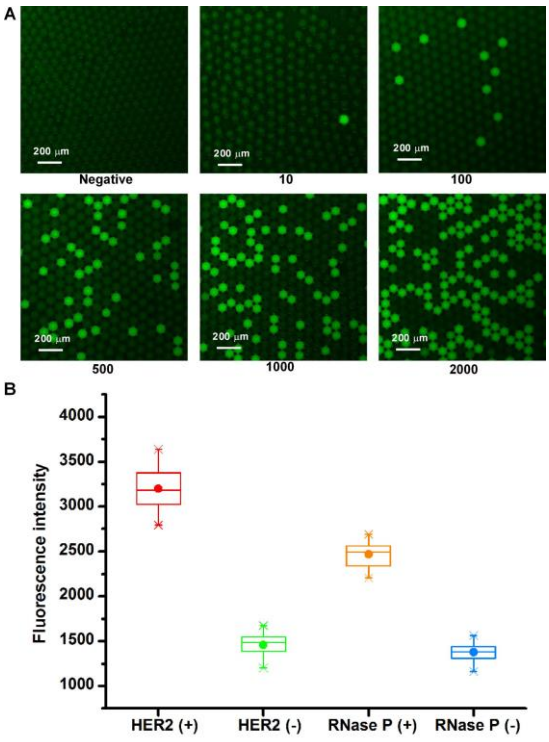

Fig 2. Assessment of *HER*2 status in breast cancers with SE-ddPCR. (A) Typical fluorescence imaging of droplet arrays after PCR with a serial dilution of *HER*2 templates ranging from 2000 copies/μL to 10 copies/μL. There was no template introduced in the negative control; (B) Statistics of binary digital PCR detecting *HER*2 and reference gene (*RNase* P).

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