Spinning micro-pipette liquid emulsion generator for single cell whole genome amplification

Zitian Chen,[a,b] Yusi Fu,[a,b] Fangli Zhang,[a,b] Lu Liu,[a,b] Naiqing Zhang,[a] Dong Zhou,[a] Junrui Yang,[c] Yuhong Pang,[a,b] and Yanyi Huang*[,a,b,d]

[a] Biomedical Institute for Pioneering Investigations via Convergence (BIOPIC), School of Life Sciences, and College of Engineering, Peking University, Beijing, China
[b] Beijing Advanced Innovation Center for Genomics (ICG), Peking University, Beijing, China
[c] School of Electronics Engineering and Computer Science, Peking University, Beijing, China
[d] Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

* Corresponding author. E-mail: yanyi@pku.edu.cn

Many on-chip approaches that use flow-focusing[1-3] to pinch the continuous aqueous phase into droplets have become the most popular manner to provide monodisperse emulsion droplets. However, not every lab can easily adapt microfluidic workflow into their familiar protocols. Here we develop an off-chip approach, spinning micro-pipette liquid emulsion (SiMPLE), to generate highly stable monodisperse water-in-oil emulsion using a moving micropipette to disperse the aqueous phase in an oil-filled microcentrifuge tube. Compared with on chip approaches, this method provides a simpler way to produce picoliter-size droplets in situ with no dead volume during emulsification.

In the previous work, we demonstrated that through microfluidic chips monodisperse droplets-based eMDA can dramatically reduce the amplification bias while retaining the high accuracy of replication, which enables simultaneous identification of both small CNVs and high confidence SNVs from a single human cell[4]. The key point of this method is the high-quality of emulsion generation. With SiMPLE, the single-cell emulsion whole genome amplification was also performed to demonstrate that this novel method can seamlessly integrate with experimental operations and supplies that most researchers have been familiar with. Meanwhile, SiMPLE generator has effectively lower the technical difficulties in those applications relied on emulsion droplets.

A schematic view of the device design is illustrated in Figure 1. In the setup, a hydrophobic coated glass micropipette (inner diameter of the tip at the level of 10 μm) push the aqueous solution out to form monodisperse droplets while spinning. The droplets formation process is shown in Figure 2 with a few specific droplets indicated helping to understand. We performed emulsion whole genome amplification (eWGA) and analysed sequencing results of single mouse embryo stem cells (Figure 3), the distribution of the read coverage shows greatly improved evenness for the SiMPLE-eWGA approach.

Compared to other emulsion generation methods typically based on microfluidic approaches, this SiMPLE generator greatly simplifies the experimental set-up and operation procedure, and rules out sample loss or contamination during liquid transfer. In addition, the size of monodisperse picoliter droplets can be precisely regulated by the flow rate of the continuous phase and the motion velocity of the micropipette tip. With further improvements in the engineering of multiple micropipette platforms and integration with other biological assay chemistries, we believe this technique will become achievable in more emulsion-based reactions in biological and chemical research studies.

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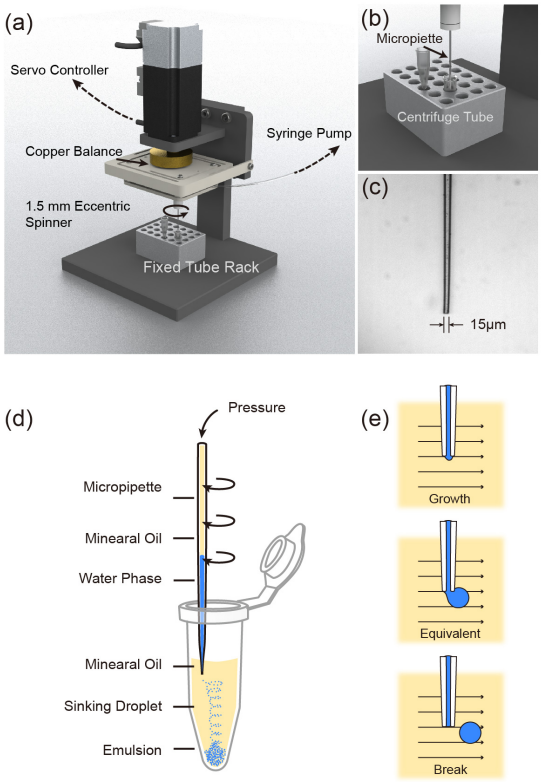

Fig. 1 Schematic illustration of a SiMPLE generator. (a) The major components of the set-up. (b) A glass micropipette with its tip in oil to produce w/o emulsion droplets. (c) A microphotograph of the glass micropipette tip. (d) SiMPLE generated aqueous droplets, with densities higher than that of oil, sinking to the bottom of the centrifuge tube. (e) Process of droplet formation.