Extraction of Droplets for Bioanalysis Based on Superhydrophobic Hole

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Abstract: Superhydrophobic surfaces, which have the excellent characteristics of self-cleaning, low-adhesion and non-wetting, have been widely used in the field of biochemical analysis by limiting sample loss. In this paper, a method to generate droplets of appropriate volume by superhydrophobic holes was proposed. First, the super-hydrophobic surface with super-hydrophobic holes was fabricated by laser etching and low surface energy modification. Then, the possible states of droplets were analyzed. In addition, the effect of droplets size and hole size on the states of droplets were studied comprehensively. This method of droplets generation based on superhydrophobic holes has great potential applications in step-by-step biological experiments.

1. Introduction

Droplets-based microfluidics are more suitable for step-by-step biological analysis than continuous flow microfluidics because of its easy operability. Single nutrient droplet is easier to be operated following the procedure of test[1,2]. In the process of nutrient droplets transfer, keeping the nutrient droplets volume enough and constant is a fundamental requirement. But obtaining a suitable volume of nutrient droplets and realizing the lossless transfer of droplets remain a problem.

Super-hydrophobic surfaces (SHS), which have the water contact angle larger than 150°, offer an attractive means to develop the self-cleaning applications because of their lower adhesion in recent years[3-5]. Droplets on the SHS can easily slide off when the surfaces are slightly tilted, and the SHS are regarded as the ideal surfaces for biochemical analysis with specific requirements. For example, the hydrophobicity of substrate surfaces can reduce the loss of nutrient solution, which can significantly affect the activity and quantity of cells. However, the uniform SHS do not limit the rolling of the droplets, resulting in uncertainty of the droplets on the open surface. Although the SHS with super-hydrophilic region can efficiently constrain the rolling of the droplets, the hydrophilic region can still retain liquid after the droplets are transferred[6,7].

To solve aforementioned problems, we used SHS with holes to extract a suitable volume of the nutrient droplets. The effects of different parameters on the droplets states were studied, including the diameter of the holes and the volume of the droplets. Potential biochemical experiments can be performed in situ, and the nutrient droplets will not remain on the surface after being transferred.
2. Experimental

2.1. Fabrication of the SHS with holes
An aluminum plate with thickness of 0.3 mm was first etched by a laser marking system (SK-CX30, Shanghai Sanke Laser Technology Co., China) at 24 W power, 20 kHz frequency, 1064 nm central wavelength, 50 mm shift of scanning lines, and 200 mm s\(^{-1}\) traverse speed. Then, the same laser marking system and processing parameters were used to drill holes with different diameters ranged from 1.5 mm to 2.1 mm on the plate, and the interval distance of two adjacent holes was 0.1 mm. After immersing in 1 wt% solution of fluoroalkylsilane (FAS, C\(_8\)F\(_{13}\)H\(_4\)Si(OCH\(_2\)CH\(_3\)\(_3\)), Degussa Co., Germany) in ethanol for 1 h, the SHS with holes was obtained, as shown in figure 1.

![Figure 1. The fabrication processes of SHS with super-hydrophobic holes.](image)

2.2. Drop trapping experiments
The prepared surfaces were placed on a water-filled pool so that the bottom surface of the aluminum plate contacted the water surface, as shown in figure 2. Droplets with different volumes were dropped on the holes, and then the states of droplets were recorded by a digital camera (DSC-RX10 III, Sony, Japan).

![Figure 2. Schematic diagram of the experimental device.](image)
3. Result and Discussion

3.1. Possibility analysis of droplets states

![Diagram showing droplets states on superhydrophobic holes](image)

Figure 3. The states of droplets on superhydrophobic holes. (a) $V > V_{\text{Critical}}$; (b) $V < V_{\text{Critical}}$.

Critical volume ($V_{\text{Critical}}$), which refers to the boundary where the contact angle of droplets remains constant or changes continuously, divides the states of the droplets on a superhydrophobic hole into two kinds, as shown in Figure 3. One is that the volume of droplets larger than $V_{\text{Critical}}$ [figure 3(a)], another is the opposite [figure 3(b)]. For the former, the contact angle $\theta$ of the droplets is always constant, and it also the contact angle of the droplets on a uniform SHS without hole, which is independent of the radius $R$ of the droplets. For the latter, droplets are always spherical, and the contact angles varies with the volume of droplets. Under the action of Laplace pressure, the droplets will trap in the hole with a certain depth $h$ for both cases. Once the droplets trapping depth greater than the depth of the hole $H$, i.e., the thickness of the aluminum plate, the droplets will merge with the water in the pool. However, when the droplets trapping depth less than the depth of the hole $H$, the droplets will be suspended on the superhydrophobic holes.

3.2 The influences of droplets size and hole size on the droplets states

In order to investigate the influences of droplets size and hole size on droplets states, the drop trapping experiments were carried. Superhydrophobic aluminum plate with superhydrophobic holes was placed on a pool filled with water, then a water droplets was dropped on the superhydrophobic hole and see whether the droplet can be absorbed by the water in the pool. The states of different droplets on holes with different diameters are shown in figure 4, it can be seen that the droplets trapping depth increase with the increase of hole diameter. The droplets with the volume of 29.2 μL suspend on a hole of 1.7 mm in diameter and come into contact with the water through the hole of 1.8 mm. When the hole diameter was constant, the droplets state will change with the increase of the volume, which is from the initial collapse state to the suspending state. Figure 5 shows the influences of the droplets size and hole size on droplets trapping depth. It can be seen that increasing the volume of the droplets or decreasing the diameter of the hole will be a choice to obtain the suspending droplets. In general, the minimum
volume of droplets suspended on a hole with a particular diameter is obtained easily, which will play a guiding role in the biochemistry analysis.

Figure 4. The states of different droplets on super-hydrophobic holes with different diameters (The pictures in the dotted line show that the droplets are just beginning to contact with the water).

Figure 5. The influences of the droplets size and hole size on droplets trapping depth.

4. Conclusion
In summary, a method to generate droplets of appropriate volume by superhydrophobic holes was proposed, which could be used for extraction and lossless transfer of droplets. The suspending droplets can be obtained by increasing the volume of the droplets or decreasing of the hole diameter. For the same volume of droplets, the droplets trapping depth increase with the increase of hole diameter. Our method shows promising application prospects in the extraction and lossless transfer of nutrient droplets in multi-step biochemistry experiments.
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