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Suspending nanoliter droplet arrays for cell capture and copper ion stimulation

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ABSTRACT

In this paper, we developed a microdevice with raised cylinder arrays for capture of *Chlorella vulgaris* cells and kinetic analysis of stimulation. The cell solution was injected into the chip and held in the gaps between the opposite raised cylinders, forming 0.314 nL volume of suspending droplets. The number of the captured cells in each nanoliter suspending droplet could be controlled within five under our experiment conditions. When the stimulation reagent of copper ion (Cu^{2+}) was injected into the chip and contacted with the suspending droplets, gradient concentration of Cu^{2+} stimulation to the captured *C. vulgaris* cells by free diffusion was formed. The bioabsorption kinetic process of *C. vulgaris* cells under continuous Cu^{2+} stimulation was investigated and the two-step bioabsorption process was revealed clearly. The Cu^{2+} toxicity accumulation effect on the cell was also studied by monitoring the fluorescence of cellular chlorophyll.

1. Introduction

Researches on biological cells have developed from a large cell population to individual cells. In conventional experiments, one challenge is how to manipulate and separate numerable cell from a large population cells. The development of microchip technologies allows capture, precise control and analysis of individual cells at the single-cell level. The microscale cell analysis in microchips allows investigating the function of microenvironmental change at the single-cell level. There is a growing interest for single-cell separation and analysis [1–4]. Different methods have been reported for cell or particle capture based on the miniaturization technology. The physical restriction methods use variations of surface topography to separate particles from a flow and immobilize them on certain sites [5–7]. However, controlling precision deposition of particle seems to be very difficult. The dielectrophoretic trapping method depends mainly on differences of permittivity and conductivity between cells and the liquid medium, which vary as a function of the frequency of the applied field; the particle is either attracted toward the higher electric field or pushed away. Electric cages can be built that, irrespective of flow, enable single particles to remain freely suspending [8]. The energetic field capture methods include magnetic trapping [9], acoustic trapping [10] and laser tweezer [11,12]. Moreover, the long time exposure to energetic field may be detrimental to cells and destroy the activity of cells. There is also microgel trapping method which can pattern the cells in three dimensional (3D) crosslinked microgels [13]. However, the UV radiation for crosslinking may induce the damnification of cells and it is difficult to feed the cell and remove the egesta of the cell because the network structure of the microgels blocks the transmission of macromolecules.

As we know, algae are always used as the vivo indicator of heavy metal pollution. The conventional researches on the metallic biosorption process were based on large cell populations which neglected the diversities of physiological information between cells. Most of bulk-population researches were focused on how that external environmental factors such as pH value [14], water hardness, speciation of the accumulated metal [15–17] and organic matters in the water [18–21] mattered. Due to the difficulties of controlling the location of individual cell, the kinetic process of biosorption for single cell was rarely researched. The microfluidic technique makes it possible to manipulate the single-cell. For example, Peng et al. [22] utilized a three-dimensional flow control concept to study the kinetics of intracellular metabolism and calcium mobilization of single yeast cell stimulated by glucose and pH changes.

In this paper, we demonstrated a microdevice with protrudent circular plot arrays to capture and retain cells. The suspending nanoliter cell droplets could be retained in the microchip by surface tension and no additional equipments such as laser tweezer, magnetic fields or standing-wave acoustic equipment were needed. The suspending droplet provided a stably survival place for cells because there is no shearing effect which is inevitably induced by the liquid flow. For the retained *Chlorella vulgaris* cells in the nanoliter droplets, the Cu^{2+} stimulation kinetic process and Cu^{2+} toxicity accumulation on the celluar chlorophyll of cell at single cells level were investigated. The two-step bioabsorption process was validated by the sensitive quenching response of the quan-

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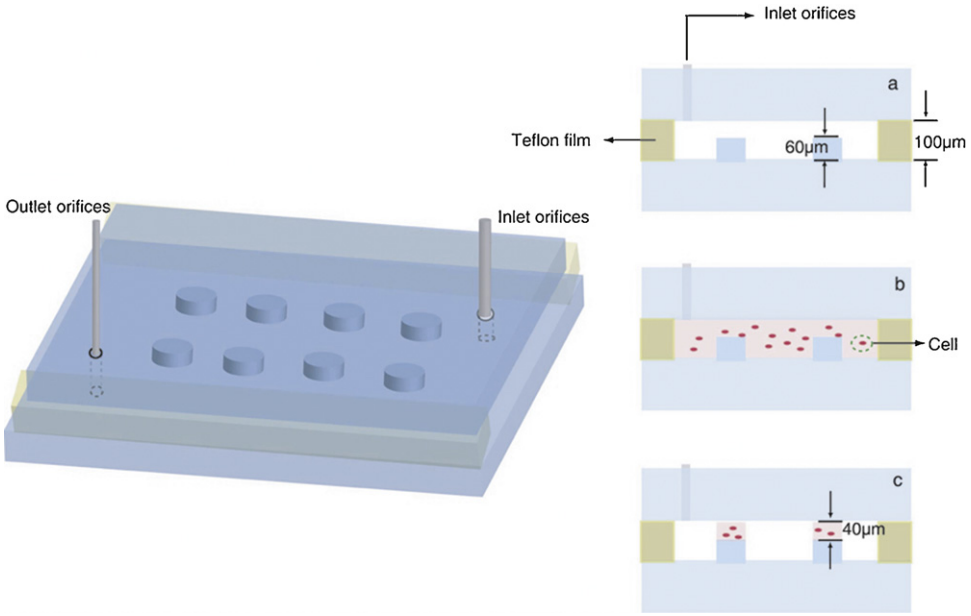

Fig. 1. Design of the microfluidic device and the storing process of *C. vulgaris* cells. (a) Structure of the device: three parts are needed to form the microfluidic chip, the chip with protrudent circular plot arrays, the Teflon film (100 μm thickness) and the cover plate with inlet and outlet orifices. (b) Side and amplificatory elevation of the chip. The process shows how the suspending nanodroplet was stored in the chip.

tum dots CdS labeled both on the membrane and interior of the cell, the Cu^{2+} toxicity accumulation to the chlorophyll in the *C. vulgaris* cell was also revealed by the fluorescence signal. This method integrated single-cell retention, gradient concentration of chemical stimulation, on-line qualitative analysis onto one microdevice.

2. Materials and methods

2.1. Microchip fabrication and cells retaining in the chip

Traditional photolithography and wet chemical etching techniques were used to fabricate the glass chip (shown in Fig. 1). The glass plates used to fabricate chips were purchased from Changsha Shaoguang Chrome Blank Co., Ltd. (Changsha, China). The process is described as follows. Firstly, the photomask with designed pattern was put onto a glass plate (1.0 mm × 1.5 mm) which was precoated with chromium and photoresist, then they were exposed under UV light for about 7 min. Following UV exposure, the glass plate was developed with 0.5% NaOH solution and then etched in 5% HF solution for 30 min to fabricate the protrudent circular plot arrays with the same dimension of 60 μm height and 100 μm diameter. Finally, a blank glass slide was used as a cover plate and assembled together with the etched glass plate by clamps. A piece of Teflon film (0.1 mm × 1.5 mm) was sandwiched between the two plates to form a confined gap. The inlet and outlet orifices for solution were mechanically drilled on the cover plate. As shown in Fig. 1a, the thickness of the Teflon film was 100 μm so the height of the gap between the protrudent circular plot and the cover plate was 40 μm (subtract 60 from 100 μm). The diameter of the columnar gap was 100 μm, the same with the glass protrudent circular plots. The distance between the circular plots in the array was 0.2 mm and a typical array of 8 plots was selected.

C. vulgaris cells and culture solution used in the experiments were provided by Ocean University of China (Qingdao, China). Firstly, the suspending cell solution was introduced into the chip from the inlet orifice by a micropump at a rate of 0.5 μL/min and the whole interspace between the two glass plates was filled as shown in Fig. 1b. Then the redundant solution was sucked out from the chip by vacuum pump at a rate of 0.1 μL/min and the suspending droplets were stored in the gap between the plot-cover plates as presented in Fig. 1c. The volume of the suspending droplet was calculated as 0.314 nL according to 100 μm diameter of the assay and 40 μm height of gap. The number of cells captured in each suspending droplet was counted under a microscope. We repeated this cell storage process three times under the same condition to test the reproducibility.

2.2. Trypan blue coloration to monitor the activity of the C. vulgaris cell in the chip under gradient Cu^{2+} stimulation

Trypan blue was purchased from Sigma (St. Louis, MO, USA) and sodium chloride was purchased from Beijing Chemical Plant (Beijing, China). The 0.05 g trypan blue, 0.18 g sodium chloride were added into 10 mL cell solution. Copper sulfate was purchased from

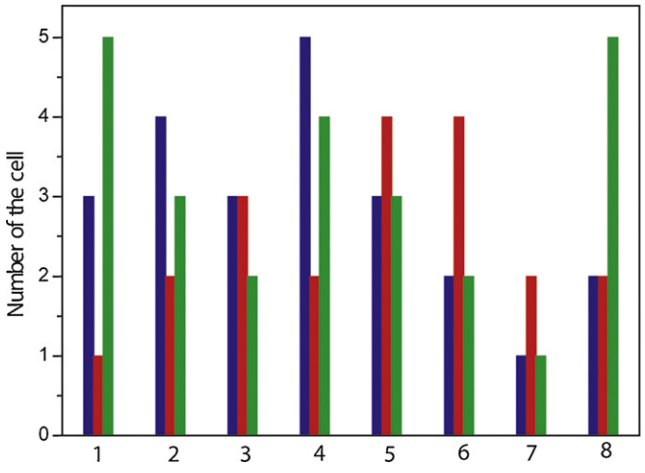

Fig. 2. The capture efficiency of the cells in the 8 plot array. The numbers of the cells in each plot was counted under a photo-microscope. The cell solution at concentration of 10^7 cells/mL was injected into the microchip at a velocity of 500 nL/min and then was drawn out by vacuum pump at a rate of 100 nL/min. The blue rectangle stands for the number of the first capture run, the red and green rectangles stand for the second and third capture runs, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)