Diffusion-based concentration control in microcavities during long time period by programmed syringe pumps

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We propose a programmed syringe pumps system for diffusion-based concentration control in microcavities. Fluorescein is used to demonstrate the influence of different durations of unvarying injecting speed and different sizes of microcavities on the concentration control and distribution in the microcavities. With longer duration of unvarying injecting speed and smaller size of microcavities, the concentration control of the microcavities will be more consistent with the input signal. Using this system, we can precisely control sudden as well as gradual change of the microenvironment of cells within a period from several minutes to days by re-setting parameters of the program. As a demonstration, we apply this system to culture budding yeast cells in the microfluidic device and follow the growth status in precisely controlled nutritional environment. This system will be useful for high-throughput cell stimulation and differentiation studies.

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1. Introduction

Microfluidic devices are becoming popular tools for cell studies because of their significant advantages in multiplexing, real time monitoring, and single cell resolution [1–4]. Among them, the diffusion-based microcavities system for cell culture that reported earlier is becoming a useful tool in biological study [5]. The device makes it easy to change the cell culture media within minutes without washing the cells out of the microstructures. However, an automatic control system is still needed to control the microenvironment of the microcavities during a long period. Compared to the integrated micro valve [6,7], the syringe pump is a commonly used and easily operated tool for injecting media into microfluidic system. For most cell studies, the required change time of microenvironment is longer than minutes or even hours. Hence several programmed syringe pumps may easily realize the microenvironment control during long period of cell culture and cell studies.

In this paper, we propose a programmed syringe pumps system for diffusion-based concentration control in microcavities. Triangle signal is used as an input to test the response in the microcavities with different sizes. The microcavities environment-controlling period ranging from minutes to hours can be easily realized by re-setting the program. As an example, we cultured the budding yeast cells in the microcavities device and study the cell growth rate in a changing environment using our system. The system will be useful for automatic microenvironment control for cell studies in near zero-flow condition.

2. Experimental

The mask of diffusion-based concentration control microfluidic chip is shown in Fig. 1a. The width of the main channel is 200 µm, the width of channels in the mixing area is 50 µm, and the side length of different microcavities is 100 µm, 150 µm, 200 µm, 250 µm, respectively. The width and length of the junction between the microcavities and the main channel is 50 µm and 40 µm, respectively. The images in this work were performed by an inverted optical microscope (Olympus IX 71) connected with a digital camera (Panasonic Super Dynamic CCD). A computer-controlled syringe pump (TS-18, Longer-Pump Company) system was used to inject solutions into inlets of the device through tubes. The budding yeast strain used in this study is YCT50 (MAT a his3.A met3.A ura3.A leu2.A ura MYO1::MYO1-GFP::HIS3[pGREG506 ADH1pr-MCM-mCherry]). Cells growing exponentially in synthetic liquid medium (SC his- ura-) were loaded into the microfluidic device [4].

As shown in Fig. 1a, different solutions are injected into the chip through Inlet\textsubscript{1} and Inlet\textsubscript{2} by programmed syringe pumps. The
middle area of Fig. 1a is the section for mixing of different solutions. The diffusion coefficient \( D \) of fluorescein is about \( 5.0 \times 10^{-6} \text{cm}^2/\text{s} \), which is close to that of small organic molecular. The channel width of the mixing section is 50 \( \mu \text{m} \). So the total mixing time through diffusion is about \( t \approx L^2/D \approx 5 \text{s} \). In the experiment, the flow rate in the channel is set to 60 \( \mu \text{l/h} \); the area of the mixing cross-section is \( 50 \mu \text{m} \times 10 \mu \text{m} \); thus the flow velocity is about 3 mm/s. For fully mixing through diffusion, the length of the mixing channel is set to 15 mm. We use Image J (an image processing program) to analyze the intensity distribution in the channel. Fig. 1b shows the microscopic pictures of fluorescein mixing result in the front, middle, and end of the mixing area. Fig. 1c is the corresponding fluorescence intensity of the white light marked in the Fig. 1b. After the mixing process, the intensity of the main channel in the microcavities area remains even.

When the mixed solution flows through the microcavities area, solute will exchange between the main channel and microcavities by diffusion. By controlling the ratio of the injection speeds of two kinds of solution, the solute concentration of microcavities can be controlled through the exchange with the main channel in the microcavities area.

3. Results and discussion

3.1. The concentration control of the main channel in microcavities area with programmed syringe pumps

The variation of fluorescence intensity of fluorescein was used to identify the concentration variation of the main channel. We injected 100 \( \mu \text{M} \) fluorescein in Inlet\(_1\), and dd-water in Inlet\(_2\). The injection speed of the syringe pumps is programmed as a function of time shown in Fig. 2a, so that the concentration of fluorescein in the main channel of the microcavities area should be varied with time as shown in Fig. 2b. Fig. 2c shows the concentration variation when the duration \( (\Delta t) \) of each flow rate is set to be 5 s, 15 s, 60 s, 120 s, and 1 h.

Because of the elasticity of the connecting tube and PDMS, pressure change of the syringe pump during the period of flow rate change will introduce a delay time to the flow. If duration \( (\Delta t) \) is smaller than the delay time, there will be little response to the programmed pumps. Fig. 2c shows that the concentration of the main channel demonstrates little response to change of the injection speed when each speed is kept for only 5 s \( (\Delta t) \). Longer duration of unvarying injection speed will help the concentration to be consistent with the injection speed change. Tens of seconds should be the minimal \( \Delta t \) that allowed the system to follow the designed pattern of concentration. When \( \Delta t \) is set to 120 s, concentration of the main channel in the microcavities area is a triangle wave. When \( \Delta t \) is long enough, each constant flow rate will cause a step of the concentration variation of the main channel. By inserting more unvarying injection speeds and diminishing the duration of each flow rate, the change of concentration will be smoother as shown in the last figure of Fig. 2c.

3.2. Delay of concentration variation in the microcavities to the main channel

The effect of the delay time that cause by the elasticity of the connecting tube and PDMS is different for different size of microcavities. To make sure the concentration variation is controlled efficiently by the programmed syringe pumps, one has to choose a proper size of the microcavities. In the experiments, we find that there are nearly no delay of concentration variation in the microcavities of 100 \( \mu \text{m} \) side length to the expectant variation. But it is different for the microcavities of 250 \( \mu \text{m} \) side length: the delaying time is 15 s, 20 s, 30 s, 40 s when \( \Delta t \) is 15 s, 30 s, 60 s, 120 s. The corresponding phase discrepancy is about \( \pi/4, \pi/6, \pi/8 \) and \( \pi/12 \).

We also conducted computer simulations and compare them with the experimental data. In the simulation, we studied the concentration variation in the microcavities of 100 \( \mu \text{m} \) and 250 \( \mu \text{m} \) side lengths with \( \Delta t \) of 15 s, 30 s, 60 s, 120 s (shown in Supplementary material). The simulation results show that the concentration variation in the microcavities of 100 \( \mu \text{m} \) side length is almost the same with the expectant variation. In the microcavities of 250 \( \mu \text{m} \) side length, the delaying time is 15 s, 25 s, 35 s, 40 s when \( \Delta t \) is 15 s, 30 s, 60 s, 120 s, respectively. All these results are consistent with our experimental observations, see Fig. 3. We thus conclude that with larger microcavities, the phase discrepancy between input signal and microcavities response will be larger; and with larger \( \Delta t \), this phase discrepancy will be smaller.

3.3. The concentration spatial inhomogeneity of the microcavities

Since the solute diffuses from the main channel to the microcavity through the connection, there should be concentration difference inside the microcavity. An expression of the standard deviation of concentration \( \Delta C \) over the average of concentration \( C \) is used to illustrate the non-uniform extent in the microcavities. As shown in Fig. 4a, with \( \Delta t \) of 60 s, we find the fluorescein concentration in 100 \( \mu \text{m} \)-sized square microcavities remains almost even, \( \Delta C/C \) being about 0.03 during the whole period. But \( \Delta C/C \) is nearly 0.08 in 250 \( \mu \text{m} \)-sized microcavities. As shown in Fig. 4b, with the same 150 \( \mu \text{m} \)-sized microcavities, \( \Delta C/C \) decreases from 0.038 to 0.028 when \( \Delta t \) is increased from 15 s to 120 s. This trend of \( \Delta C/C \) as a function of or sizes of microcavities is confirmed by our computer simulation, see Fig. 4. The quantity of \( \Delta C/C \) of the experimental result is a little larger than that of the simulation, which may be caused by the noise of CCD.

3.4. Cell culture in controlled concentration environment

To evaluate the our system, we culture budding yeast cells in the microcavities device with the nutrition solution concentration controlled as a function of time and follow the growing status for several hours. Ten percent concentration of the media (SC His-Ura-) was injected in Inlet\(_1\) and dd-water in Inlet\(_2\). Change the ratio
of injection speeds per 5 h to control the concentration in the main channel down from 8% to 4% and 2%, as shown in the inset of Fig. 5a. 150 µm-sized square microcavities were used to ensure there is sufficient space for cell growth [4]. The growth curve of cells in selected five cavities was counted per hour and shown in Fig. 5a. The increased cell number (ΔN) in the 5 h (the duration

Fig. 2. Concentration variation of the main channel in the microcavities area: (a) the injection speed of syringe pumps as a function of time. A cycle is divided into eight steps, and one step corresponds to one Δt. In each step, the sum of speeds of dd-water and fluorescein equals 60 µl/h; (b) the expectant concentration variation of fluorescein in the main channel of the microcavities (c) with different Δt the response of the main channel with time. For each sub-picture, we capture a picture of the main channel every 5 s, and analysis the intensity using Image J.

Fig. 3. Concentration variation in different sizes of microcavities (100 µm and 250 µm) with different Δt.
time of each concentration) over the total cell number ($N$) is used to illustrate the cell growth rate. As shown in Fig. 5b, cell number increases by 5.5, 1.4 and 0.07 times in 5 h when the concentration is 8%, 4% and 2%, respectively.

In the example, since the budding yeast cell cycle is about 2 h, we set the duration of each concentration 5 h. This control system should still be efficient to deal with other faster biological process studies as cell stimulation.

4. Conclusion

We present a simple method for automatically controlling the environment in microcavities through a programmed pump system. We character the response time for triangle signals in different sizes of microcavities. In addition, the geometry distribution in the microcavities is demonstrated. This control system should be able to control the microenvironment of other cell culture system as well as microcavities system. It may push forward the automatic control of cell culture or high-throughput cell analysis system.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mee.2009.11.090.

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