HYDROGEL-FREE AND PUMP-LESS MICROFLUIDIC DEVICE FOR BACTERIAL CHEMOTAXIS UNDER CHEMICAL GRADIENT

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ABSTRACT

We present a static microfluidic device without use of hydrogel barrier for the generation of chemical gradient. The microfluidic device allows for the analysis of bacterial chemotaxis without use of hydrogel, membrane, and pump. We demonstrate a chemotactic response to attractant and repellent by measuring of dynamic change of bacterial population in detection area. In this chemotaxis assay, bacterial chemotaxis obviously shows different behaviors as chemoeffectors and concentrations.

KEYWORDS

Bacterial Chemotaxis, Microfluidic Device, Chemical gradient.

INTRODUCTION

Chemotaxis is physical phenotypes of organism for the determination of moving direction by sensing external stimuli. Under chemical gradient, this process of chemotaxis is operated by cooperation of chemoreceptors, signal transduction pathway, and molecular motor. In general, planktonic bacteria are randomly moving by rotation of their flagella between clockwise (CW) and counterclockwise (CCW) directions. Specific chemical gradient can frequently trigger bacterial switching movement of their flagella from CW to CCW. For example, they are biased to the CCW for running motility or CW for tumbling motility. Therefore, biased motility of bacteria in running leads to move toward the high concentration in attractant gradient or low concentration in repellent gradient. Likewise, generation of chemical gradient represents a powerful environment model to study bacterial chemotaxis responsed to chemoeffectors.

There are several traditional methods for bacterial chemotaxis using chemical gradient such as swam plates and capillary assay. Another conventional method has analyzed the flagella motion in chemical gradient by monitoring of body rotation on attached state of bacteria. However, these techniques have several limitations in unstable chemical gradient, low sensitivity of bacteria, and longer analytical time. Recently, microfluidic device has become a powerful tool for generating a stable chemical gradient due to no turbulent flows in low Reynolds number.[1] In addition, fluid flow and channel dimension in microfluidics can be easily defined for accurate measurement of chemical gradient as well as observation of bacterial response. However, delicate control of flow in microchannels is critical factor for studying swimming bacteria because the high flow velocity drifts all bacteria or misunderstand the bacterial chemotactic behavior. To compensate the disadvantage of continuous dynamic methods, static microfluidic device integrated with hydrogels for the diffusion of chemicals are proposed.[2] However, hydrogel based microfluidic approach for the analysis of chemotaxis is inherently needed for complex procedure, external flow control system, and relatively long experiment time due to poor diffusion through hydrogel pore. Here, we present a static microfluidic method without hydrogel barrier and external pressure for the generation of chemical gradient and bacterial chemotaxis assays.

RESULTS AND DISCUSSION

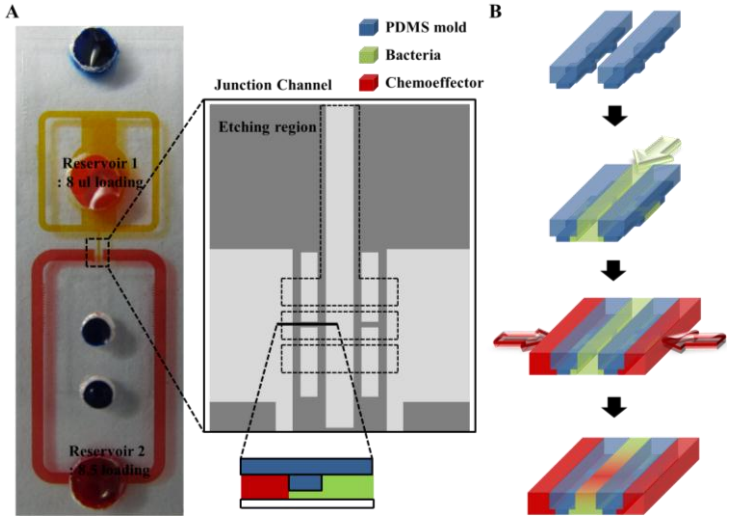

Figure 1. Schematic diagram of microfluidic devce. (a) Design of total microfluidic device and junction channels gradient. Dashed line is etching regions to fabricate different height. (b) Experiment procedure for generation of chemical gradient.

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Microfluidic design for the generation of chemical gradient and bacterial chemotaxis assay is depicted as shown in Figure 1A. Two reservoirs for sample loading and three ventilations are respectively positioned, and junction channel with different height is fabricated by multi-layer photolithography to control fluids. These structures are essential to stop fluid flow by surface tension. The microfluidic device contains hydrophobic PDMS and hydrophilic glass acts as modulator for flowing and stopping liquids in microchannels without external pressure. Experimental procedure for our device is shown in Figure 1B. First, bacterial solution is loaded in reservoir 1 and the solution is introduced into microchannel by capillary action of hydrophilic glass. Next, chemical solution is loaded in reservoir 2 when the flow of bacterial solution is stopped by surface tension at junction channels. Two solutions are directly contacted at junction channels which molecules are diffused to detection channel from source channel of two sides. Finally, chemical concentration gradient is formed at detection channel and bacteria can respond.

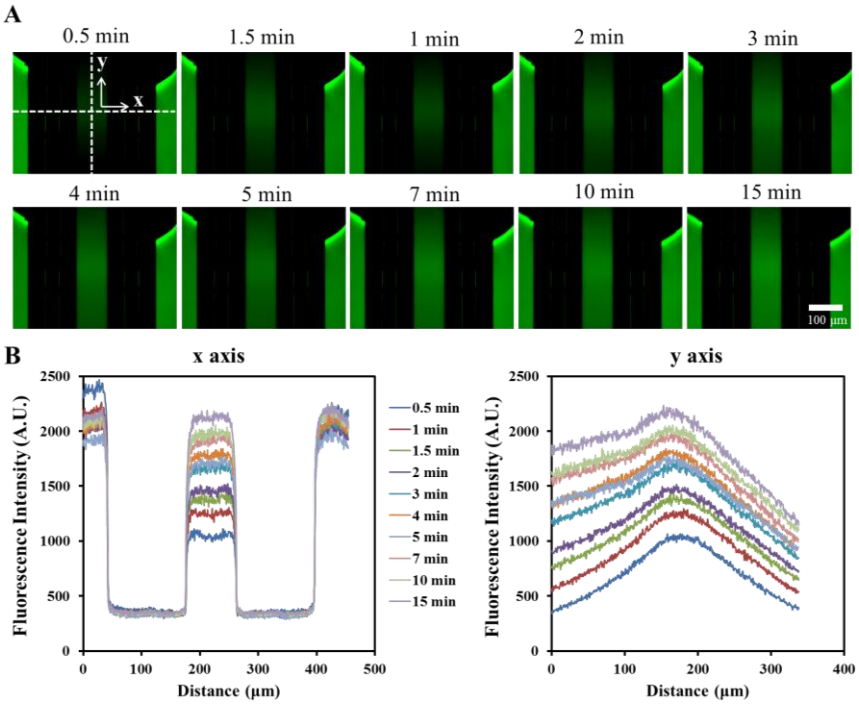

Figure 2. Characterization of chemical gradient at junction channels. (a) Time-lapse images for monitoring of dynamic diffusion behaviors. (b) Quantitative data for x and y axis at junction channels.

First, we demonstrate a chemical diffusion and formation of chemical gradient in detection regions using fluorescence dye as visualization agent (Figure 2). HEPES buffer and fluorescein (100 μM) dye are loaded in each reservoir 1 and 2, and then we have monitored a dynamic change of fluorescence intensity by fluorescence microscopy and measured to quantify x and y axis fluorescence intensity via line profiling (Figure 2A). Fluorescence images show a chemical diffusion dynamics which concentration in detection channel is increased as function of time. In addition, line profiling analysis of fluorescence along the x and y axis indicates the quantitative information for chemical diffusion dynamics (Figure 2B). In quantitative data of x axis, fluorescence intensities of both sides (source channel) retain a similar concentration during 15 min. Meanwhile, fluorescence intensities in middle region (detection channel) are increased in 15 min and reach to maximum concentration at 15 min. The concentration profiles of y axis as function of time show a linear formation of chemical gradient in detection region. Thus, we demonstrate that our static microfluidic device rapidly generates the chemical gradient within 15 min.

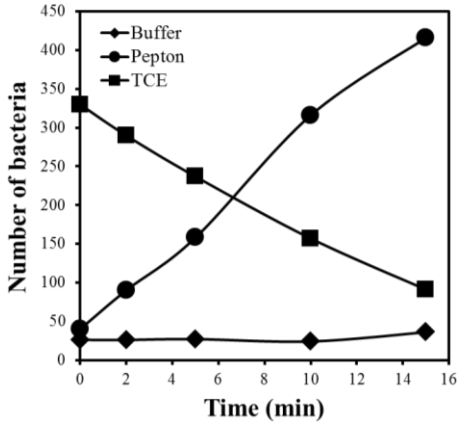

Figure 3. Chemotactic response of PAO1 for attractant (1% pepton) and repellent (10 mM TCE).

Next, we used our static microfluidic device to monitor bacterial chemotaxis response to for chemoeffectors. As proof of concept, buffer as a control, 1% pepton as attractant and 10 mM trichloroethylene (TCE) as repellent are evaluated for response of *Pseudomonas aeruginosa* (PAO1) (Figure 3). To quantify the bacterial population, we define a detection area (40 μm × 60 μm) and measure bacterial population as function of time (Figure 3b). First, we monitored the individual bacteria behaviors by the phase contrast optical microscopy to immediately measure dynamic change of bacterial population's response. In the case of pepton, as expected, bacterial population continuously accumulates in chemical entrance region (relatively high concentration region), indicating that the bacteria sense chemical gradient of pepton as an attractant (Figure 3B). For analysis of repellent, we have loaded high density of bacteria to monitor moving away from high concentration of repellent in detection channel. We also identify the decreasing of bacterial population in detection channel (Figure 3B). Importantly, increase and decrease of bacterial number is obviously observed within 2 min indicating that PAO1 is rapidly sense the chemoeffector. In the case of HEPES buffer as control, bacterial population is not changeable, indicating that bacteria are freely move in the microchannel without influence of bacterial motility. Thus, we successfully demonstrate a chemotactic response to attractant and repellent by monitoring of bacterial population in detection area.

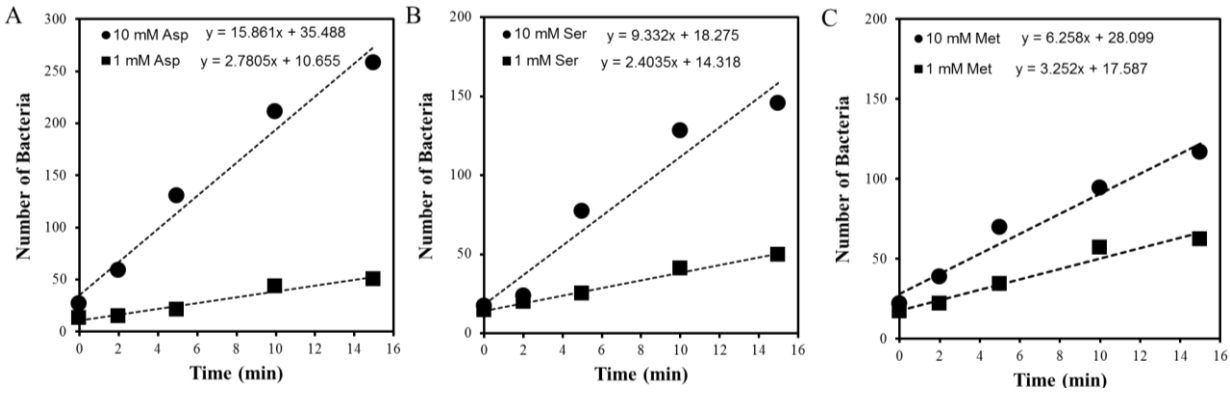

Figure 4. Evaluation of bacterial chemotaxis for three types of amino acids as concentration (each 1 mM and 10 mM). The dashed lines are a linear regression.

To extend our developed microfluidic device, we have examined the three types of amino acids (Figure 4). Bacterial chemotaxis obviously shows different behaviors as amino acids and concentrations. Meanwhile, in present of chemoeffector gradient, we found that accumulation rate of bacterial number (slope of linear regression) in detection area reveal the different magnitude of chemotactic response as introduced initial concentrations which indicate response magnitude of bacterial chemotaxis. Thus, these results confirm that the microfluidic device can easily monitor the change of bacterial population in specific region although it is dynamic and sensitive behavior of bacteria in rapid change of external environment.

CONCLUSION

A number of analysis tools have been used for study of bacterial chemotaxis. This device should be rapid and convenient to provide quantitative information of bacterial chemotaxis responded in various chemoeffectors for biologist. In this study, we demonstrate that developed microfluidic device is rapid and convenient for analysis of bacterial chemotaxis including short analysis time, hydrogel-free and pump-less system. In addition, static microfluidic device can measure and compare a chemotactic behavior via monitoring of change of bacterial population. Our device can study chemotactic behavior for several chemoeffectors related in bioremediation, human disease and marine food. In conclusion, the static microfluidic device can monitor and analyze a bacterial chemotaxis under the stable chemical gradient without hydrogel barrier and external equipment.

ACKELEDGEMENT

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