STABLE GENERATION OF MULTIPLE CHEMICAL GRADIENTS USING *IN-SITU* FORMED NANOPOROUS MEMBRANES

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ABSTRACT

We developed a gradient generator of multiple chemicals using self-assembled particles in microchannels. The porous membranes based on the assembled particles allow the stable gradient generation without any convection flow disturbance and leakage. Moreover, they are more robust in dry environment and have long-term storage potential compared to hydrogel based membranes. Dynamic spatial control and the parallel preferential chemotaxis assay of *salmonella typhimurium* were successfully demonstrated. Our proposed devices are expected to be utilized for mimicking the real microenvironment for cells and various drug screening.

KEYWORDS

Multiple chemical concentration gradients, *In-situ* formed membrane, nano-assembly, bacteria, and chemotaxis

INTRODUCTION

This paper reports a microfluidic platform for stable generation of multiple chemical gradients using *in-situ* formed nanoporous membranes and describes the control of chemotactic response of bacteria with the proposed system. Many types of devices have been developed for chemotaxis study [1, 2], and among them, diffusion based devices have several advantages, such as, enabling mimicking *in-vivo* environment and generating stable, viscous shear stress-free chemical gradients. The conventional diffusion based devices can make a one-directional chemical gradient in the cell chamber only [2]. However, cells are exposed to various attractants and stimulants, and therefore, it is needed to study under similar environments. Here, we propose the microfluidic device for multiple chemical gradients and show its potential by monitoring motion of GFP-strained bacteria.

EXPERIMENT

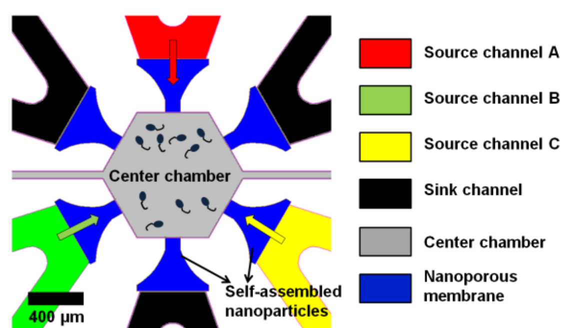

Figure 1. Schematic views of the principle idea.

Figure 1 shows the schematic view of principle idea. There exist source channels to supply the molecules to the center chamber and on the right opposite side, there are sink channels to remove the same quantities of molecules in the center chamber. Moreover, the center chamber is fully separated from source and sink channel by the diffusible porous membrane which is formed by nano-interstices of self-assembled polystyrene particles. Therefore, if the 100 % and 0 % of concentration is introduced by withdrawing the flow at source and sink channel, respectively, stable chemical gradients can be preserved in the center chamber without any external flow disturbance.

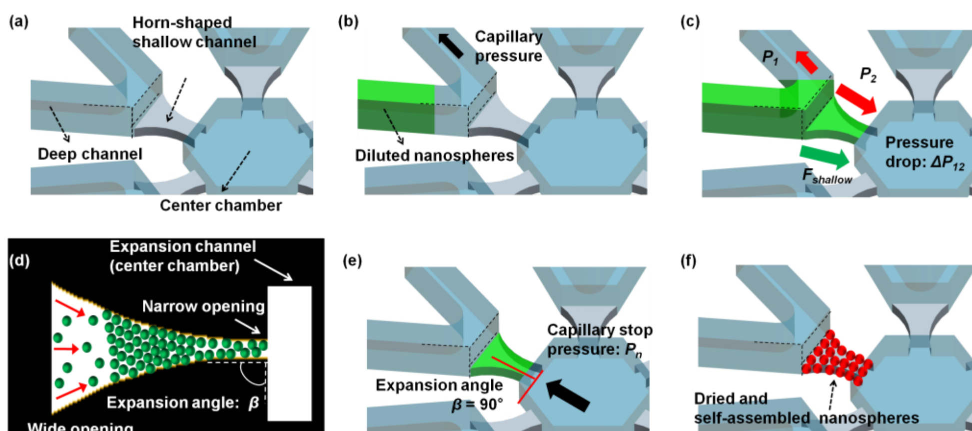

Figure 2. Schematic illustration of the fabrication process

Figure 2 shows the fabrication process for the *in situ* formation of porous membranes using the self-assembly of particles within the PDMS channel: (a) The PDMS device with shallow and deep channels was fabricated as described above. (b) Diluted nanospheres (300 nm) were introduced into the deep-channels by capillary pressure. (c) The diluted nanospheres at the intersection between shallow and deep channel experience a sudden pressure drop (ΔP_{12}), which tries to drag the solution in the deep channel into the shallow channel ($F_{shallow}$). (d, e) In the designed horn-shaped channel, since more evaporation and capillary pressure were induced at the narrow opening, all particles were moved toward the narrow opening. However, the diluted nanospheres could not move forward at the interface between the narrow opening and the expansion channel (center chamber) because the capillary stop pressure was maximized at the expansion angle β around 90°. (f) During the solution was dried out, microspheres were self-assembled, unidirectionally and nanopores were formed from nano-interstices in these closed-packed nanoparticles, only within the shallow channel. (g) Finally, we annealed the self-assembled particles for 1 h at 90°C.

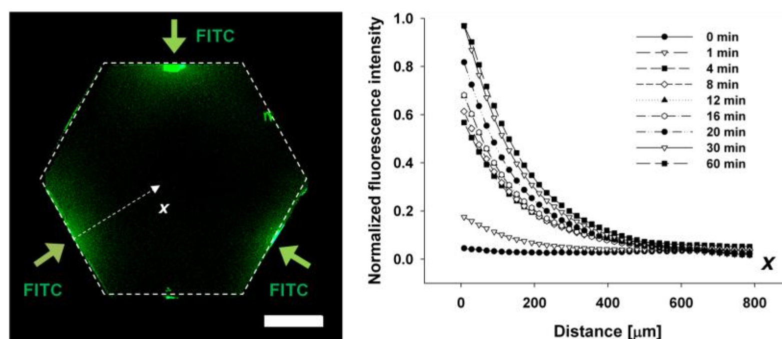

Figure 3. Generation of gradients at different directions and its corresponding plots (scale bar: 200 μm)

The generation of concentration gradients at different directions was visualized using fluorescence dye (FITC, MW 389, diffusivity: 0.49×10^{-9} m^2/s) and its corresponding plots are shown in Figure 3.

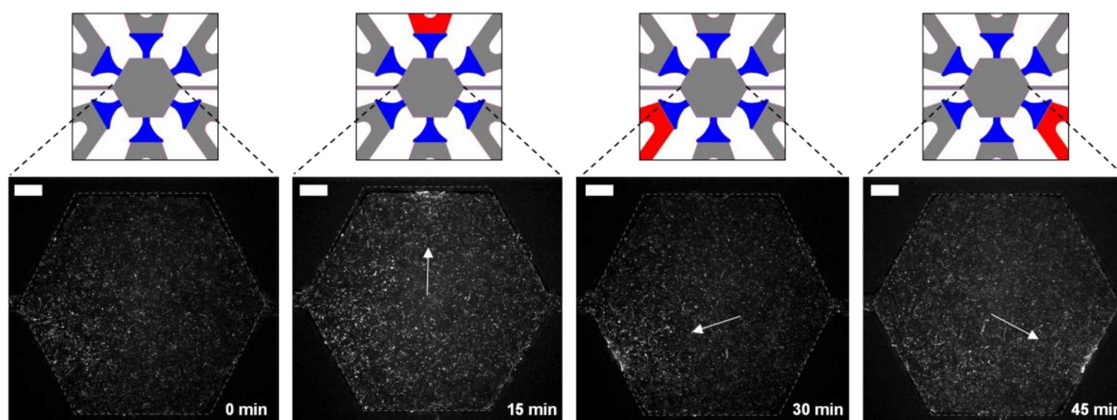

Figure 4. Device demonstration by sequentially introducing the chemo-attractants and observing the movement of bacteria depending on the direction of chemical gradients (scale bars: 100 μm)

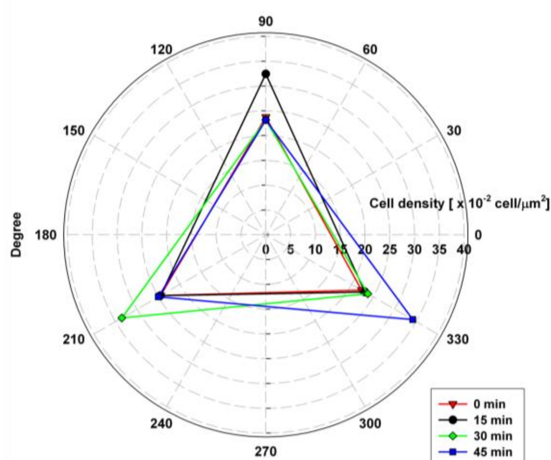

Figure 5. Cell density sequentially changes according to the chemical gradients.

The movement control of bacteria was demonstrated by sequentially introducing the chemo-attractants in every 15 min and observing the movement depending on the direction of chemical gradients (Figure 4). The cell density increased toward the high chemical gradient and figure 5 depicts the corresponding polar plots.

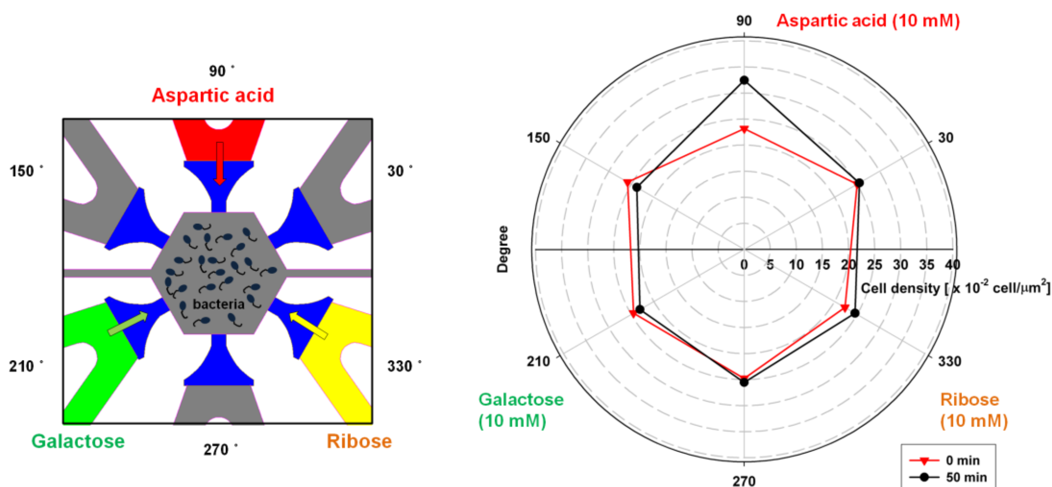

Figure 6: Polar plots when three different chemo-attractants were introduced

The experiment for the preferential chemotaxis of bacteria toward three different chemo-attractants was demonstrated (Figure 6). The same molarity of aspartic acid, galactose and ribose were introduced from different channels and, after 50 min, more bacteria migrated toward the 90° as depicted in Figure 6 because their chemoreceptors seem more sensitive to the aspartic acid.

We believe that proposed system can be a useful tool for parallel and rapid characterization of bacterial responses to various chemical sources, screening of bacterial cells, synthetic biology, and understanding many cellular activities.

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