BACTERIAL CELL-TO-CELL COMMUNICATION ASSAYS IN A MICROFABRICATED CONCENTRATOR ARRAY DEVICE
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ABSTRACT
We present a microfluidic device for monitoring cell-to-cell communication by motile bacterial cells a chip. The device can accumulate motile bacterial cells to desired destinations with required cell densities in a controllable manner by using the arrowhead-shaped ratchet structures that only allow uni-directional movement of the cells so that the device can provide physically separated but chemically connected microenvironment, facilitating the cell-to-cell communication assay. Since the cell signaling molecules diffuse freely between chambers, the device makes it possible to quantify the effect of the spacing distances and the density of cells on the cell-to-cell communication of synthetically engineered bacterial cells.

KEYWORDS: Cell-to-Cell communication, Synthetic Biology, Microfabrication, Ratchet

INTRODUCTION
It is essential to understand coordinated microbial cell-to-cell communication for the further advancement of microbiology and synthetic biology so that the microbial cell-to-cell communication has been attracting but intriguing research area.[1] Recently, artificially programmed two genotypes of cells (sender and receiver cells) have been engineered thanks to the advance of synthetic biology and it was demonstrated that the sender and receiver cells communicate with each other.[2] However, the spatial resolution of cell patterning has been limited due to the manual seeding of synthetically engineered bacterial cells onto distinct areas of a cell culture plate. Using our novel ratchet structures, we can enhance spatial resolution and controllability of cell-to-cell communication assay. In addition, Simple fabrication by conventional soft-lithographic technique allows further development of the concept to the multi cellular communication assay. Therefore, we believe that the structure of the device and the approach presented in this work not only would be a novel, useful tool for other microbiology and synthetic biology but also could spawn many biotechnological applications.

EXPERIMENTAL
As shown in Fig. 1., Two main channels are used for cell loading for two specific cell types in right and left concentrators respectively. A pair of the left and right concentrator is connected via open microchannels of which distance is defined as \( d_c \) that ranges from 300 µm to 8000 µm in this work. To accumulate motile bacterial cells with desired density, the device employs previously developed and well characterized arrowhead-shaped ratchet structures.[3] The connecting microchannels, referred to be “bridge channels”, allow for cell signaling molecules excreted by the cells in the right concentrator to diffuse freely to the cells in the left concentrator while preventing any convective flow by minimizing the pressure difference between the main channels.

Figure 1: (A) Schematic of the open channel based microfluidic concentrator array device for synthetic bacterial cell-to-cell communication. Motile bacterial cells are concentrated by the arrowhead-shaped ratchet structures in the left and right concentrators after being loaded into the main channels that are connected with each other via bridge channels. (B)
The bridge channels integrated with the arrowhead-shaped ratchet structures to prevent the concentrated cells from escaping from the concentrator. (C) The plain bridge channels connecting a pair of concentrators allows cell to move freely between concentrators. However, cell signaling molecules diffuse from one concentrator to the other regardless of the ratchet structures in the bridge channels.

RESULTS AND DISCUSSION

To test our device, we monitored the fluorescent intensities of the GFP- and RFP-expressing cells in the left and right concentrators with time, respectively. As shown in Fig. 2(C), both the fluorescent intensities of the GFP- and RFP-expressing cells gradually and continuously increase with time. The same result is found in the quantification of the qualitative results as shown in Fig. 2(D). Since the number of the cells in the concentrators almost linearly increases with time, if the concentrator does have the bridge channel with ratchet structure as shown in Fig 2(A), it is possible to adjust the number/density of the cells in each concentrator.

Figure 2: (A) A concentrator with the ratcheting structures in the bridge channels and its fluorescent image when GFP-expressing cells are loaded for concentration in the left main channel. The inset shows the SEM image of the part of the concentrator. (B) A concentrator with the plain bridge channels and its fluorescent image when the same GFP-expressing cells are loaded in the left main channel. The inset shows the SEM image of the part of the concentrator. (C) The time lapsed image sequences of the green fluorescent intensities from the GFP-expressing cells in the left concentrator and the red fluorescent intensities from the RFP-expressing cells in the right concentrator. (D) Quantification of the concentration results of (C). The number of the GFP- and RFP-expressing cells in the left and right concentrator, respectively, is almost linearly proportional to the concentrating time.

We also tested the device in terms of long-term cell growth and the cross-contamination of cells between the left and right concentrator due to the migration of cells along the bridge channels with time, prior to cell-to-cell communication experiments. As shown in Fig 3, growth of cells were well maintained during the experiments and the migration between chambers were minimized in the presence of the ratchet structures.

Figure 3: (A) Cell growth was monitored by measuring the number of the GFP-expressing cells in the left and the RFP-expressing cells in the right concentrator with time after cell were concentrated. Both the GFP- and RFP-expressing cells
grew well in the device. (B) Quantification of (A). Cell growth was well maintained during the experiment. (C) The quantification of the number of cells that crossed the middle line of the bridge channels over time.

For the cell-to-cell communication experiment, we loaded the RCs that produce GFP when it induced by signaling molecule AHL in the left main channel while the SCs that produce AHL constitutively in the right main channel so that the cells were concentrated in the left and right concentrator for about 40 min, respectively. Since the device enabled us to perform the cell-to-cell experiment with and without the ratchet structures in the bridge channels at a time, we obtained the fluorescent images from the concentrators that are differently spaced. We chose the \(d_c=300 \mu m\) for the fast diffusion of AHL from the SCs and the early induction of the RCs by the AHL while the \(d_c=4000 \mu m\) for the slow diffusion and late induction. In addition, for an intermediate level of diffusion and induction, the \(d_c=1000 \mu m\) was chosen as well. Fig. 4(A) shows the fluorescent intensities were measured from the concentrators with the ratcheting and the plain bridge channels with time, respectively. In the absence of the ratchet structures, fluorescence intensity of cells were close each other regardless of the spacing distances while the intensities affected by the spacing distances in the presence of the ratchet structures. Therefore, we demonstrated that the concentrator array device can facilitate the cell-to-cell communication assays.

![Figure 4](image)

Figure 4: (A) The fluorescent intensities of the RCs and SCs for the ratcheting bridge channels in various spacing distances. (B) The fluorescent intensities of the RCs and SCs in the left concentrator for the plain bridge channels in various spacing distances (C) The quantification of the (A) shows that the RCs at the moderate spacing distance \((d_c=1000 \mu m)\) shows stronger fluorescent intensities than others \((d_c=300 \mu m and 4000 \mu m)\). (C) The quantification of the without ratchet structure shows that the RCs at difference spacing distances shows almost similar fluorescent intensities with time.

CONCLUSION

We demonstrate that the device can adjust the density of cells by controlling the concentrating time so that it can make it easy to investigate the effects of the spacing distance affecting diffusion time and induction timing on the cell-to-cell communication. Since the device consists of a pair of concentrators with a wide range of spacing distances, we found that the spacing distance play a crucial role in the cell-to-cell communication because both the diffusion of the produced AHL by the SCs and the induction of the RCs are affected by the channel length.

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