Electronic Supporting Information for Microfabricated Ratchet Structure Integrated Concentrator Arrays for Synthetic Bacterial Cell-to-Cell Communication Assays

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Control experiment between RFP-expressing cells and RCs

As control, we conducted an experiment similar to that shown in Fig. 4 in the paper by loading the RCs and the RFP-expressing cells that did not produce AHL. As shown in Fig. S1 in OSI, no fluorescent signals were detected in the left concentrator where the RCs were concentrated while the RFP intensities continuously increased with time because the RFP cell grew well in the right concentrator. We quantified the GFP signals of the left concentrator in Fig. S1(B) and the RFP signals of the right concentrator in Fig. S1(C). As compared to the cell-to-cell communication experiment shown in Fig. 4(C), the fluorescent intensities were close to a noise level because no AHL was induced into the RCs. Instead, the RFP signals showed a gradual increase with time, implying the good growth of the RFP-expressing cells in the concentrator. From this result, it was confirmed that the culture condition was well maintained during the control experiment. Further, the experiment with the ratchet bridge channels showed greater fluorescent intensities than that with the plain bridge channels. This could be attributed to the migration of the cells from the right to the left concentrator, affecting the number of initially concentrated cells in the right concentrator leading to a different final number of cells in 8 h as characterized in the cell growth experiment (Fig. 3(A)). However, no green fluorescent intensities were detected in the left concentrator, meaning that no cell-to-cell communication occurred.

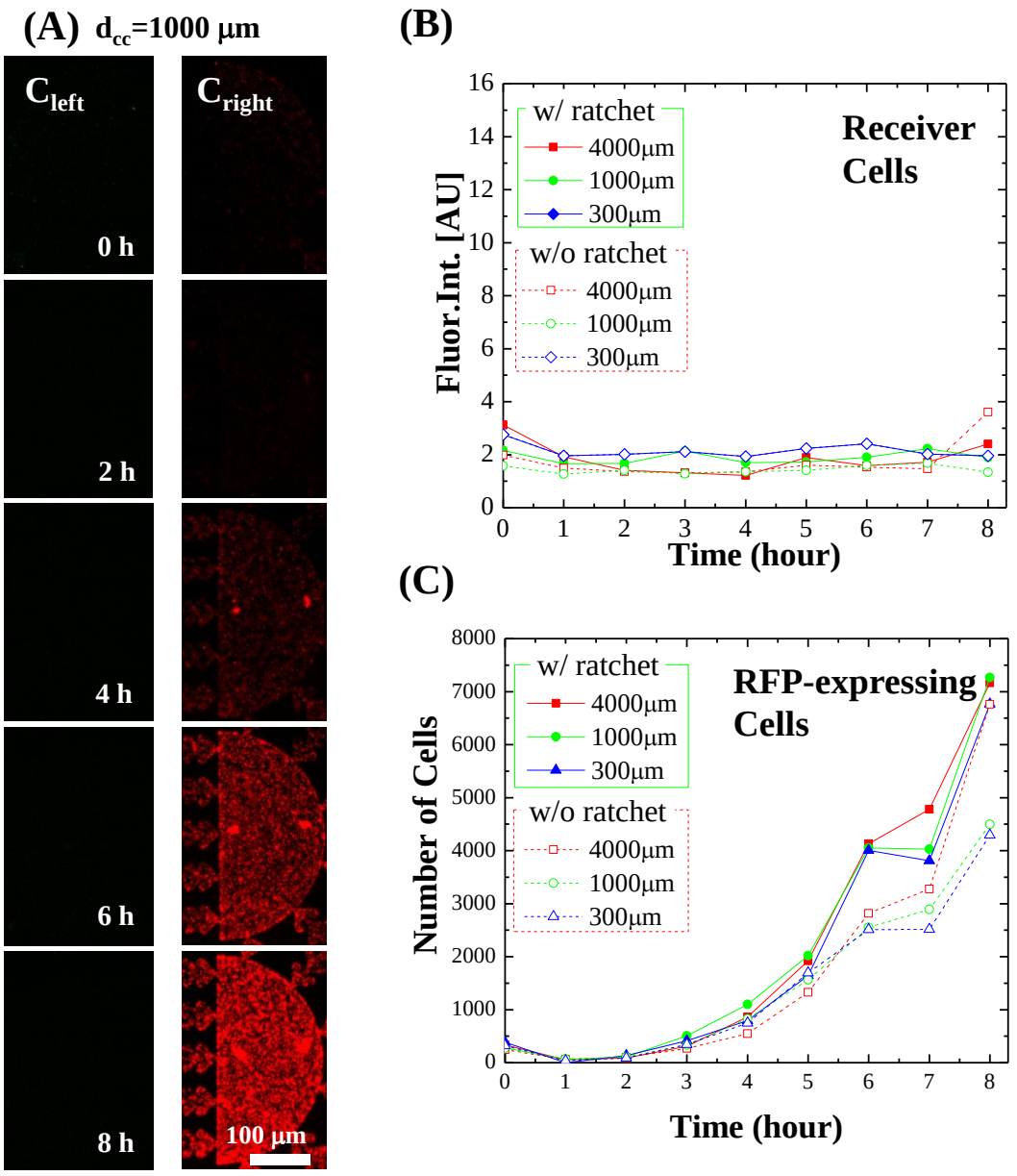

Figure S1 Control experiment using the same concentrator array device. The RCs in the left concentrator show no fluorescent intensities because the RFP-expressing cells in the right concentrator do not produce any AHL. (B) Quantification of the green fluorescent intensities of the RCs in the left concentrator. (C) Quantification of the RFP-expressing cells in the right concentrator shows gradual increases in the fluorescent intensities with respect to time, implying that the culture condition is well maintained during the control experiment.

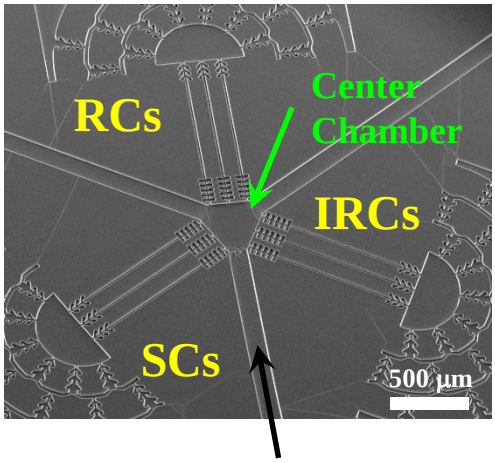

Figure S2 SEM image of a cell-to-cell communication assay device. Three concentrators are connected to the centre chamber so that the device is suitable for multi-cellular communication assays among three types of cells. Moreover, three agarose injection channels can be used for filling the centre chamber with agarose hydrogel to completely block a physical cross-contamination of cells but allow a chemical diffusion of cell-signalling molecules.

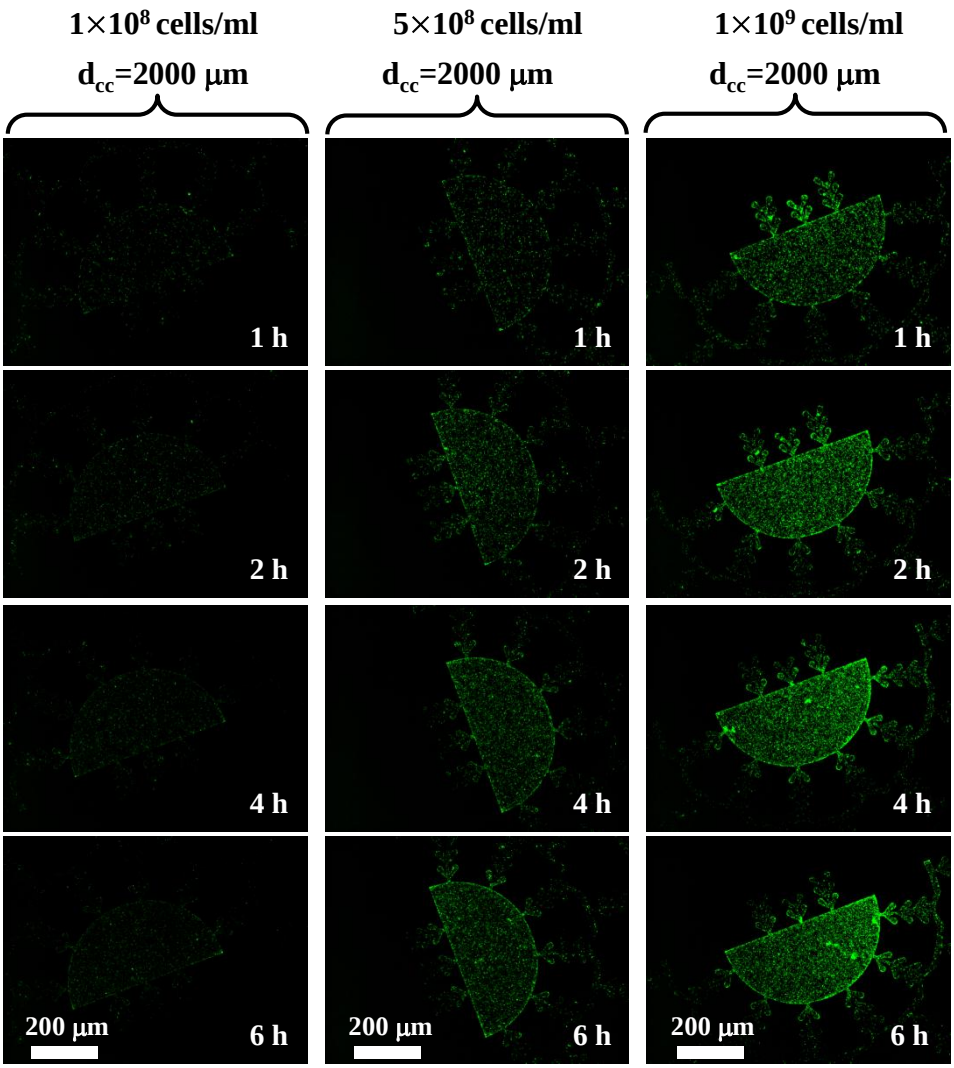

Figure S3 IRCs are concentrated in the concentrator with different initial cell densities such as 10^8 cells/ml, 5×10^8 cells/ml, and 10^9 cells/ml, corresponding to approximately 200, 1000, and 2000 cells in the concentrator, respectively, while the initial cell density of the SCs is about 10^9 cells/ml, corresponding to 2000 cells in the concentrator.

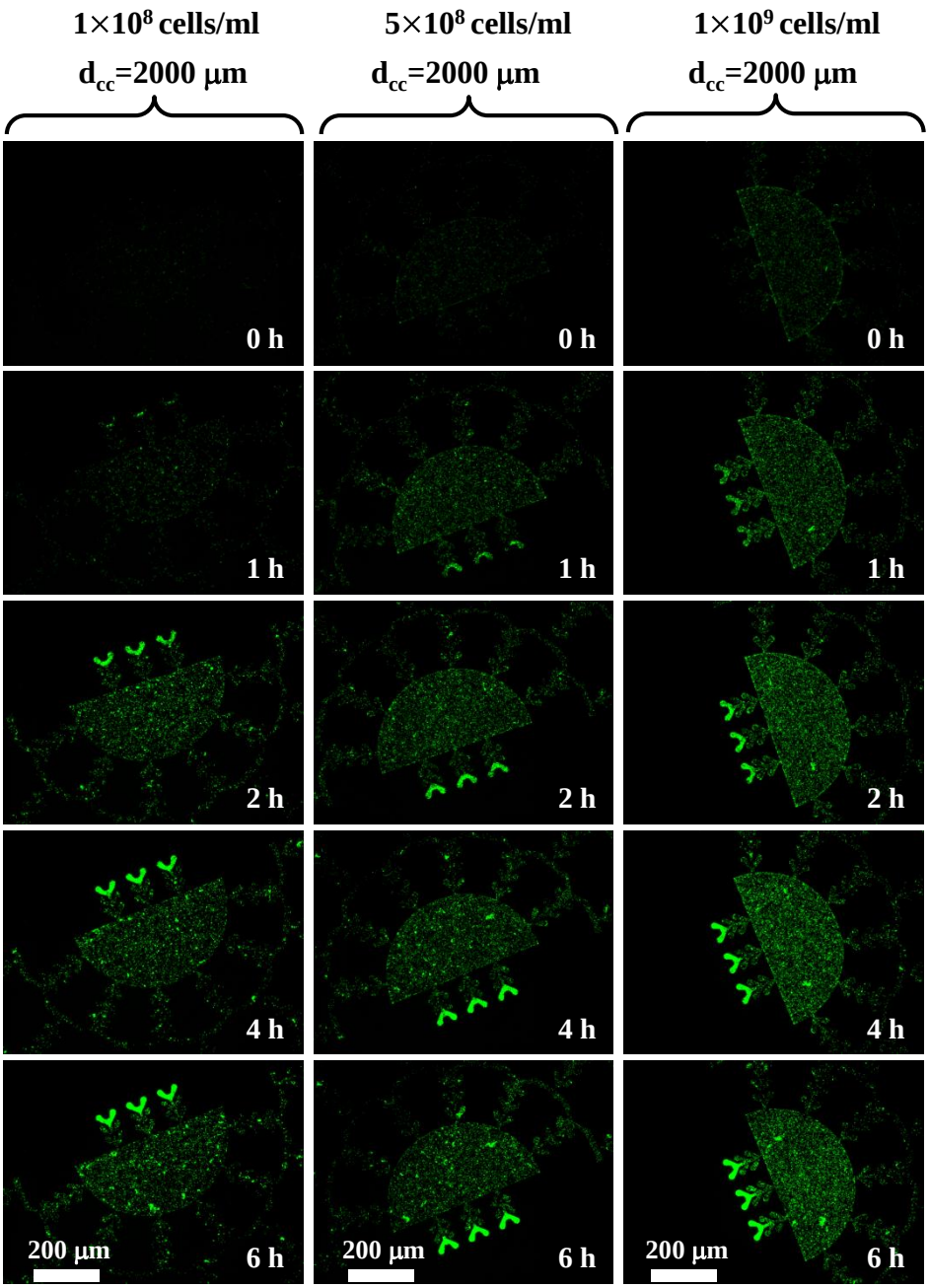

Figure S4 RCs are concentrated in the concentrator with different initial cell densities such as 10^8 cells/ml, 5×10^8 cells/ml, and 10^9 cells/ml, corresponding to approximately 200, 1000, and 2000 cells in the concentrator, respectively, while the initial cell density of the SCs is about 10^9 cells/ml, corresponding to 2000 cells in the concentrator.

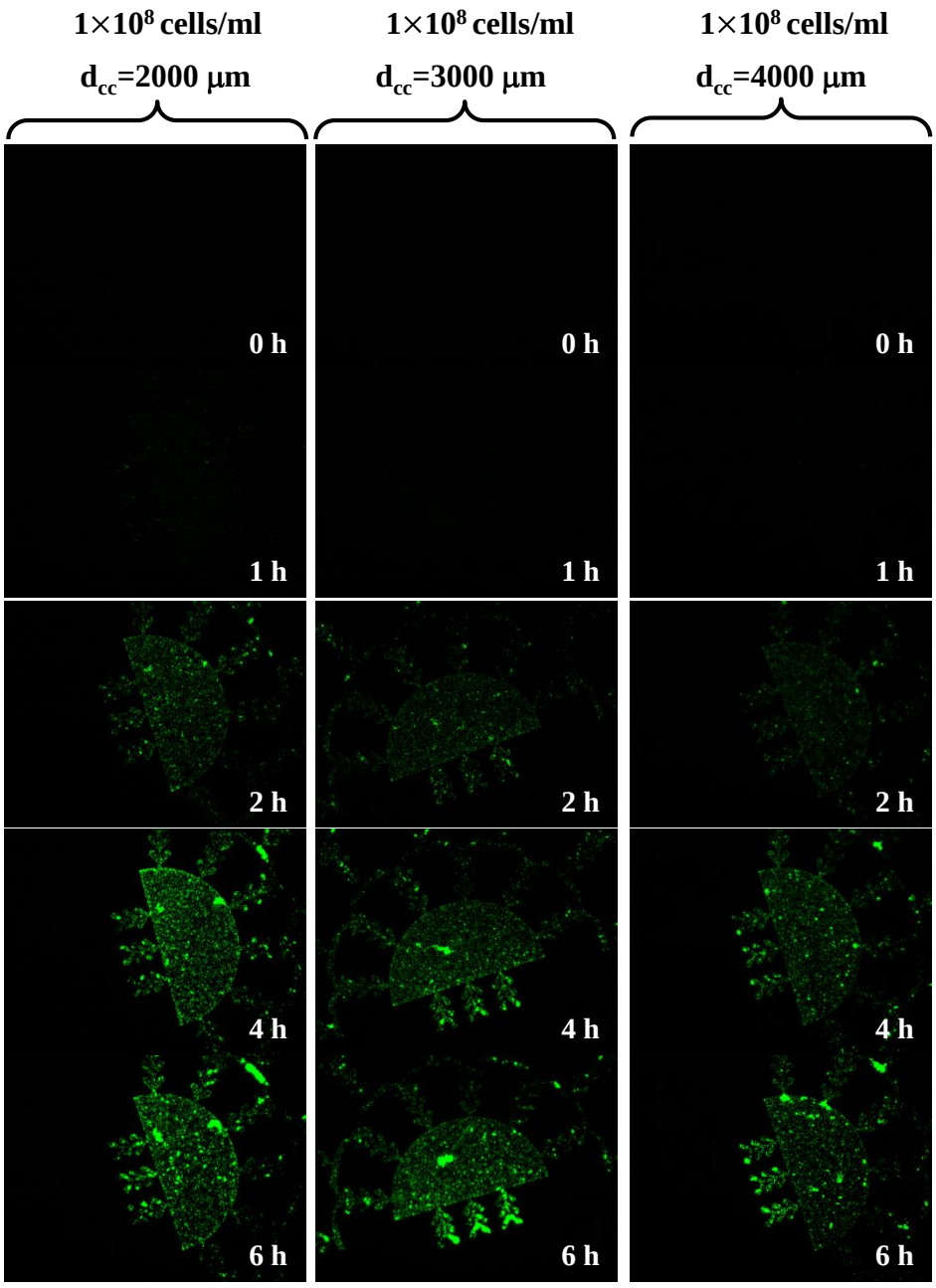

Figure S5 RCs are concentrated in the concentrator with a constant initial cell density of 10^8 cells/ml (about 200 cells in the concentrator) but varying distances from a concentrator to a concentrator via the centre chamber such as 2000 μm, 3000 μm, and 4000 μm. The RCs show a gradual increase in the fluorescent intensities with respect to time but the closer the distance between the RCs and the SCs is, the earlier the fluorescent intensities appear to increase.

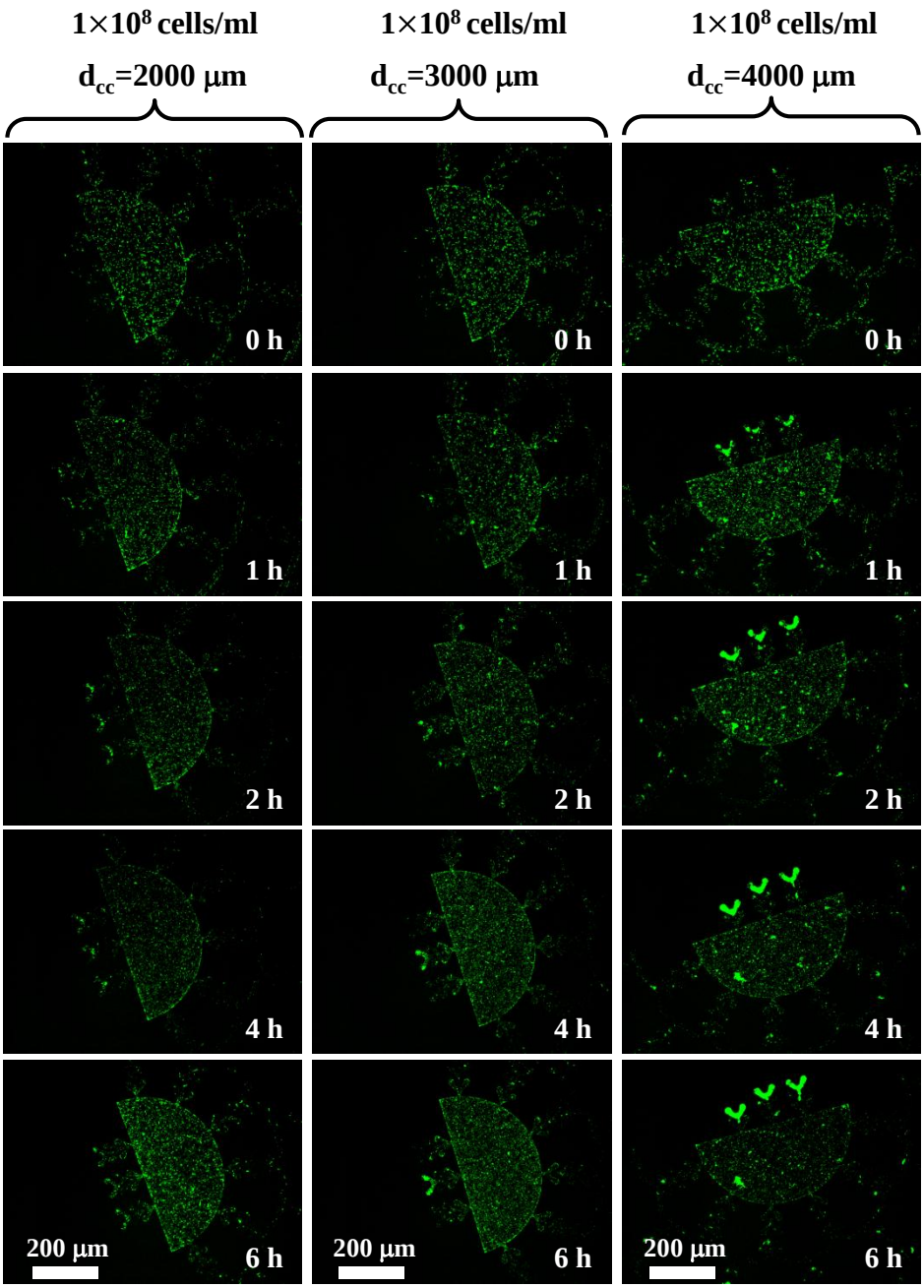

Figure S6 IRCs are concentrated in the concentrator with a constant initial cell density of 10^8 cells/ml (about 200 cells in the concentrator) but varying distances from a concentrator to a concentrator via the centre chamber such as 2000 μm, 3000 μm, and 4000 μm. The closer the distance between the IRCs and the SCs is, the earlier the fluorescent intensities appear to decrease.