

Microfluidic application-specific integrated device for monitoring direct cell-cell communication via gap junctions between individual cell pairs

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Direct cell-cell communication between adjacent cells is vital for the development and regulation of functional tissues. However, current biological techniques are difficult to scale up for high-throughput screening of cell-cell communication in an array format. In order to provide an effective biophysical tool for the analysis of molecular mechanisms of gap junctions that underlie intercellular communication, we have developed a microfluidic device for selective trapping of cell-pairs and simultaneous optical characterizations. Two different cell populations can be brought into membrane contact using an array of trapping channels with a $2\ \mu\text{m}$ by $2\ \mu\text{m}$ cross section. Device operation was verified by observation of dye transfer between mouse fibroblasts (NIH3T3) placed in membrane contact. Integration with lab-on-a-chip technologies offers promising applications for cell-based analytical tools such as drug screening, clinical diagnostics, and soft-state biophysical devices for the study of gap junction protein channels in cellular communications. Understanding electrical transport mechanisms via gap junctions in soft membranes will impact quantitative biomedical sciences as well as clinical applications. © 2005 American Institute of Physics. [DOI: 10.1063/1.1938253]

Molecular-level gap junction protein-based communication between adjacent cells is essential for the proper function of living tissue. It has been noted for over 40 years that membrane contact with neighboring cells can cause changes in morphology, gene expression, and growth.¹ These processes have been found to be important in multiple physiological functions including transmitting action potentials in cardiac myocytes,² various roles in the immune system,^{3,4} neural activity,^{5,6} and proper organ development.⁷⁻¹¹ Impaired cell-cell communication has been implicated in numerous diseases, and is correlated with most forms of cancer.^{12,13} While there exists a large body of literature on observations of direct cellular communication in almost all types of cancer, the molecular processes of how this regulates oncogenesis is still poorly understood.

The development of an improved *in vitro* method to monitor cellular communication on the molecular level can greatly increase the rate of discovery in this field. Currently, the most widely used method assays the transfer of fluorescent dye between a labeled cell and an unlabeled cell in membrane contact.¹⁴ This is generally accomplished by labeling one population of cells with a diffusible dye and culturing them in the presence of an unlabeled population of cells. While this experimental approach has proven reliable, information about single-cell transfer kinetics is lost due to the difficulty of manipulating the time and location of cell-cell contact.

Microfabrication technology offers a promising route for developing cell-based analytical tools. In previous work, we demonstrated that pressure-driven single-cell trapping could be achieved using microfluidic channels with a cross section much smaller than the size of a mammalian cell for patch-clamp applications.¹⁵ In this letter, we present a microfluidic application-specific device capable of trapping multiple cell-pairs for the simultaneous optical observation of functional gap junction intercellular communication. This design has the advantages of ensuring each cell is in membrane contact with only one other cell, creating selective contact between two different populations of cells, and increasing the overall throughput with parallel arrays of sites. The device operation is adaptable with current cellular monitoring techniques, providing a versatile platform for increasing the efficiency of cell-cell communication research.

Microfluidic devices were designed to trap individual cells within one cell diameter of an adjacent cell (Fig. 1). A single pair of trapping sites was designed to be separated by $20\ \mu\text{m}$ on opposite sides of a microfluidic channel. Since the cell line used in this letter averaged $12\ \mu\text{m}$ in diameter, this was wide enough to allow cells to flow through the channel unhindered, but narrow enough to ensure membrane contact when the cell-pairs were trapped across from each other. The design can easily be modified for cell lines with different diameters by varying the distance between trapping sites. The cell-trapping design consisted of two different heights of channels molded in polydimethylsiloxane (PDMS). The main microfluidic channel had a height of $50\ \mu\text{m}$ and a width of $20\ \mu\text{m}$ while the 37 pairs of cell-trapping channels had an opening of $2\ \mu\text{m}$ by $2\ \mu\text{m}$ located at the floor of the

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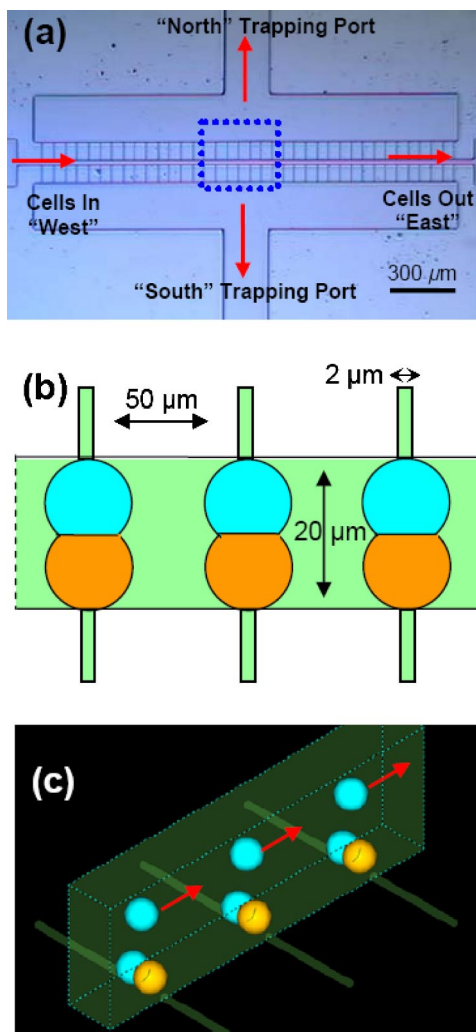


FIG. 1. (Color online) Layout of microfluidic cell-trapping device. (a) Microscope image of the arrayed device. The four inlets are referred to as north, south, west, and east according to the depicted layout for clarity. Cells are flowed through the main channel from west to east. Trapping is controlled by altering fluid flow using valves connected to the four outlets. (b) The trapping design allows two independently controlled sets of channels for localizing different cell populations either to the north or south trapping sites. (c) Three-dimensional schematic view depicting channel geometry, trapped cells, and cell flow.

main channel. Because the depth of the main channel was $50\ \mu\text{m}$, cells were able to freely flow through the channel without displacing trapped cell-pairs (Fig. 2). The cell-trapping array had four fluidic access ports connected to control valves. As depicted in Fig. 1, the west and east ports were used for flow of cells into the device, and the north and south ports controlled the corresponding row of cell-trapping sites. All cell-trapping channels were patterned on the same depth plane. Independent control of the two trapping ports (north and south) allowed selective trapping of one cell population on a specific side and a separate population at the opposing trapping channels.

The microfluidic application-specific devices were fabricated using soft-lithography technology and replicate molding. SU-8 negative photoresist (Microchem Corp.) was used as the master mold. A $2\ \mu\text{m}$ SU-8 2002 layer was patterned on a silicon substrate to define the cell-trapping channels. A second $50\ \mu\text{m}$ SU-8 2050 layer was then spin coated on top of the cell-trapping channels. The $20\ \mu\text{m}$ wide channel and other flow channels were photolithographically defined.

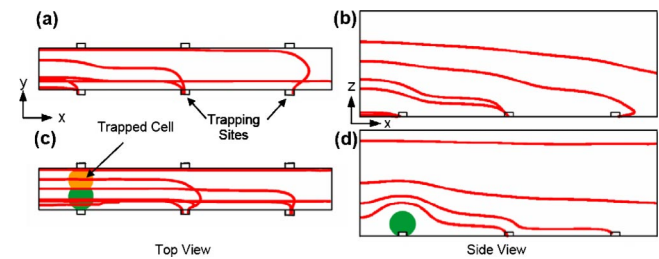


FIG. 2. (Color online) Flow simulation of trapping design. Three-dimensional finite element simulations depict streamlines during cell trapping along the south sites for (a) top view and (b) side view. Trapping of cell-pairs in the channel does not inhibit subsequent cells from finding downstream sites (c, d). Flow is from left to right.

PDMS (Sylgard 184, Dow Corning Corp.) was prepared according to the datasheet provided by the vendor and patterned devices were bonded to a glass slide. The coated cover glass formed the floor of the channels and provided a surface for cell adhesion and culture after trapping.

Device operation was tested using two mouse fibroblast (NIH3T3) cell populations: one fluorescently labeled with calcein AM ($2\ \mu\text{M}$, 15 min) and the other unlabeled. The selective cell-trapping device was used to demonstrate trapping of the fluorescent population on one row of sites in membrane contact with the unlabeled cells (Fig. 3). The west and east valves were initially open with the north and south valves closed. Labeled cells were introduced from a syringe connected to the west port. As the cells entered the device, the east valve was closed and the south valve opened to atmospheric pressure. This directed the flow of cells into the trapping sites at the south of the device. Because cells became trapped on the floor of the device, there was still enough space in the channel for other cells to flow through the main channel without disturbing trapped cells. After flushing the nontrapped cells from the device using sterile cell culture medium, a second set of cells (not labeled) was trapped on the north of the device by opening the corresponding valve to atmospheric pressure while leaving the opposing valve open.

Dye transfer experiments were conducted using tissue-cultured fibroblasts (NIH3T3). This cell line has been shown to be capable of dye transfer when cells are in membrane contact.^{16,17} The intracellular fluorescent dye calcein AM is permeable through cell-cell junctions and was used to study dye transfer.¹⁷ The AM ester is advantageous for this assay since it is transported into the cells from solution and converted to a fluorescent moiety that cannot diffuse out through the plasma membrane. After fluorescent and nonfluorescent cell-pairs were selectively trapped, the device was placed in a standard cell culture incubator ($37\ ^\circ\text{C}$, 5% CO_2) to promote dye diffusion. Dye transfer was observed between 3 and 16 h incubation for cells in membrane contact, but not for cells that were not in contact (Fig. 4).

While dye transfer is the most general method for investigating cell-cell communication, this device can also be applied for other experiments such as subcellular protein localization and electrophysiology. The expression and trafficking of fluorescently labeled proteins have been used to study intercellular communication on the molecular level.¹⁸ Additionally, measuring electrical activity between cells that are in membrane contact can provide vital information on how ions and action potentials are propagated directly between

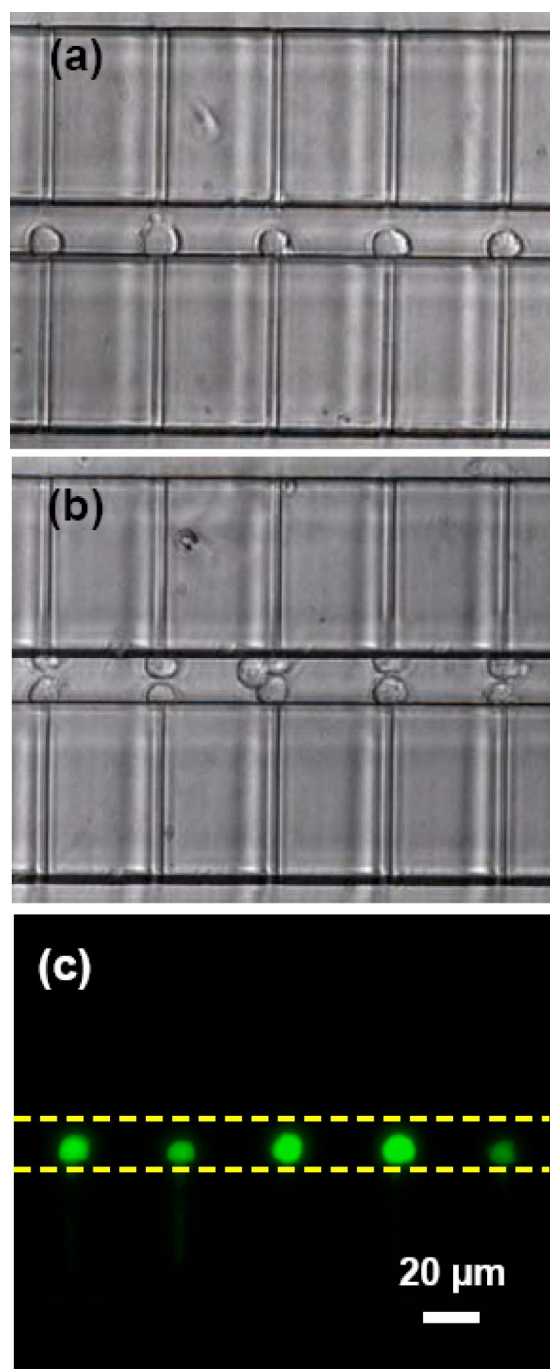


FIG. 3. (Color online) (a) Phase contrast image of labeled NIH3T3 fibroblasts trapped along the south sites. (b) A second population of fibroblasts was subsequently trapped on the north sites without displacing the original cells. (c) Fluorescence image with the same field of view as (b) depicting the selective trapping of two cell populations. The cells trapped on the south sites were previously labeled with calcein AM, while the second population of cells was unlabeled. Dotted lines indicate the boundary of the main channel.

cells.^{19,20} Our lab has previously demonstrated the capability of performing patch-clamp measurements through microfluidic trapping channels.¹⁵ This raises the possibility of performing multiple on-chip electrophysiology experiments for intercellular communication via gap junctions in an integrated microfluidic device. The study of how electrical signals propagate in living cells is of great interest to the biophysics community. Further development of the microfluidic application specific design presented here might prove valu-

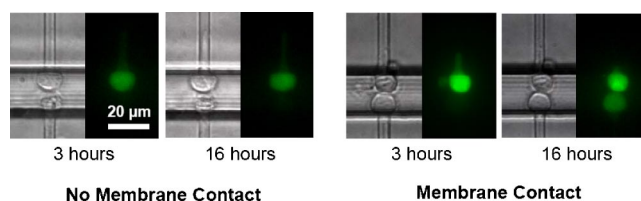


FIG. 4. (Color online) Diffusion of intracellular dye between NIH3T3 fibroblasts in membrane contact. The north cell in both cell pairs was initially labeled with calcein AM, while the south cell was not. When the two trapped cells were not in membrane contact (left), no dye transfer occurred. When membrane contact was present (right), fluorescent dye was able to transfer to the adjacent cell within 16 h. Phase contrast and fluorescence images are depicted for the same field of view.

able for obtaining information on the physics of electrical signal transmission across cell-cell gap junctions as well as behavior of biological materials.

This work describes the initial characterization and feasibility of using an arrayed microfluidic PDMS device for the study of intercellular communication between single cell pairs. Direct cell-cell communication via gap junctions is crucial to a large number of physiological functions with implications in numerous diseases. We hope to provide a tool to aid research on the molecular mechanisms that underlie intercellular communication in various tissues as well as cell-based biophysical studies. We are currently implementing a system to continuously monitor cells in real time while providing a stable culture environment within the microfluidic device. This should enable more detailed study of the kinetics of direct intercellular gap junction communication and how it is altered in disease.

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