Partial transfection of cells using laminar flows in microchannels

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This manuscript describes a convenient method for partial transfection using a Y-shaped microchannel polydimethylsiloxane (PDMS)-glass chip and on-chip cationic lipid-mediated transfection. Enhanced green fluorescent protein genes (pEGFP-N2) were introduced into the COS-7 cells cultured in half of the channel, while red fluorescent protein genes (pDsRed-N1) were introduced into the cells cultured in another half of the channel. This on-chip partial transfection technique provides an avenue for the spatial control of transfection. It is possible to use this technique to perform parallel transfection on chips in order to study cell behaviors under two or more gene transfections in the same culture. © 2011 American Institute of Physics. [doi:10.1063/1.3643827]

I. INTRODUCTION

Transfection describes the introduction of a foreign gene (such as plasmid DNA or siRNA constructs) into eukaryotic cells. It is used to analyze and study the functions of genes, regulatory sequences, and metabolism in living cells, thus making it an essential tool for life science research. Several methods based on biological, chemical, and physical principles are available for transfection. However, the limitation of traditional cell culture vessels causes the transfection of exogenous molecules to target all the cells in a culture. Additionally, there is a need to express two or more different genes in different groups of cells in the same culture to perform a comparison of their phenotypes.¹ Microinjection and gene guns are both excellent ways of delivering DNA to a specified target, but both are limited in terms of the number of cells. Thus, there is a need to develop new techniques for spatial control over the transfection of a particular group of cells in the same culture.

Microfluidic chips are especially suited to perform biological experiments at the cellular level because the scale of channels is commensurate with that of the cells.² Transfection has previously been performed on chips, mostly with the electroporation method.^{1,3–5} For instance, a microelectrode array is utilized to induce electroporation in a targeted group of cells for site-specific transfection.¹ In addition, cationic lipid-mediated transfection has been studied on PDMS-glass microchannels.⁶ Compared with on-chip electroporation, transfection with chemical reagents is easier to perform because no additional equipment or microelectrode fabrication is required. A chemical

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method can likewise be used in various microchannel structures. In this paper, we describe partial transfection of adherent cells on a Y-shaped channel microfluidic chip. This method relies on the fact that flows in microchannels are generally laminar because of their low Reynolds numbers.^{7,8} This feature has been applied to selectively treat cellular microdomains as well as pattern cells and their environments,^{9–11} study cellular chemotaxis using microgradient generators,^{12,13} and fabricate a wound edge in a cultured cell sheet for an on-chip cell migration assay.¹⁴

In this paper, COS-7 cells were cultured in PDMS-glass microfluidic chips with a Y-shaped channel structure. Two transfection mixtures—one includes pEGFP-N2 plasmids carrying enhanced green fluorescent protein and the other includes pDsRed-N1 plasmids carrying red fluorescent protein—were infused into the two inlets of the chip, respectively. A two-syringe infusion pump was used to drive the liquids and maintain the laminar flow in the microchannel. The effect of partial transfection was investigated.

II. EXPERIMENTAL

A. Design and fabrication of the microfluidic chip

The Y-shaped channel chip has two inlet channels converging into a single main channel. Two different sizes of channels were used. Channel I is 1 cm long, 60 μm high, and 900 μm wide, while channel II is with 3 cm long, 80 μm high, and 2 mm wide. The microchannel structure was fabricated in PDMS (Sylgard 184, Dow Corning) by rapid prototyping and replica molding techniques.¹⁵ Briefly, the negative relief of PDMS was formed by curing the prepolymer (Sylgard 184, Dow Corning) on a silanized Si master having a positive relief of the channels formed in photoresist (SU-8 2100, MicroChem) on its surface. Inlets were drilled using a blunted and beveled syringe needle. The PDMS replica was exposed under UV light, bonded with clean slide glasses, and pressed to achieve an irreversible seal.¹⁶ The chips are shown in Figure 1.

B. Formation of laminar flow in the microchannel

A two-syringe infusion pump (KDS200, KD Scientific) was used to infuse the liquids into the channel and maintain the laminar flows. In order to confirm laminar flow formation inside the channel, 300 μl of Opti-MEM (Invitrogen, Carlsbad, CA) with or without methyl blue was, respectively, loaded by a 1 ml syringe. The two syringes were mounted on the infusion pump. Flexible plastic tubes were used to connect the syringes and the inlets of the chip.

C. Cell culture

Prior to cell seeding, the chip was autoclaved for 20 min at 121 $^{\circ}\text{C}$. Next, a 0.01% (w/v) Poly-L-Lysine (PLL, Sigma) solution was injected into the channels using a 1 ml syringe to

FIG. 1. Photo of the Y-shaped channel PDMS-glass chips. The chip has two inlet channels converging into a single main channel. The dimensions of the main channels are: channel I—1 cm long, 900 μm wide, and 60 μm high, channel II—3 cm long, 2 mm wide and 80 μm high, respectively.

promote cell adhesion. After 5 to 10 min of incubation, the PLL was removed using the syringe, and the chip was dried in the clear hood at room temperature (RT). COS-7 cells were dissociated from the 25 cm² tissue culture flask (Corning) using trypsin-EDTA (Gibco) and then resuspended in 1 ml of the DMEM with 20% fetal bovine serum (FBS). The number of cells was $0.5\text{--}1 \times 10^7/\text{ml}$. Thereafter, the cell suspension was carefully injected into the channel and the chip was incubated at 37 °C with 5% CO₂. After the cells adhered to the glass surface, the medium was replaced every 6 to 8 h. Cells were ready for transfection when they attained about 80% to 90% confluency.

D. Partial transfection

Enhanced green fluorescent protein report plasmid pEGFP-N2 and red fluorescent protein report plasmid pDsRed-N1 were amplified in *Escherichia coli*, purified using an Endo-free Plasmid Midi Kit I (Omega), and then diluted to a 0.5 µg/µl concentration, respectively. LipofectamineTM 2000 (Invitrogen, Carlsbad, CA) was used for transfection. The on-chip partial transfection protocol is shown in Fig. 2. First, two transfection mixtures were prepared. In a tube, 2 µl of plasmid pEGFP-N2 was diluted in 50 µl of Opti-MEM and mixed gently. In another tube, 2 µl of Lipofectamine was diluted with 50 µl of Opti-MEM and mixed gently as well. After 5 min of incubation at RT, the diluted DNA was combined with diluted Lipofectamine, mixed gently, and incubated at RT for 20 min. Then, 200 µl of DMEM with 20% FBS was added. The same process is followed in preparing the transfection mixture included plasmid pDsRed-N1. Each transfection mixture was a little over 300 µl. Second, two 1 ml syringes were used to load the two mixtures, respectively, and were then mounted on the two-syringe infusion pump. Flexible plastic tubes were used to connect the two syringes and the two inlets, respectively. The chip was then placed in the 5% CO₂ incubator at 37 °C. The transfection mixtures were infused continuously for 4 to 6 h. The flow rate was set at 0.1 µl/min. Then, the pump was stopped and the fresh medium (DMEM with 10% FBS) was infused. Finally, the chip was placed in the incubator with the medium changed every 6 to 8 h until observation. The control experiment was done as follows: first, load the two transfection mixtures (one for each syringe); second, connect the two syringes and the two inlets; third, use the pump to inject the two transfection mixtures into the channel; fourth, immediately stop the pump and place the chip in the incubator; and finally, change medium 4–6 h afterwards. The gene expression results were

FIG. 2. Protocol of the partial transfection.

inspected and recorded by a fluorescent microscope (Ti-s, Nikon, Japan) and a CCD camera (Ds-Ri1, Nikon, Japan).

E. Transfection efficiency and cytotoxicity analysis

Each channel was divided into the upper half and the lower half; the transfection efficiencies of the pEGFP-N2 and pDsRed-N1 in the upper half channel and the lower half channel were calculated, respectively. The transfection efficiency was calculated following Kim's method⁴ by counting the number of EGFP/DsRed expressing cells in the total cells from four randomly captured microscope field images. When calculate the cell viability, control channels without transfection were used. Cells were seeded and cultured in both channels with or without transfection. Dead cells would be washed out of the channels during every medium changes. The cell viability was evaluated by counting the number of cells remained in the transfected channel from four randomly captured microscope field images and comparing it with the number of cells remained in the control channel. All the experiments were carried out more than three times.

III. RESULTS AND DISCUSSION

Because the Reynolds numbers of flows in microchannels are normally very small, the flows are laminated (laminar flow), i.e., the liquid in the channel flows in parallel layers with no disruption between the layers.^{17,18} Laminar flow in our microfluidic channels with flow rate at 0.1 $\mu\text{l}/\text{min}$ could be clearly seen under an optical microscope when the two inlets were filled with pure water and an aqueous solution of methyl blue, respectively (Fig. 3(a)). The clear interface between the two streams of solutions disappeared after the pump was stopped (Fig. 3(b)).

Figure 4 shows the transfected COS-7 cells 20 h after transfection in channel I (Figs. 4(a)–4(c) and 4(i)–4(l)) with 10 $\times$ objective magnification and channel II (Figs. 4(e)–4(g)) with 4 $\times$ objective magnification. Low magnification objective was used in the latter case so that the entire width of the channel could be fit into one view. Figures 4(a) and 4(e) were recorded under the bright field and Figs. 4(b), 4(c), 4(f), 4(g), and 4(i)–4(l) were recorded under the fluorescence field. The cells successfully transfected with plasmid pEGFP-N2 emitted green fluorescence when they were excited by a blue light source (Figs. 4(b), 4(f), 4(j), and 4(l)), and those successfully transfected with plasmid pDsRed-N1 emitted red fluorescence when they were excited by a green light source (Figs. 4(c), 4(g), 4(i), and 4(k)). Without flow, the transfection of pDsRed-N1 and pEGFP-N2 is random (Figs. 4(i) and (j)). Figs. 4(k) and 4(l) show the results of the control experiment; without flow, the transfection reagents (both the green and red ones) can diffuse into the other half of the channel. To show the partial transfection result more clearly, Fig. 4(d) is the merged image of Figs. 4(b) and 4(c), and Fig. 4(h) is the merged image of Figs. 4(f) and 4(g). A white dashed line was drawn in each merged image to indicate the center of the microchannel. The cells that emitted green fluorescence were in the upper half of the channel, while the ones that emitted red fluorescence were located in the lower half of the channel. During the 4 to 6 h when the two transfection mixtures were being infused in the

FIG. 3. Visualized laminar flow profiles in the microchannel. (a) The flow rate was set at 0.1 $\mu\text{l}/\text{min}$. (b) The interface disappeared in several minutes after the pump was stopped.

FIG. 4. Transfection of COS-7 cells in the channels, 20 h after transfection. (a)-(d), (i)-(j) channel I and the objective magnification was $10\times$. (e)-(h) channel II and the objective magnification was $4\times$. (a) and (e) Bright field image; (b), (f), (j), and (l) fluorescence microscopic image excited with blue light; (c), (g), (i), and (k) fluorescence microscopic image excited with green light; (d) merged from (b) and (c), and (h) merged from (f) and (g). The white dashed line was drawn as the midline of the channel. (i) Transfected with plasmid pDsRed-N1 and (j) transfected with plasmid pEGFP-N2 without flow. (k) and (l) control experiment. After the pump was immediately stopped, the plasmids diffused to the other half of the channel.

FIG. 5. Partial transfection efficiencies in channel I and channel II, and the transfection efficiencies in channel I without flow, 20 h after transfection. The unit of flow rate is $\mu\text{l}/\text{min}$.

microchannel as laminar flows, the cells in the upper half of the channel only made contact with plasmid pEGFP-N2, while those in the lower half of the channel only made contact with plasmid pDsRed-N1.

The transfection efficiencies of the pEGFP-N2 and pDsRed-N1 in the upper half and the lower half channel were calculated and shown in Fig. 5. All the cells in the field (X) and cells with fluorescence (Y) were counted and the transfection efficiency was calculated as $Y/X \times 100\%$. Data shown are the mean $\pm$ SD values obtained from three independent transfection experiments. The results show that in all cases, the transfection efficiency of the cells is $\sim 20\%$ – 35% of both transfection reagents. There is no significant difference in transfection efficiency in these three cases, indicating that channel size and flow play little role in the transfection efficiency of the cells. The data also show that in both channels I and II, with laminar flows, over 90% of the transfected cells by either reagent is located in the correct stream of that reagent. There is a small amount of transfected cells (less than 10%) that are in the other side of the channel (“wrong” side). Three possible reasons could have possibly caused this small percentage of transfected cells. The first reason is that the transfection reagents have diffused into the other flow stream. Because the transfection molecules are relatively large and they quickly pass the study area in the flow (~ 1 – 20 min depending on the size of the channel), their diffusion can affect the cells in the proximity of the center line (the Peclet numbers in our experiments are over 1000 by assuming the mass diffusion coefficient of our transfection plasmid as $1 \times 10^{-7} \text{ cm}^2/\text{s}$). But there are still a few transfected cells that are located into the other side far from the center line. The second is that these cells have contacted the transfection reagent by accident before laminar flow is established (it takes 1–2 min to establish laminar flow in the channel with syringe pump). The third possible reason is that the cells have migrated from one side to the other side during the time span from transfection to imaging collection. Because that the numbers of the red and green cells at their “wrong” sides are similar in each experiment, we consider that cell migration plays a more important role (the second reason would cause systematic bias in the number of red and green cells at the “wrong” sides). Interestingly, a few cells near the center line were transfected by both reagents (emitting both red and green fluorescence) (Fig. 4(d)). Overall, our experimental results clearly demonstrate the applicability of our microfluidic device for partial transfections simultaneously in the same channel.

The viability of the COS-7 cells in Channel I was $13.26\% \pm 1.48\%$, lower than that when the cells were transfected under the same conditions without flow.⁶ In the partial transfection experiment, flow is required to establish and maintain the interface between two transfection mixtures; hence, the cells in the channel have to be washed by the transfection mixtures for 4 to 6 h. During this period, shear stress is constantly present within the microchannel and may result in cell damage.^{19–21} The transfection reagent or transfection reagent–nucleic acid complexes likewise cause toxic effects on transfected cells. Compared with the transfection without flow, long-time cytotoxic reagents washing may cause relatively serious harm to the cells. There were very few cells remained in channel I when the flow rate was greater than $0.5 \mu\text{l}/\text{min}$. Thus, a low flow rate ($0.1 \mu\text{l}/\text{min}$) was used in this paper. In the wider channel (channel II) where the linear flow rate is lowered with the same volume flow rate as that in channel I, the cell viability is significantly increased to $31.05\% \pm 1.75\%$.

In the traditional experiment, the transfection mixture is added into the cell culture vessel and the medium is replaced after 4 to 6 h. During the 4 to 6 h that the DNA-lipid complexes make contact with the cells, the medium is localized within the vessel. Similarly, the transfection mixture is also localized within the channel when chemical transfection is performed on chips.⁶ Fluid flow is important for cell culture on microfluidic devices. In this study, the results indicated that transfection efficiencies on cells under flow in microchannels are similar to that in static solutions. However, fast flow may damage and detach the cells from the channel walls, causing lower viability of cells than that in static solutions.

The potential application of this partial transfection technique is connected to the conduct of parallel transfection and control experiments in the same culture on chips. It is difficult to transfect two or more different genes in different groups of cells in the same culture using a traditional cell culture vessel. As such, the study of gene expression comparison has to be performed in several cell culture vessels, such as the wells of a 24-well plate. However, it is difficult to avoid the differences between the cells in different wells, which will thus influence the reliability of the study. In contrast to traditional vessels, the reagents with different plasmids are infused into one channel during the on-chip partial transfection experiment, wherein the cells are strictly cultured under the same conditions. In this paper, plasmid pEGFP-N2 and pDsRed-N1 were used to demonstrate the effect of partial transfection. In further practical studies, more inlets can be designed to introduce different plasmids. Therefore, it is possible to study cell behaviors under two or more genes affection in the same culture, increasing the reliability of the experiment.

IV. CONCLUSIONS

In summary, we have demonstrated a convenient and robust microfluidic device for the partial treatment of mammalian cells with transfection mixtures using laminar flows in microchannels to deliver DNA to selected groups of the cells. This experiment cannot be achieved using traditional cell culture vessels. Compared with electroporation chips for spatially controlled transfection, partial transfection with chemical reagents in PDMS-glass microchannels is simpler and easier to perform. This is because the PDMS-glass chip is easy to fabricate and no microelectrode array and electrode control system are required. Moreover, this technique can easily be adapted to transfect multiple different genes in parallel in one microchannel. Therefore, it is possible to study cell behaviors under two or more gene transfections in the same culture to ensure an accordant cell state and to increase the reliability of the parallel experiments.

This study used Lipofectamine 2000 and COS-7 cells; nevertheless, the method proposed here is general for common transfection reagents and mammalian cells. A two-syringe infusion pump was used to infuse liquids and maintain laminar flows, but other liquid pumping methods can also be used. Because of difference among different types of cells, the effects of flow rate and the concentration of transfection reagents should be investigated in detail for each cell type.

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- ¹T. Jain and J. Muthuswamy, *Lab Chip* **7**, 1004 (2007).
- ²G. M. Walker, H. C. Zeringue, and D. J. Beebe, *Lab Chip* **4**, 91 (2004).
- ³Y. S. Shin, K. Cho, J. K. Kim, S. H. Lim, C. H. Park, K. B. Lee, Y. Park, C. Chung, D. C. Han, and J. K. Chang, *Anal. Chem.* **76**, 7045 (2004).
- ⁴J. A. Kim, K. Cho, Y. S. Shin, N. Jung, C. Chung, and J. K. Chang, *Biosens. Bioelectron.* **22**, 3273 (2007).
- ⁵S. K. Kim, J. Y. Kim, K. P. Kim, and T. D. Chung, *Anal. Chem.* **79**, 7761 (2007).
- ⁶L. Li, Y. Nie, D. Ye, and G. Cai, *Lab Chip* **9**, 2230 (2009).
- ⁷J. P. Brody, P. Yager, R. E. Goldstein, and R. H. Austin, *Biophys. J.* **71**, 3430 (1996).
- ⁸D. J. Beebe, G. A. Mensing, and G. M. Walker, *Annu. Rev. Biomed. Eng.* **4**, 261 (2002).
- ⁹S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, and G. M. Whitesides, *Nature (London)* **411**, 1016 (2001).
- ¹⁰S. Takayama, J. C. McDonald, E. Ostuni, M. N. Liang, P. J. A. Kenis, R. F. Ismagilov, and G. M. Whitesides, in *Proceedings of the National Academy of Sciences of the United States of America* **96**, 5545 (1999).
- ¹¹S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, and G. M. Whitesides, *Chem. Biol.* **10**, 123 (2003).
- ¹²N. L. Jeon, H. Baskaran, S. K. W. Dertinger, G. M. Whitesides, L. Van de Water, and M. Toner, *Nat. Biotechnol.* **20**, 826 (2002).
- ¹³G. M. Walker, J. Q. Sai, A. Richmond, M. Stremmer, C. Y. Chung, and J. P. Wikswo, *Lab Chip* **5**, 611 (2005).
- ¹⁴F. Q. Nie, M. Yamada, J. Kobayashi, M. Yamato, A. Kikuchi, and T. Okano, *Biomaterials* **28**, 4017 (2007).
- ¹⁵D. C. Duffy, J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides, *Anal. Chem.* **70**, 4974 (1998).
- ¹⁶L. Chen, J. C. Ren, R. Bi, and D. Chen, *Electrophoresis* **25**, 914 (2004).
- ¹⁷P. J. A. Kenis, R. F. Ismagilov, and G. M. Whitesides, *Science* **285**, 83 (1999).
- ¹⁸G. Batchelor, *An Introduction to Fluid Mechanics* (Cambridge University Press, Cambridge, UK, 2000).
- ¹⁹J. Zhou, K. Ren, W. Dai, Y. Zhao, D. Ryan, and H. Wu, *Lab Chip* **11**, 2288 (2011).
- ²⁰C. Couzon, A. Duperray, and C. Verdier, *Eur. Biophys. J.* **38**, 1035 (2009).
- ²¹X. Zhang, P. Jones, and S. J. Haswell, *Chem. Eng. J.* **135**, S82 (2008).