MICRODEVICE FOR CELL MIGRATION ASSAYS USING REVERSE-TRANSFECTION

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ABSTRACT

A microdevice for cell migration assays using reverse-transfection (RTF) was presented. The device consisted of multiple nanoliter scale chambers, which separate individual RTF spots to prevent cross-contamination of reagents and cells among neighboring RTF spots. The microdevice was designed to use forces generated by surface tension to seed cells in the chambers in a simple manner. We demonstrated that the migration of cells was significantly suppressed by the transfection of anti-paxillin siRNA using the microdevice. This approach may provide a promising platform for robust and reliable RTF assay systems for various biological analysis applications.

KEYWORDS

reverse transfection; cell migration; PDMS; paxillin.

INTRODUCTION

Cell migration is essential for both physiological and pathological processes, including wound healing, inflammation, and cancer invasion and metastasis [1]. High-throughput screening of the genes responsible for cell migration is therefore desirable for elucidating the mechanisms underlying these processes. The reverse-transfection (RTF) technology is a microarray-driven gene expression system, in which plasmid DNA and/or siRNA previously spotted on the surface of a slide glass are reverse-transfected locally into adherent cells. This is a powerful tool for screening hundreds of functional genes [2]. However, a potential drawback is that, as the RTF spot density increases, the potential for cross-contamination of regents and cells among neighboring spots also increases [3]. To address this issue, we fabricated a microdevice that separates the spots using nanoliter scale chambers. We examined whether the microdevice can be used for the simultaneous seeding of cells and the subsequent evaluation of migration-related genes.

EXPERIMENTS

The microdevice consisted of two microchannels, eight chambers, and RTF spots placed in each chamber (Figure 1). An RTF mixture was prepared by mixing siRNA (QIAGEN), plasmid-encoded yellow-green fluorescence (Venus) protein, Lipofectamine 2000 Reagent (Life Technologies), gelatin (Sigma), fibronectin (Research Institute for Functional Peotides, Yamagata, Japan), and Dulbecco's modified Eagle's medium (Sigma). The RTF mixture (~10 nl) was spotted and arrayed on a glass slide coated with type I collagen using a microarray printer (KCS-mini; KUBOTA Comps, Osaka, Japan). A PDMS structure was fabricated by replica molding using a template formed using a thick-film photoresist (SU-8; Microchem, Newton, MA, USA). The PDMS substrate was then placed on the collagen-coated glass slide after the precise alignment of the spots and chambers under a microscope. The plasmid-encoded fluorescence protein was used to visualize the transfected cells. NBT-L2b cells, typical invasive cancer cells, derived from bladder carcinoma of *rattus norvegicus* were used. The cells were suspended in a culture medium (MEM; Sigma) supplemented with 10% fetal bovine serum (ICN Biomedical Inc., OH) and antibiotics at a density of 1×10^6 cells/ml. The cell suspension was introduced into the multiple chambers simultaneously by using a pneumatic controller connected to the ends of the microchannels. After 24 h of culture, the migration of cells was tracked by taking phase-contrast and fluorescence images for 1 h at 5-min intervals. The migration speed of cells in each chamber was quantified by analysis of these time-lapse images using ImageJ software.

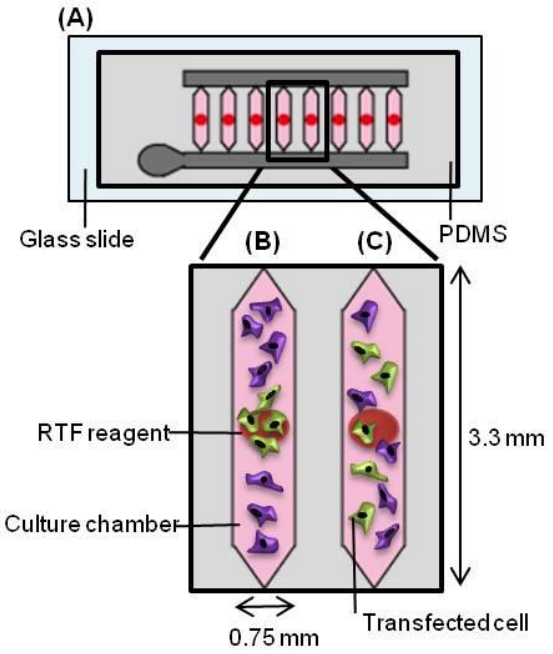

Figure 1: (A) Microdevice for cell culture. (B,C) Representative schematics of cell distributions in the chambers when cell migration is inhibited by the interference with a transgene (B) or when a transgene is not involved in migration (C). Transfected and non-transfected cells are indicated with yellow-green and purple, respectively. Red regions indicate spots of the RTF mixture reagent.

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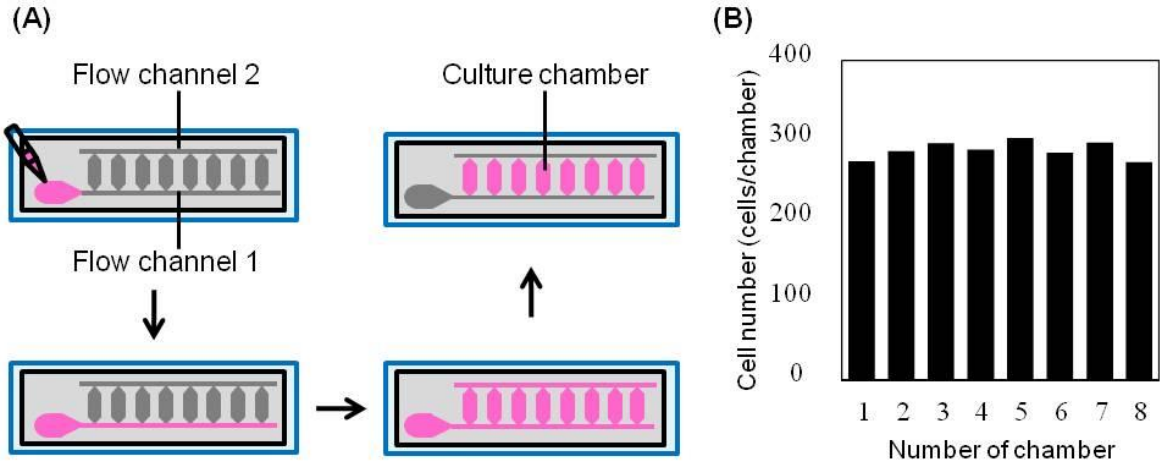

Figure 2: (A) Steps for simultaneous cell seeding to the multiple chambers. (B) Comparison of the cell densities seeded into the chambers.

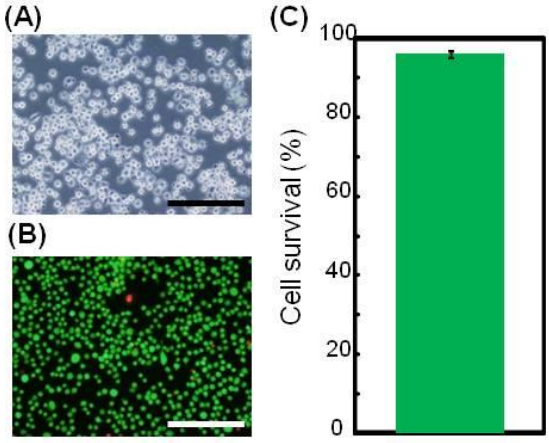

Figure 3: (A) Phase-contrast image of cells in the chamber after 24 h of culture. Scale bar, 200 μm. (B) Live/dead fluorescent staining of cells at 24 h of culture. Scale bar, 200 μm. Viable cells were identified by the green cytoplasmic fluorescence, whereas the dead cells showed red nuclear fluorescence. (C) Cell viability after 24 h of culture in the chamber.

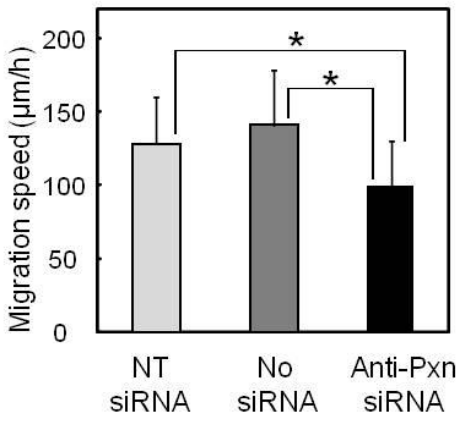

*Figure 4: Effect of anti-Pxn siRNA on migration of NBT-L2b cells. Cell migration was evaluated after transfection of anti-Pxn siRNA and non-target siRNA (NT siRNA), and without transfection (No siRNA). * indicates $p < 0.01$ by the Mann-Whitney u-test.*

RESULTS AND DISCUSSION

The microdevice was designed so that cells adhering to RTF spots in the chambers were transfected with siRNA and plasmid-encoded fluorescence protein during culture (Figure 1). The plasmid was used to visualize the transfected cells. Because cells uptake siRNA and plasmid at virtually the same time, fluorescently labeled cells can be assumed to be siRNA-transfected cells. When cells are transfected with siRNA targeted to genes essential for positively regulating cell migration, the distribution of the cells will be limited to close proximity of an RTF spot (Figure 1B), whereas cells transfected with siRNA unrelated to migration or without transfection will randomly migrate toward the outside of a spot (Figure 1C).

Figure 2A shows the steps for the simultaneous cell seeding of multiple chambers. The cell suspension was dropped into the solution inlet port using a pipette. Subsequently, by reducing air pressure from the end of flow channel 1, the solution was introduced into flow channel 1. All the chambers were then filled with the solution by applying a negative air pressure to the end of flow channel 2 for a few seconds. Finally, by applying a positive air pressure to the end of flow channel 1, the remaining excess solution was extracted from flow channel 1. As expected, the cell suspension was readily delivered to all the chambers, and it did not pass through the outlet of the chambers. This precise control of a cell suspension solution was realized by taking advantage of differences in surface tension. After the solution enters the chambers, the small rhombus structure at the end of each chamber effectively prevents overflow of the solution into flow channel 2. In this region, because surface free energy increases rapidly, the solution does not flow spontaneously. Figure 2B shows the number of cells introduced into each chamber. Cells were

uniformly seeded into the chambers, again because the cell seeding can be completed readily and rapidly owing to the precise handling of a solution. The viability of the cells in the chambers was evaluated after 24 h of culture with a live/dead fluorometric assay using fluorescein diacetate and ethidium bromide (Figure 3). With this assay, viable NBT-L2b cells were identified by green cytoplasmic fluorescence, whereas dead cells showed red nuclear fluorescence. As shown in Figures 3B and 3C, more than 95% cells remained viable after 24 h of culture, suggesting that the microdevice provides a favorable cell culture environment.

To demonstrate that the microdevice can be used for cell migration assays, paxillin (pxn) was used as a model target gene. Pxn is a focal adhesion-associated adaptor protein and is essential for cell migration. Anti-Pxn siRNA interferes with the expression of the Pxn gene and suppresses cell migration. We prepared an array of RTF spots containing anti-Pxn siRNA, as well as siRNA without target (NT siRNA) and without siRNA (No siRNA) as controls. Transfection efficiency was quantified by counting the number of cells expressing a transfected fluorescent protein. The efficiency was ~30%, which was sufficient for the statistical evaluation of cell migration. After 24 h of culture NBT-L2b cells were tracked through a time series of phase-contrast and fluorescent images of the entire region of each chamber at 5-min intervals for 1 h. Although there was no significant difference in the migration between the NT siRNA and No siRNA samples, migration was significantly suppressed by transfection with anti-Pxn siRNA as compared with the controls (Figure 4, $P < 0.01$).

CONCLUSION

We fabricated a microdevice to facilitate RTF of microarrayed siRNA and validated the technique through evaluation of genes closely related to cell migration. To prevent cross-contamination of reagents and cells among RTF spots, individual spots were confined inside microchambers. To facilitate cell seeding into the multiple chambers, the chamber structure was designed to use forces generated by surface tension for precise handling of the cell suspension solution. NBT-L2b cells were introduced into the chambers, reverse-transfected with siRNA, and cultured under a favorable condition over 24 h. RTF with anti-Pxn siRNA significantly inhibited the migration of NBT-L2b cells. This approach may contribute to a better understanding of fundamental cell biology and may facilitate the development of a tool for screening and evaluating migration-related genes.

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