Effects of Cell Density and Coculture on Cell Traversal through Channels

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Physical confinement in extracellular matrix and presence of different cell types play important roles in regulating cell migration, which is related to cancer metastasis and wound healing. Conventional cell migration studies using Transwell assays do not allow for real-time monitoring of cell migration dynamics. In this study, platforms consisting of microwells and connecting channels were fabricated to investigate the migration behaviors of nasopharyngeal epithelial (NP460) and carcinoma (NPC43) cells under confinement.

Figure 1 presents the fabrication technology for the polydimethylsiloxane (PDMS) platforms with microwells and connecting channels. The microwells were 100 × 100 μm^2 with 20 μm wide, 50 μm long channels, replicated from a patterned silicon (Si) stamp. NP460 and NPC43 cells were seeded onto the platforms, and their migration behaviors were monitored for 16 h using time-lapse imaging.

Figure 2 (a) demonstrates the influence of cell density in the microwells on the traversing probability of monocultured NP460 cells. As the number of cells in microwells increased, the traversing probability initially increased and subsequently reached a plateau. This could be attributed to enhanced contact inhibition from more cell-cell contacts at higher cell densities, which cause NP460 cells to migrate from neighboring cells and promote longer-range migration. As shown in Fig. 2 (b), the traveling radius of NP460 cells in microwells increased with cell density.

Compared with monoculture of 1 to 6 NP460 cells in microwells, higher traversing probability was found when cocultured with NPC43 cells, as shown in Fig. 3 (a). Figure 3 (b) demonstrates the distribution of cocultured NP460 and NPC43 cells at the beginning and after 16 h. NPC43 cells formed clusters on the bottom surface following homotypic cell contacts, displacing NP460 cells toward less occupied peripheral regions and increasing their likelihood of reaching the channel openings. These findings showed that both cell density and the type of neighboring cells influenced nasopharyngeal epithelial cell migration through channels. The proposed high-throughput microwells with connecting channels allow real-time monitoring of cell migration in confinement and offer insights into NPC cell migration behaviors.

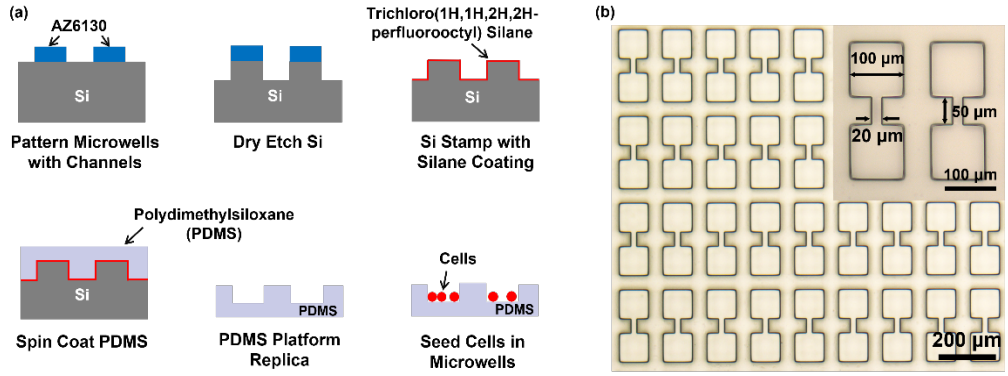

Figure 1: (a) Fabrication technology for PDMS microwells with channels for cell culturing. (b) Array of microwells with channels. Microwells were 100x100 µm^2, and channel width was 20 µm with length of 50 µm.

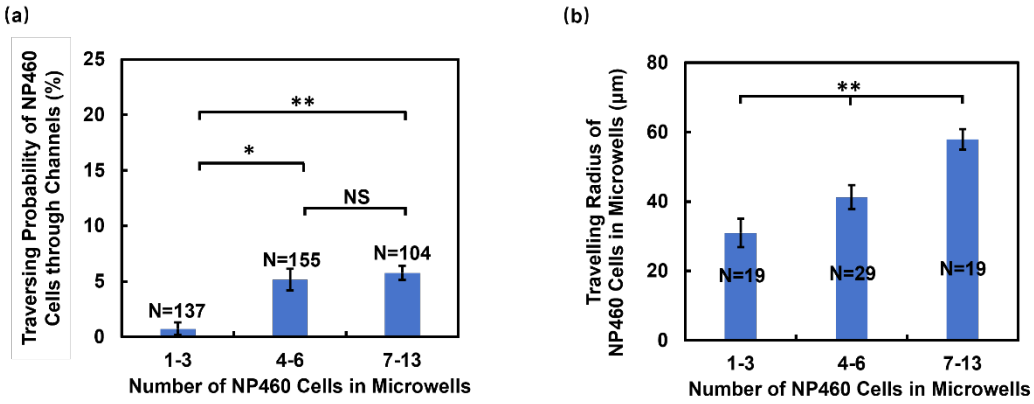

Figure 2: (a) Traversing probability of monocultured NP460 cells through 20 µm wide, 50 µm long channels increased with cell density in microwells. (b) NP460 cells had longer traveling radius with increasing cell density in 100x100 µm^2 microwells. Traveling radius was defined as maximum distance reached by cells from their initial positions. One-way ANOVA and Tukey's *post hoc* test with *p <0.05, **p <0.01, and NS - not significant with p >0.05.

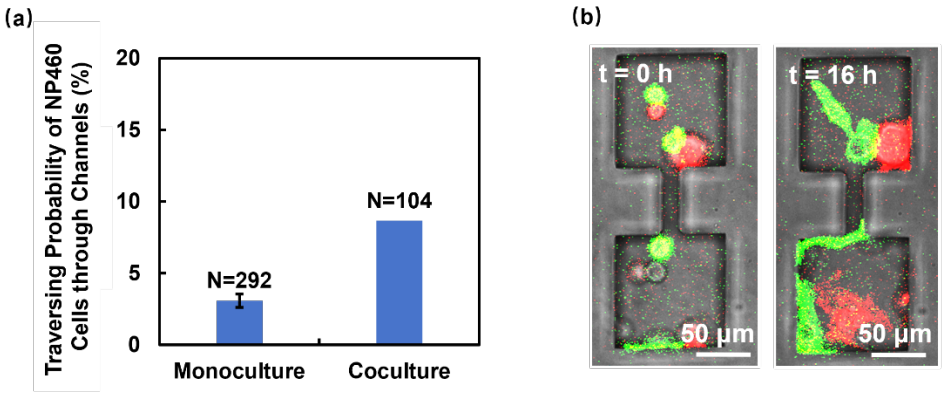

Figure 3: (a) Traversing probability of NP460 cells through 20 µm wide, 50 µm long channels increased when cocultured with NPC43 cells. (b) Distribution of cocultured NP460 (green) and NPC43 (red) cells in microwells with channels.