# Flower Microchannel Device for Cancer Cell Migration Study

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## Introduction

The majority of cancer related fatalities are the consequences of secondary cancer caused by metastasis (over 90%), of which migration mechanisms are among the highest interests in the field of cancer research. Unlike the more common methodologies for cell migration studies such as Scratch assay and Boyden chamber-transwell assay, microfluidic based devices have shown numerous advantages including real-time and long-term monitoring, reduction of reagent consumption, as well as chemo-attractant independence. In our study, we present a novel microfluidic design called Flower Microchannel device for studying cancer cell migration, in which various dimensions of microchannels are incorporated into one device allowing researchers to target different aspects of tumor cell migration processes such as cellular expression, transition at interface between 2D vs. 3D, transition of various migration modes, or cellular responses to anti-cancer drug. In addition to serving as a model system for studying tumor cell migration, the devices are aimed to improve the limitations of time consuming experiments and large standard deviations that remain an intrinsic drawback of many microfluidic devices.

## Materials and Methods

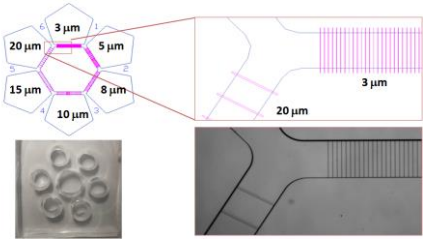


**Figure 1.** Multi version of Flower device. Center reservoir (cell seeding) is connected to the other 6 satellite reservoirs via 6 different microchannel widths: 3, 5, 8, 10, 15, 20µm (channel length is 500µm).

*Fabrication of Flower Microchannel device:* Using AutoCAD software, the flower device was designed to have a central reservoir surrounded by six satellite reservoirs and microfluidic channels connecting the two types of reservoirs together. The channel's pattern included two versions, tapered and multi (Figure 1). In a tapered device, channel's widths reduced gradually along the length of the channel from 20 to 15, 10, 8, 5µm in order for users to monitor the cellular and molecular transition of a single cell at different spatial confinements. In a multi device, channel widths were unchanged along the length of the channels, but six different widths 20, 15, 10, 8, 5, 3µm were placed toward the six different satellite reservoirs. As the channel width decreased from 20 to 3µm, we adjusted the number of channels to12, 16, 24, 30, 48, and 80, respectively, so that the total cross sectional area remained the same. The multi device allowed users to target different migratory behaviors of cancer cells in multi-dimensional confinements at the same time.

The standard photolithography method was used to prepare a silicon wafer having double deposited layers of photoresist corresponding to the channel and reservoir layer. The thicknesses of these two layers were 3-15 and 100µm, respectively. The wafer was used for soft lithography to created Polydimethylsiloxane (PDMS) devices. Central and satellite reservoirs of PDMS devices were punched by 8 and 6mm biopsy punch, respectively. The devices were then decontaminated with 70% ethanol, and assembled onto glass surfaces.

*Migration study of hGBM:* Vigorous migration of hGBM via various microchannels was conducted. Devices were laminin coated and cells were cultured at a density of $5x10^4$ cells per device with the seeding volume at 50µl (n=4). Changes of cellular morphology during migration were observed. In order to gain better insight of the brain tumor cell interactions in the brain tissue during migration, further experiments using tapered devices to monitor hGBMs co-cultured on top of cortical neurons or fibroblasts were conducted.

*Migration study of different genotypes*: Similar multi devices were prepared with the exception that the cells used were mouse astrocytes with three different genotypes: single mutation *p53-/-* (P53), double mutation *p53-/- pten-/-* (Pten), and double mutation *p53-/- pten-/-* added oncogene *Braf* (Braf). Quantitative analysis of cells completely migrated to the satellite reservoirs were performed to compare the migration ability of different cells via various dimensional constrictions.

## Results and Discussion

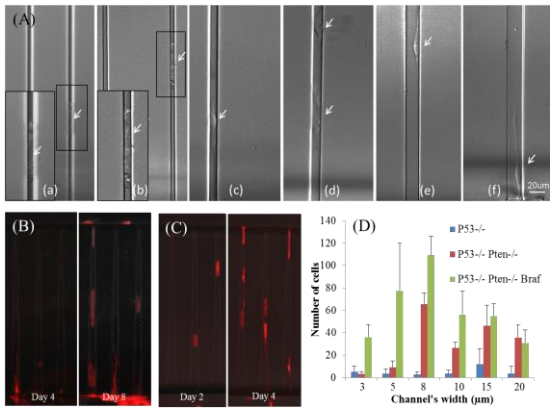


**Figure 2.** (A) hGBM migrating via 6 different microchannels: Ameboid mode of migration via the confined microchannels (3 and 5µm) and Mesenchymal mode of migration via the wide microchannels (15 and 20µm). Co-culture of hGBM on top of cortical neurons (B) and fibroblasts (C). (D) Migration of different genotypes of mouse astrocytes via 6 different channels. n=4/condition.

By using our device, it was possible to visualize the transitions of different modes of cell's migration inside varied-width channels (Figure 2A) corresponding to the expression of cytoskeleton filaments such as actin and microtubules. Moreover, results of co-culture experiments showed not only the ability of hGBMs to migrate together with other cell types *in vitro,* but also the guidance effect of either cortical neurons or fibroblast on migration. This suggested the device can mimic *in vivo* and clinical conditions in which brain cancer cells move in the white matter track or when they encounter the invasion of fibroblasts after surgery.

Furthermore the multi flower devices also allowed us to quantify the migratory ability of different genotype mouse astrocytes (Figure 2D). The number of Pten that completely migrated was on average 7 times larger than single mutation P53 when cells moved through 10, 15, and 20 µm channels. However, Pten had to struggle in more confined channels, 3 and 5 µm; and the addition of oncogene *Braf* resulted in an approximately 9 times increase of cells migrated. This was consistent with the fact that more cancer-related genetic events can cooperate to promote cancerous migratory behavior.