**Abstract**

The fabrication and operation of a gradient-generating microfluidic device for studying cellular behavior is described. A microfluidic platform is an enabling experimental tool, because it can precisely manipulate fluid flows, enable high-throughput experiments, and generate stable soluble concentration gradients. Compared to conventional gradient generators, poly(dimethylsiloxane) (PDMS)-based microfluidic devices can generate stable concentration gradients of growth factors with well-defined profiles. Here, we developed simple gradient-generating microfluidic devices with three separate inlets. Three microchannels combined into one microchannel to generate concentration gradients. The stability and shape of growth factor gradients were confirmed by fluorescein isothiocyanate (FITC)-dextran with a molecular weight similar to epidermal growth factor (EGF). Using this microfluidic device, we demonstrated that fibroblasts exposed to concentration gradients of EGF migrated toward higher concentrations. The directional orientation of cell migration and motility of migrating cells were quantitatively assessed by cell tracking analysis. Thus, this gradient-generating microfluidic device might be useful for studying and analyzing the behavior of migrating cells.

**Protocol**

**A. Microfabrication of the gradient-generating microfluidic device**

1. The Si wafer is treated with reactive oxygen plasma (5 min at 30W, Harrick Scientific, NY).
2. Negative photoresist (SU-8 50, Microchem, MA) is spin-coated at 1000 rpm for 1 min on a Si wafer.
3. The wafer is soft baked at 65°C for 10 min and subsequently at 95°C for 30 min on a hotplate.
4. The wafer is exposed to UV light (200W) for 3 min through a transparency mask with a minimum feature size of 30 µm.
5. The wafer is post baked at 65°C for 1 min and at 95°C for 10 min.
6. Si master mold with 100 µm thick channels is developed using SU-8 photoresist developer.
7. The wafer containing microchannels is placed in a Petri-dish.
8. Poly(dimethylsiloxane) (PDMS) (Sylgard 184) molds are fabricated by mixing silicone elastomer and curing agent (10:1 ratio).
9. The PDMS mixture is poured onto the Si master mold.
10. The Si master mold is placed on a vacuum desiccator to remove bubbles for 10 min.
11. PDMS is cured at 70°C for 1~2 hours.
12. PDMS molds are peeled off from the Si master mold.

**B. Experimental setup**

1. Cell inlet, outlet, and infusing inlets of the PDMS-based microfluidic device are punched by using sharp punchers.
2. A device and a glass slide (2×3 inch) are irreversibly bonded by the reactive oxygen plasma (5 min at 30W, Harrick Scientific, NY).
3. Polyethylene tubing (PE 20, Becton Dickinon, MD) is inserted into the infusing inlets of the microfluidic device and subsequently connected to a syringe pump.
4. Fluorescein isothiocyanate (FITC)-dextran (MW=10kD, 10 µM, Sigma) and buffer (PBS, Invitrogen, CA) are infused into the microfluidic device to confirm stable gradients inside the fluidic device.
5. Extracellular matrix (ECM) (i.e., fibronectin) is coated inside the microfluidic device for 1 hour in incubator (37°C).
6. NIH 3T3 fibroblast cells are trypsinized and dissociated.
7. Dissociated cells are loaded into the microfluidic device (800 µm wide) at the cell density of 2×10⁶ cells/ml.
8. Media, and 50 ng/ml epidermal growth factor (EGF) is infused into a microfluidic device for generating soluble gradients using a syringe pump (0.05 µl/min).
9. Cells are real-time monitored every 5 min by using an inverted microscope (Nikon TE 2000).

**Discussion**

Cells exposed to stable concentration gradients of EGF in a microfluidic device migrated toward higher concentrations. The directional orientation of cell migration, chemotactic index, motility of migrating cells were investigated by cell tracking analysis. Therefore, this gradient-generating microfluidic platform could be useful for studying cancer metastasis, embryogenesis, and axon guidance.
References


