Video Article

A Microfluidic Device with Groove Patterns for Studying Cellular Behavior

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Abstract

We describe a microfluidic device with microgrooved patterns for studying cellular behavior. This microfluidic platform consists of a top fluidic channel and a bottom microgrooved substrate. To fabricate the microgrooved channels, a top poly(dimethylsiloxane) (PDMS) mold containing the impression of the microfluidic channels was aligned and bonded to a microgrooved substrate. Using this device, mouse fibroblast cells were immobilized and patterned within microgrooved substrates (25, 50, 75, and 100 μm wide). To study apoptosis in a microfluidic device, media containing hydrogen peroxide, Annexin V, and propidium iodide was perfused into the fluidic channel for 2 hours. We found that cells exposed to the oxidative stress became apoptotic. These apoptotic cells were confirmed by Annexin V that bound to phosphatidylserine at the outer leaflet of the plasma membrane during the apoptosis process. Using this microfluidic device with microgrooved patterns, the apoptosis process was observed in real-time and analyzed by using an inverted microscope containing an incubation chamber (37°C, 5% CO_2). Therefore, this microfluidic device incorporated with microgrooved substrates could be useful for studying the cellular behavior and performing high-throughput drug screening.

Video Link

The video component of this article can be found at http://www.jove.com/video/270/

Protocol

A. Microfabrication of the microfluidic device

1. 4-inch Si wafer is treated with reactive oxygen plasma (5 min at 30W, Harrick Scientific, NY).
2. Negative photoresist (SU-8 2015, Microchem, MA) is spin-coated at 900 rpm for 1 min on a Si wafer.
3. The wafer is soft baked at 95°C for 6 min on a hotplate and is exposed to UV light (200W) for 4 min through a mask film containing microchannels.
4. The wafer is post baked at 95°C for 6 min and is developed using SU-8 photoresist developer.
5. The photoresist patterned wafer containing microchannels is placed in a Petri-dish.
6. Poly(dimethylsiloxane) (PDMS) (Sylgard 184) molds are fabricated by mixing silicone elastomer and curing agent (10:1 ratio).
7. The PDMS mixture is poured onto the Si master mold and is placed on a vacuum desiccator to remove bubbles.
8. PDMS is cured at 70°C for 1~2 hours.
9. PDMS molds are then peeled off from the Si master mold.

B. Assembling the device

1. 40 μm thick top fluidic channels and 40 μm thick bottom microgrooved channels (25, 50, 75, and 100 μm wide) are obtained from two different Si master molds.
2. Channel inlet and outlet of the fluidic device are punched.
3. Fluidic channels and microgroove channels are irreversibly bonded by the reactive oxygen plasma (5 min at 30W, Harrick Scientific, NY).
4. Extracellular matrix (ECM) (i.e. fibronectin) is coated inside microfluidic device for 1 hour in incubator (37°C).

C. Cell seeding and experimental setup

1. NIH-3T3 mouse fibroblasts are cultured in a tissue culture flask using Dulbecco's Modified Eagle's Media (DMEM) containing 10% Fetal Bovine Serum (FBS).
2. Cells are trypsinized and dissociated.
3. Dissociated cells are loaded into the microgroove channels at the cell density of 3×10^6 cells/ml (Figure 1).

Figure 1

4. 2 mL media, 100 mM H_2O_2, and apoptosis assay (20 µL Annexin V and 40 µL propidium iodide, Invitrogen, CA) are infused into a channel using a syringe pump (1 µl/min).
5. Cells are real-time monitored by using an inverted microscope (Nikon TE 2000).

Discussion

Cells were immobilized and patterned within microgrooved substrates in a microfluidic device. The apoptosis process of cells exposed to hydrogen peroxide was observed in real-time and analyzed by using Annexin V and propidium iodide. Thus, this microfluidic device containing microgroove channels could be useful for high-throughput drug screening.

References

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