Supporting Information for

Assembly of Controllable Cell Gradients Directed by Three-Dimensional Microfluidic Channels

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The original cell density of homogeneous cell suspension affect the generated cell gradient profile

Cell gradient profile is dependent on the original cell density in the solution. We further studied how the original cell density affects the profiles of the cell gradients. In these experiments, a 3D stair-shaped microfluidic chip was used with a linear profile (step rise, 90 μm). Cell suspensions with a wide range of cell density (from 6×105 cell mL-1 to 4.5×106 cell mL-1) were loaded separately into the microchip to generate cell gradients. Results were shown in the Figure S2. Linear profiles were generally observed for cell suspensions of various concentrations. Higher the concentration, larger the slope was found for a wide range of original cell density. It must be noted that the experimental profile slightly departed from the theoretical profile for an extremely high concentration of cell suspension, which might result from the limited space in the microchannel and cell aggregation (Figure S2).

Redistribution of cell gradient after long-term culture

Another study has also been carried out to investigate whether long pre-incubation affect the cell gradient profile. After linear cell gradient was generated inside the channel, cells were pre-incubated for 24 hours. Cell densities were measured in each step of the stair-shaped microchannel at four time point, 0, 6, 12 and 24 hours. Results were shown in the Figure S3. As a result, the cell gradient profiles were greatly changed after long-term incubation, which might result from the proliferation and migration of cells.

To further investigate the redistribution of cell gradient, cells were cultured in a FBS-free medium for 24 hour prior to generation of cell gradient. Cell gradient was also incubated in a FBS-free environment to ensure no cell proliferation. Thus, cell

gradient could be redistributed only by cell migration. Cell densities were measured in each step of the stair-shaped microchannel at four time point, 0, 6, 12 and 24 hours. Results were shown in the Figure S4.

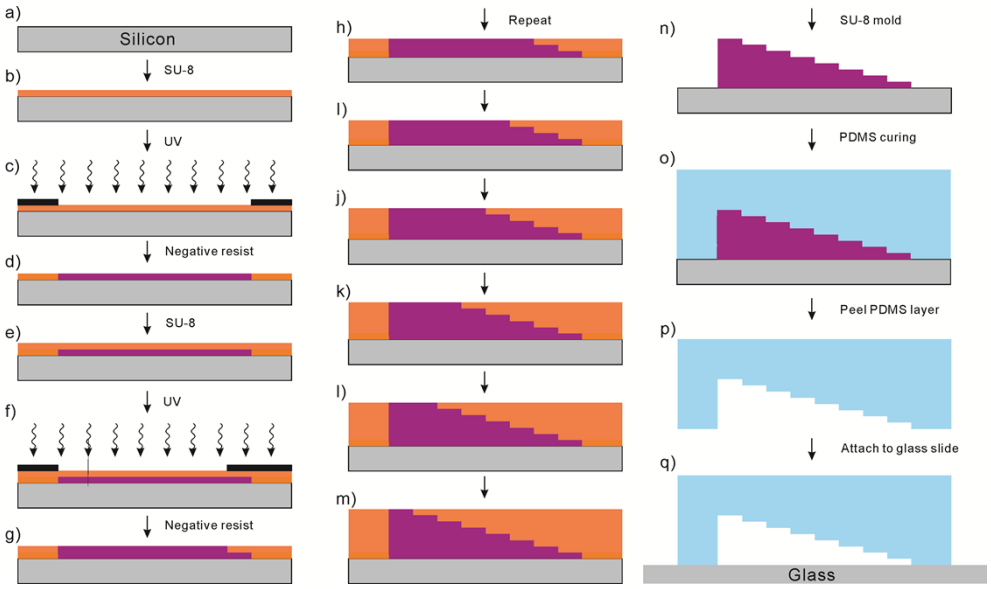

Figure S1. The fabrication process for the 3D PDMS microchannels. a-m) Fabrication of the SU-8 mold using soft lithography. n-q) Fabrication of the 3D PDMS microchannels using rapid prototyping method.

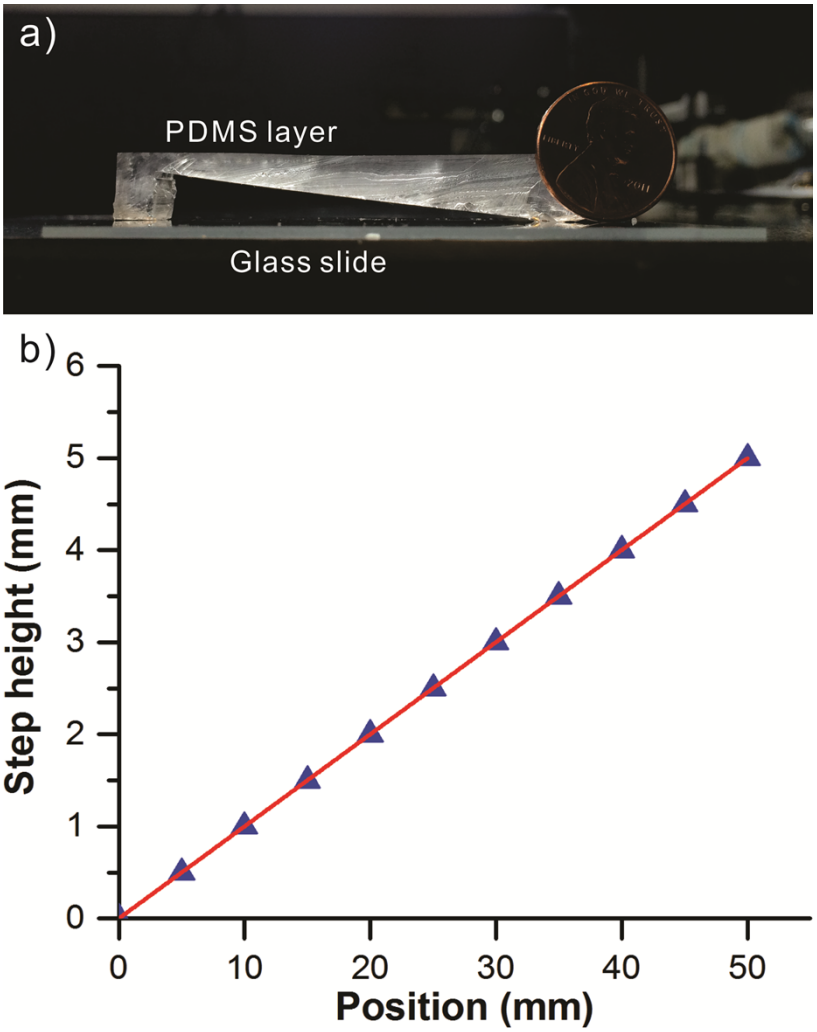

Figure S2. Microchannel fabricated by casting 3D printed mold. a) Cross-section of the channel with smooth linear profile. b) The heights of the microchannel at different positions.

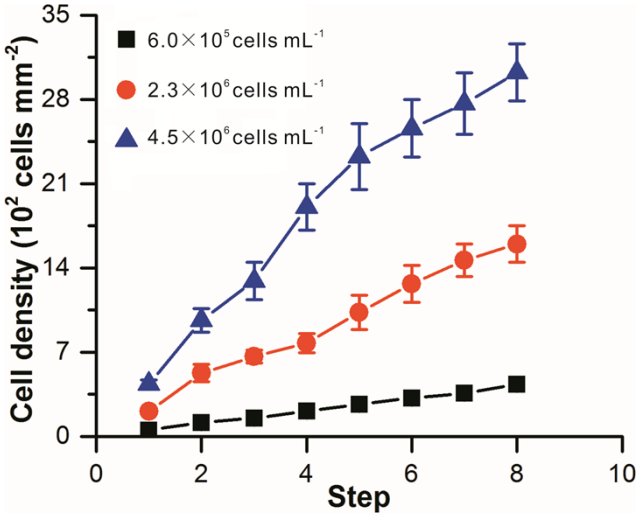

Figure S3. Generation of linear cell gradient with cell suspension of various original densities.

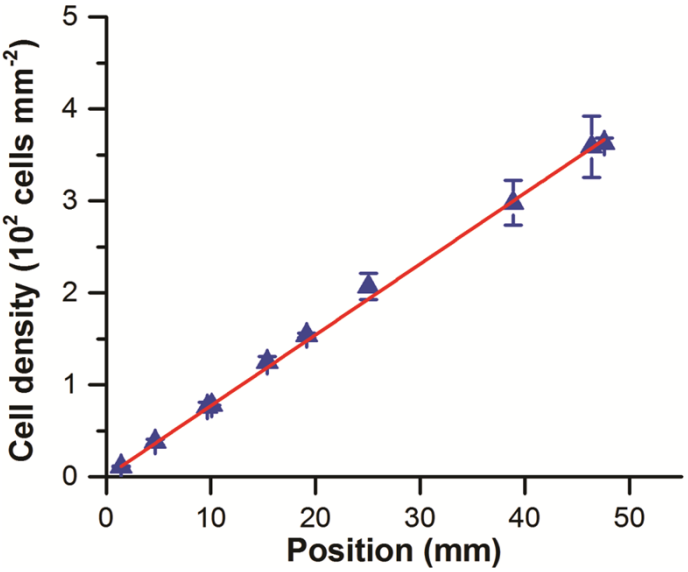

Figure S4. Cell densities at different positions along the microchannel. The microchannel was fabricated by casting 3D printed mold. The positions were selected randomly.

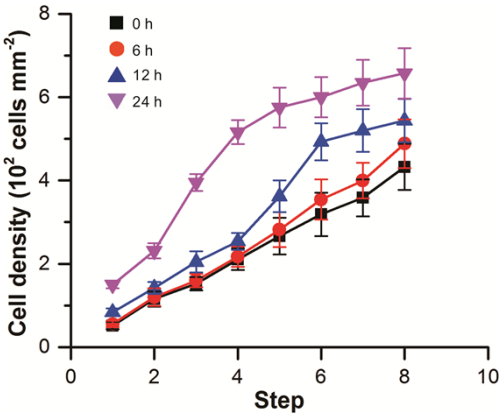

Figure S5. Redistribution of cell gradients. Cell densities were measured in each step of a stair-shaped microchannel with a linear profile (step rise, 90 μm) at four time point: a) 0 hour, b) 6 hour, c) 12 hour, d) 24 hour.

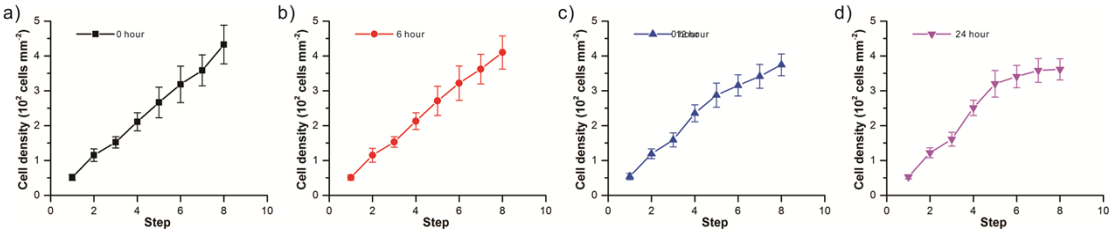

Figure S6. Redistribution of cell gradients by cell migration. Cell densities were measured in each step of a stair-shaped microchannel with a linear profile (step rise, 90 μm) at four time point: a) 0 hour, b) 6 hour, c) 12 hour, d) 24 hour.