Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Information

Obtain Smaller Microstructure with Oxygen Plasma

High resolution photolithography is required to achieve the 3 µm trapping gap, which usually demands for more sophisticated masks such as Chrome mask. Here we have developed a cost effective way to reach lower dimension with only Mylar mask using oxygen plasma etching. After photolithography, the width of gap was 10 µm as provided with the Mylar mask. Then the wafer was exposed to oxygen plasma for 3 mins at 0.12 Torr oxygen pressure and 120 Walt power level followed by 5 mins at 0.5 Torr oxygen pressure and 40 Walt power level, repeating twice. The chemical reactions between reactive oxygen and SU-8 polymer generated decomposed versatile species from surface. Then, the versatile species could be taken away from the surface by vacuuming. The repeated different plasma energy level was used in order to achieve fine trimmed edges: higher energy level effectively etched the surface and lower energy level gave even, smoother edge. In this way, 3 µm gap structures were made. (SI Fig. 1)

Chip Preparation and Cell Trapping Procedure

Before use, the microfluidic chip was autoclaved for 25 mins. Then, the chip was placed in vacuum chamber for 15 mins and the culture media was immediately dropped on top of the inlets in order to fill the chip. The chip was incubated in cell culture incubator for 3 hours before cell loading to prepare a protein absorbed surface to help cell attachment. To avoid possible debris from culture media and cell suspension that would be trapped at the gap, the media was filtered with 0.45 µm Nylon membrane every time before use; the prepared cell suspension was stood in culture incubator for 5 mins to precipitate debris before loading.

A PDMS gasket with a length of 2.5 cm, a width of 4 mm and a height of 5 mm was manually cut to fit the 8 cell loading inlets area, and was attached to the top of the chip. The microfluidic chip was perfused with culture media for at least 10 mins at a withdrawing flow speed of 5 µL /min before cell loading. Then cells were dropped into the gasket and were uniformly distributed by gentle pippeting Fluid was driven by withdrawing through the outlet *c*, at a constant speed with a syringe pump (PHD 2000, Harvard Apparatus) interfaced to the Luer ports via Tygon tubing (ID 1.3 mm) . During cell loading, the chip was observed under the microscope, and was placed on a hot plate with a temperature of 37 °C. After loading, gasket was removed from the top and the chip surface was cleaned with media. The residue cells in the loading channels were washed away by pumping media through perfusion inlet *a1* and *a2* at the speed of 2 µL/min for less than 5 mins. The whole process of loading was done within 5 mins.

After the first cell type was trapped as single cell, the chip was placed in cell culture incubator for 5 hours before the second cell type trapping. After second cell trapping, the chip was put into incubator for static culture for 4 hours. Then inlets *b1-b8* were blocked by attaching a thin layer of PDMS membrane on top. Media was loaded from inlets *a1* and *a2* through the gradient generator, by withdrawing through outlet *c* with a constant speed.

Trapping Parameter Optimization

The efficient cell trapping requires the balance of several key parameters, such as cell density, flow speed and loading time. High cell density enables shorter time of trapping, which might be beneficial for the cells to experience less time of stress, yet could also cause clogging of cells or multiple cell trapping. After optimization, a density of 2.0×10^5 cells/mL was used to achieve the highest single cell trapping efficiency and prevent clogging of multiple cells. Higher speed of loading results in faster trapping, yet the viability of trapped cells could be severely damaged due to the shear stress. Lower loading speed results in higher trapping efficiency, yet the clogging and attachment of cells could cause extra cell trapping and

the difficulties during washing step. We have optimized the loading speed for the best balance of single cell trapping efficiency and cell viability. We used the flow speed of 0.8 µL/min after dropping the cells. At the same time, the loading period should be kept as short as possible within the requirement of trapping efficiency. In our case, the trapping duration was kept less than 5 mins.

The static culture after loading is essential for the attachment and spreading of cells for their healthy growth. It provided the condition for the cells to prepare the sufficient extracellular matrix (ECM) concentration around cell and helped the cells to anchor to the surface easily. We optimized the static culture time as no more than 4 hours and no less than 2.5 hours. After cells completely attached to the bottom of the chip, continuous perfusion culture was started.

We found that the migration distance of fibroblast can be controlled by different ECM coating density. In order for fibroblast cells to attach to the culture surface, the chip was incubated with culture media for certain time period to make the glass surface hydrophilic. Because fibroblasts have high ability to secrete their own ECM, this extent of surface treatment was found enough to support them anchoring the surface and spreading out. (For other cell lines, which has less ability to secrete their own ECM, long time coating with certain type of ECM was normally necessary). We have tried different period of media incubation from 10 mins to 4 hours. With 4 hours of coating, fibroblast cells easily anchored the surface even during loading process. Thus it was difficult to wash out the extra cells. With 10 mins of coating, fibroblast cells can be easily loaded and the extra cells can be washed out, yet it took more than 5 hours for them to become the healthy spreading state. The coating time also affects the fibroblast migration. Before loading the second type of cells, with 10mins of coating, fibroblasts migrated only about 10 µm away from the gap; with 30 mins of coating, they could migrate about 50 µm away from the gap, which gave enough space for the second cell trapping. Thus, we used 30 mins of media incubation to support fibroblast migration.

Culture Condition Optimization

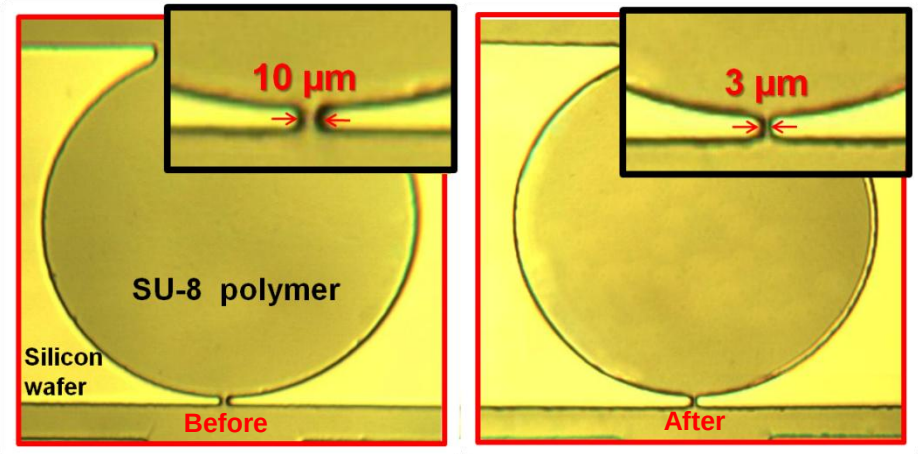
The perfusion culture flow rate was balanced between media refreshment, secretion pattern formation and shear stress. We have tested different flow rate for cell culture. It was shown that in the range of 0.1 µL/min to 1 µL/min, a range of cell types are able to grow with sufficient viability from upstream to downstream. Yet in order to ensure the best culture condition for more valid observation, we have calculated the media supply rate and shear stress upon cells in the upstream and downstream (Support Information Figure 2). Using simulation of the media distribution under a flow rate, we analyzed the media replacement of the 1^{st} chamber and the 42^{th} (the last) chamber after the same time of media supply (20 seconds).

Generally speaking, the media replacement depends on two factors, the flow rate and diffusion rate. If the flow rate is too low (e.g. 0.1 µL/min), dilution of new media from diffusion can diminish the media replacement speed; if the flow rate is high (e.g. 1 µL/min), there is not enough time for the new media to exchange in the chamber; at a certain flow rate (0.2 µL/min), diffusion and laminar flow balances to give the best media replacement efficiency. We also calculated the shear stress to cells in the culture chamber verses flow rate. The shear stress upon cells in this platform is generally small. At the flow speed of 0.2 µL/min at the withdrawing outlet *c* (0.025 µL/min in each of the 8 units), shear stress is calculated to be about 0.013 Dyne/cm^2, which are minimized as to not influence the cell viability. Therefore, we have chosen 0.2 µL/min as the culture flow speed.

With this optimized culture condition, we have tracked the growth of MEF and mESC single-cell pairs as well as single MEF (Support Information Fig. 3). For 3 days of observation, the co-cultured MEF and mESC showed consistent proliferation for up to 4^{th} generation. mESC was able to grow into big colonies with similar morphology as that of traditional dish culture. MEF, on the other hand, while proliferation rate being reduced in co-culture, kept its high mobility and viability. In the case of MEF single cell culture, it proliferated with a similar speed as reported in the literature. This result shows the ability of this device to provide reliable long-term single cell culture information.

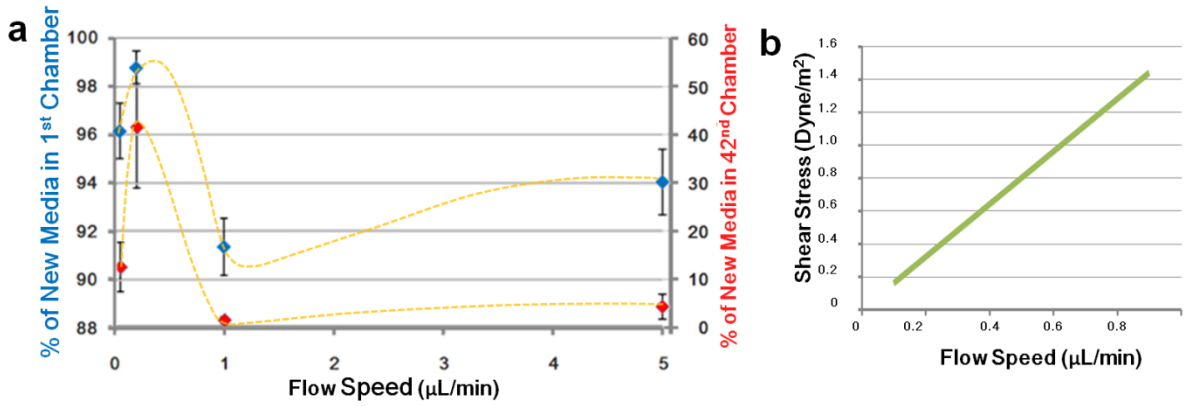
Supplementary Information Figure 1

Obtain Smaller Microstructure with Oxygen Plasma. A thin layer of SU-8 photoresist was spin-coated on silicon wafer. Then the wafer was put under UV light exposure with Mylar mask on top. SU-8 of the circle area, including the trapping junction polymerized. After developing, a 10um wide junction structure was made (left picture, “Before” oxygen plasma). The wafer was subjected to oxygen plasma to further etch the edge of the SU-8 structure, creating a 3 um wide junction as a result (right picture, “After” oxygen plasma).



Supplementary Information Figure 2

Calculation of the media refreshing efficiency of the first and last chamber along one wave-shaped channel (a). The blue data points are the percentage of new media supplied in the first chamber at different flow rate. The red data points are that of the last chamber. The flow speed on the X axis is the overall flow speed at the withdraw outlet, which supplies the media flow in all of the 8 units on the chip. For each unit (one of the 8 wave-shaped channels), the flow speed is one eighth of the overall speed. At optimized flow speed (0.2 µL/min), the media refreshing could achieve its maximum efficiency with a percentage of more than 96% new media even in the last chamber. (b) Shear stress upon cells cultured in the chamber at different flow speed of one unit. With the speed of 0.2 µL/min at the withdraw outlet, the flow speed of one unit is 0.025 µL/, shear stress is 0.013 Dyne/cm^2.



Supplementary Information Figure 3.

Long term culture of MEFs and mESCs for several generations. (a) The upper row of pictures show single-cell MEF and mESC coculture. Dotted circle in dark blue indicates MEFs and dotted circle in red indicates mESCs. During coculture for 3 days, mESC readily proliferated into colonies, and MEF maintained low proliferation rate. The lower row shows single-cell MEF been cultured alone. (b) Doubling time of mESC been cultured with MEF (blue bars) and MEF been cultured alone (red bars). The percentage of cells having a doubling time from 6 hours to 48 hours has been calculated from the collected well images. Most of mESCs have a doubling time of 20 hours and MEFs have a doubling time of 48 hours or longer.