Electronic Supplementary Information

Development of a multi-layer microfluidic array chip to culture and replate uniform-sized embryoid bodies without manual cell retrieval

Materials and Methods

Microfabrication of a multi-layer microfluidic array platform

A multi-layer microfluidic array platform containing concave microwells and flat cell culture chambers was generated by using a thin poly(dimethylsiloxane) (PDMS) membrane and negative pressure (Figure 1). A thin PDMS membrane (10 μm in thickness) was bonded onto the top of a base microchannel with oxygen plasma as we have previously described.[1-2] The base microchannels were created by negative pressure through an acrylic support hole aligned with the through-hole of the PDMS base microchannel using a vacuum pump (negative pressure of 40–50 kPa), resulting in deeper microwells (500 μm in width, 250 μm in depth). When the PDMS membrane was deflected as a round shape, it was coated with SU-8 photoresist solution, which was flattened by application of a 1-mm thick glass slide. The surface was illuminated with 365 nm UV light (6.8 mW cm^{-2} intensity) for 10 minutes to photocrosslink the SU-8 photoresist and the SU-8 layer was subsequently separated from the PDMS membrane. Similarly, shallow concave microwells (500 μm in width, 150 μm in depth) were fabricated by using negative pressure of 20–30 kPa. Flat cell culture chambers of hemi-round shape were fabricated by controlling the depth of the base microchannels, which had a depth smaller than the radius of a flat cell culture chamber (Figure 1b). Similarly, the membrane was sealed to the top of the base microchannel and air was removed through the acrylic support hole under negative pressure of 40–50 kPa. Finally, the concave microwells and flat cell culture chambers were fabricated by using two master molds. The inlets and outlets were punched into each layer for medium perfusion and cell docking.

Cell docking and computer simulation within concave microwells

The fluidic flow profiles in the microchannel were analyzed by computational fluid dynamics (COMSOL, MA). Regardless of channel depth, the flow pattern showed a curved line in concave microwells, whereas the flow pattern in the microfluidic channels was laminar. At slower medium velocity, the seeded cells tended to move to the bottom of the microwell due to gravity, followed by movement along the streamline on the bottom of the microwells. In concave microwells, the cells moved along the center streamline at the bottom. Cells could be uniformly seeded by controlling flow velocity. At fast flow speeds, the cells were not less affected by gravity and were removed from the microwells, whereas at slow flow rates, the cells easily moved to the bottom of the microwells. Cell docking was analyzed by perfusing culture medium containing 10^5 cells into asymmetric channels at flow rates ranging from 0.02 to 0.1 mL h^{-1}, with one inlet channel split into three hemi-round channels. The speed of fluid flow was controlled using a syringe pump and cell number was counted in nine microwells. Experimental results were compared with 2D computer simulations.

Cell seeding in a multi-layer microfluidic array platform

The microfluidic device consisted of two PDMS layers containing concave microwells and flat cell culture chambers. Each layer was used to assess cell docking and embryoid body (EB) differentiation. Cells, at a concentration of 5×10^6 cells mL^{-1}, were applied through a cell inlet port and perfused using a syringe pump at an average total flow rate of 0.05 mL h^{-1} for 3 minutes. The culture medium was subsequently infused using an osmotic pump, at a poly(ethylene glycol) (PEG) concentration of 0.1 M.

Embryonic stem (ES) cell culture

Murine ES cell-derived EBs were cultured for 4 days in concave microwells of a multi-layer microfluidic array platform under ES cell culture conditions as we have previously described[1] and were

replated into flat cell culture chambers treated with poly-L-lysine (PLL, 20 μg mL^{-1}) and laminin (5 μg mL^{-1}). To induce EB-derived neural progenitor cells and neuronal differentiation, the EBs replated on flat cell culture chambers were cultured with insulin/transferring/selenium/ fibronectin (ITSFn) medium using an osmotic pump for an additional 8 days.

***Immunocytochemistry*[1]**

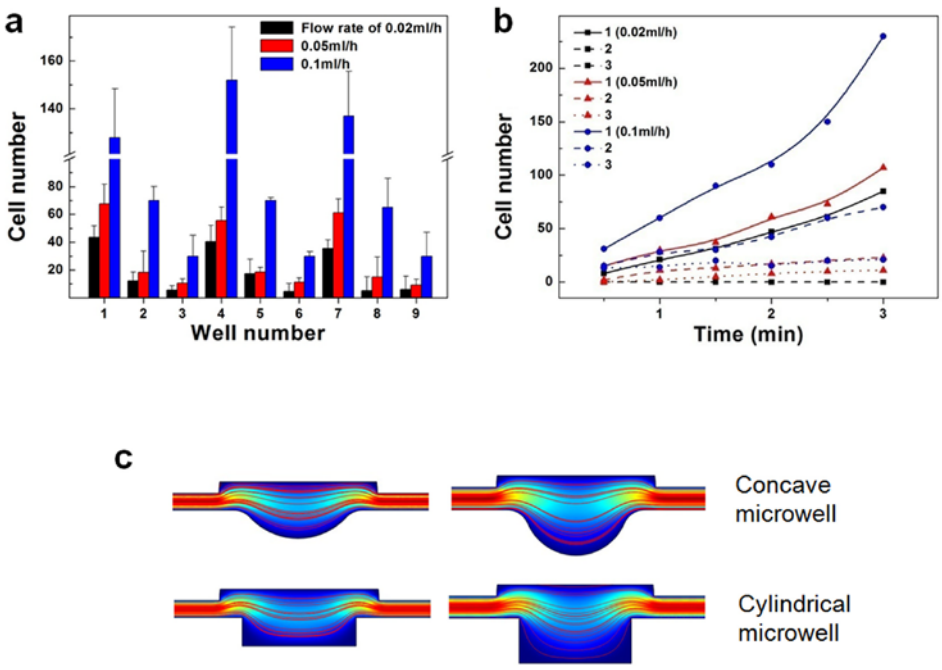
EB-derived neural progenitor cells and neurons cultured on flat cell culture chambers for 12 days were fixed for 20 min with 4% formaldehyde and were permeabilized with 0.1% Triton-X100 for 20 min. The cells were blocked with 3% bovine serum albumin, incubated overnight at 4ºC with primary antibody against neurofilaments (1:1000) and nestin (1:100), and subsequently incubated with secondary antibodies for 1.5 hours. Fluorescent images were obtained by using a fluorescence microscope (Axio observer. A1, Carl Zeiss, Germany).

References

1. Y. Y. Choi, B. G. Chung, D. H. Lee, A. Khademhosseini, J. H. Kim and S. H. Lee, *Biomaterials*, 2010, **31**, 4296-4303.
2. E. Kang, D. H. Lee, C. B. Kim, S. J. Yoo and S. H. Lee, *J Micromech Microeng*, 2010, Advanced Article

	Experimental conditions	*EB formation*	*EB differentiation*
1	**PLL + Laminin**	**- -**	**+ + + (EB adherence)**
2	**PLL + Laminin (r: 12h)**	**-**	**-**
3	**PLL + Laminin Plasma (r: 1h)**	**- - -**	**+ +**
4	**PLL + Laminin Plasma (r: 12h)**	**+**	**+**
5	**Plasma (r: 1h)**	**- - -**	**-**
6	**Plasma (r: 12h)**	**+**	**- -**
7	**PLL + Laminin Plasma (r: 24h)**	**+ + +**	
8	**Plasma (r: 24h)**	**+ + +**	
9	**Pure PDMS**	**+ + +**	**- -**
10	**Control**	**+ + (non uniformity)**	**+ + +**

Supplementary Table 1. PDMS surface conditions used to generate EBs and induce neural progenitor cell and neuronal differentiation (r: room temperature, plasma: oxygen plasma treatment, h: hour, PLL: poly-L-lysine, +: positive effect, -: negative effect).



Supplementary Figure 1. Cell docking behavior within cylindrical microwells (500 μm width, 250 μm depth). (a) Numbers of cells docked. Microwells near the inlet were designated 1, 4, and 7 and microwells near the outlet were designated 3, 6, and 9 (N=10). (b) Computer simulation-based analysis of numbers of cells. The microwell nearest the inlet was designated 1 and the microwell nearest the outlet was designated 3. (c) Simulation of stream patterns and velocity distribution within concave and cylindrical microwells.