Microfluidic Device for Stem Cell Differentiation and Localized Electroporation of Postmitotic Neurons

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Supporting Information
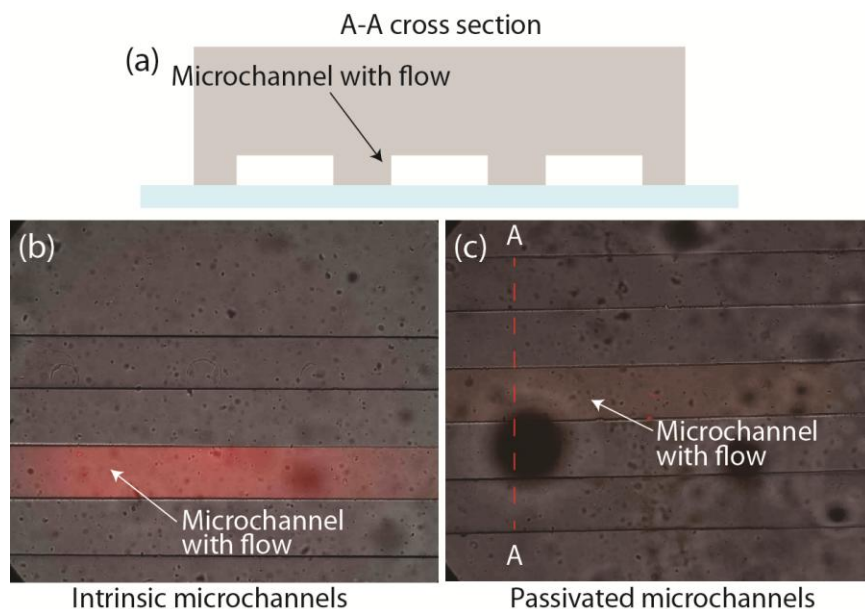
S1. Passivation of PDMS surfaces

Figure 1S Passivation of PDMS surfaces to minimize surface absorption of molecules.

To minimize absorption of molecules into PDMS, microchannels were incubated with Pluronic solution for 1 hour and thoroughly washed by circulating phosphate buffered saline solution

through microchannels. Solution with texas-red-dextran was fed through a microchannel at the center (Figure 1S) and then washed by PBS. Final bright field and fluorescent images were taken. Figure 1Sb shows merged image for intrinsic PDMS surface. Substantial fluorescence response is observed compared to the passivated microchannel in Figure 1Sc.

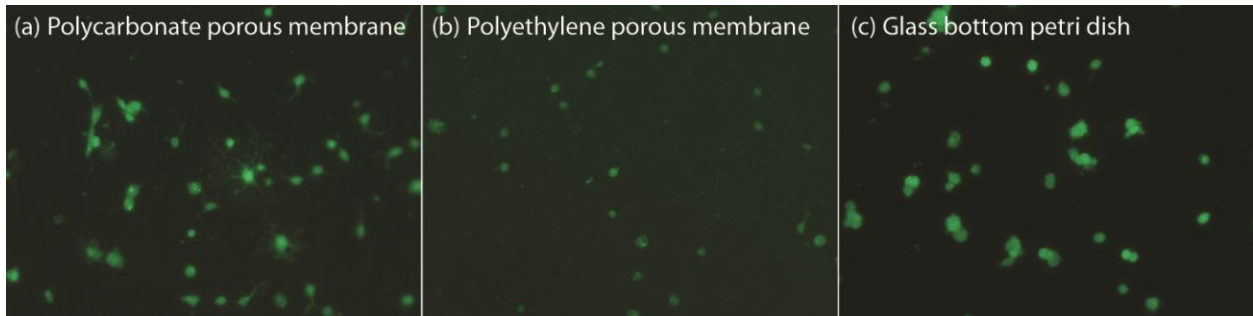## S2. Choice of materials for a porous substrate

Figure S2 Calcein AM test 24hours after cell plating on the LEPD: (a) polycarbonate substrate and (b) polyethylene substrate. The neural stem cells are plated on the same day following identical protocols, but the fluorescent images show that cells on the PC device developed axon-like structures unlike those on the PET device.

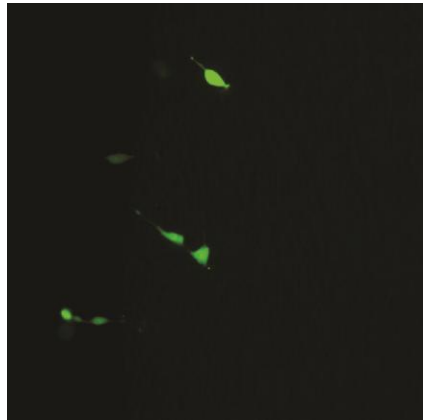## S3. Transfection of GFP plasmid into HeLa cells

Figure S3 A green fluorescent image due to GFP expression in HeLa cells. GFP plasmid at 1µg/µl concentration was delivered into HeLa cells using the LEPD with two square wave pulses of 1 sec with 1 sec interval.

S4. Size and density of substrate micro/nanochannels

Table S1 summarizes the size and density of microchannels used on various polycarbonate substrates. The fourth and fifth columns show the number of channels, which will be likely covered by each cell based on the density of channels and cell size. For neurons, we used substrates with D=0.6μm and =1.2μm while D=2μm was used for larger cells like HeLa and HT1080 cells.

Pore D (μm)	Substrate thickness (L)	Density /cm2	Neurosphere (D=5μm)	HeLa/HT1080 (D=30μm)
0.2	25	5.00E+08	98.125	3532.5
0.6	24	4.00E+07	**7.85**	282.6
1.2	24	2.20E+07	**4.3175**	155.43
2	23	3.00E+06	0.58875	**21.195**

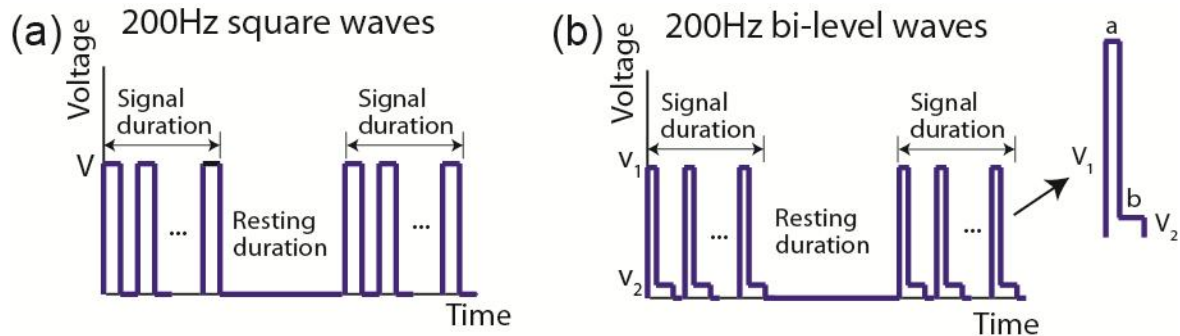## S5. Input signals for electroporation

Figure S4 Input signals for electroporation: (a) square waves and (b) bi-level waves. V in (a) is the input voltage amplitude. V_1 and V_2 are input voltage amplitudes and a and b are corresponding time periods.

For transfection of neuron, the 200Hz bi-level waves were used with V_1 = 80-100 V, V_2=10V, a=0.25ms, b=3ms, signal duration (train duration) of 0.25sec, resting time of 0.5sec, and 8 pulses.
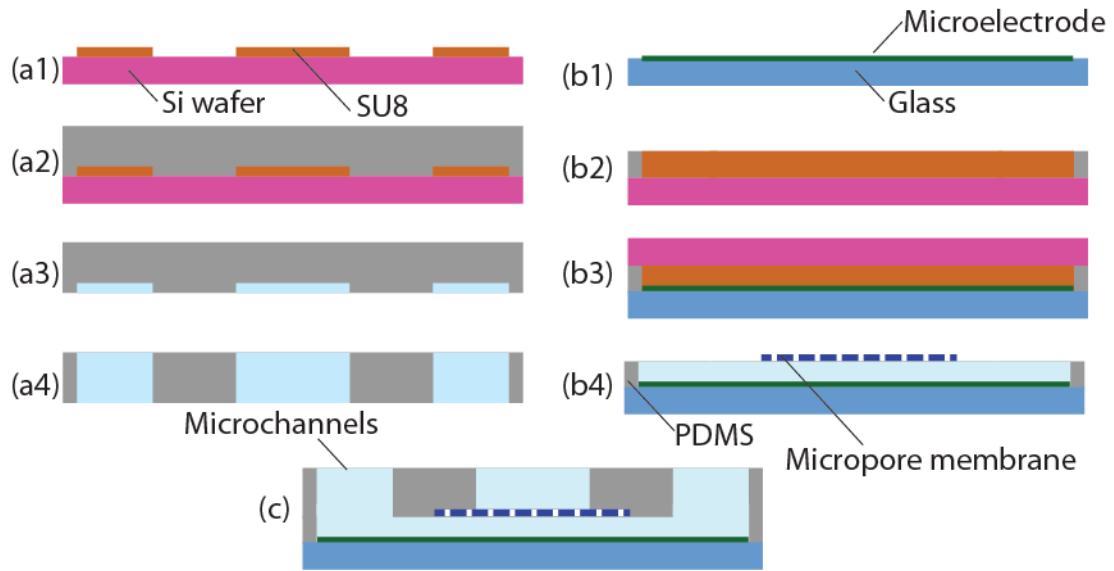
S6. Microdevice fabrication and assembly

Figure S5 Microfabrication of the localized electroporation device. (a1)-(a4) show a procedure to fabricate a top layer with a cell chamber, inlets, and outlets to and from microchannels. (b1)-(b4) show the fabrication steps used to make microchannels. The top and bottom layers are assembled with a perforated substrate, which separates the cell culture chamber from the bottom microchannel as shown in (c). In order to increase adhesion between PDMS and glass in b3, the dimensions of microelectrodes matches with that of microchannels in Figure 1b.