Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Supplementary Fig. 1: Schematic of the fabrication of the vertically-layered configuration of the co-culture microfluidic device. (a) The first PDMS layer was fabricated using a SU-8 photoresist mold and was plasma bonded to a glass coverslip. Layer I contained two cell chambers separated by a valve barrier with microchannels. Holes punched in the PDMS layer corresponding to media input and waste wells were connected to the cell culture chambers via channels. Semilunar-shaped PDMS supporters within the cell chambers provided support to the roofs of the chambers. The dimensions for the co-culture microfluidic device can be found in the Material and methods. (b) A second PDMS layer, which formed the pressure chamber, was plasma bonded to layer I. Two small holes were bored into the pressure chamber for attaching tubing that was used to inject air or water into the chamber. (c) Liquid PDMS was used as glue to attach tubing to the pressure chamber as well as four cloning cylinders to layer I to create media reservoirs. Please note the schematic is for visualization of the fabrication process and is not drawn to scale. (d) A schematic of the assembled device is shown (left panel). A photograph of the actual device, which was placed in a 100 mm tissue culture dish, is shown (right panel).

Supplementary Fig. 2: Schematic of the fabrication of the four chamber co-culture microfluidic platform. (a) The first PDMS layer, which was plasma bonded to a glass coverslip, consisted of four cell culture chambers that were separated by three valve barriers. Eight holes were punched in the PDMS layer for media input and waste wells. (b) A second PDMS layer, forming the pressure chamber, was then plasma bonded to the first PDMS layer. (c) Tubing and eight cloning cylinders, which formed the media reservoirs, were glued to the device with liquid PDMS.

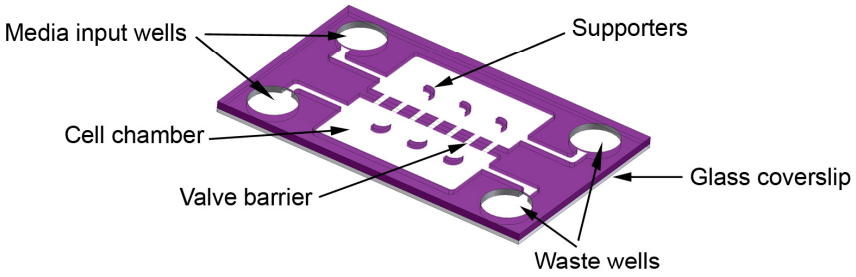
Please note the schematic is for visualization of the fabrication process and is not drawn to scale. (d) A schematic of the assembled microfluidic platform is shown (left panel). A photograph of the actual microfluidic device in a 100 mm tissue culture dish is shown (right panel).

Supplementary Fig. 3: Flow in four chamber microfluidic platforms. (a) The cell chambers were separated by activating the three valve barriers (closed conformation). The glial chambers were filled with either Alexa Fluor 488 conjugated avidin (green) or Alexa Fluor 647 conjugated ovalbumin (red). The fluorescent proteins were not observed to flow into the adjacent neuronal chambers, demonstrating that the valve barriers effectively isolated the cell chambers. (b) When the valve barriers were released (open conformation), the fluorescent proteins flowed into the adjacent chambers, indicating fluid exchange between the adjacent chambers.

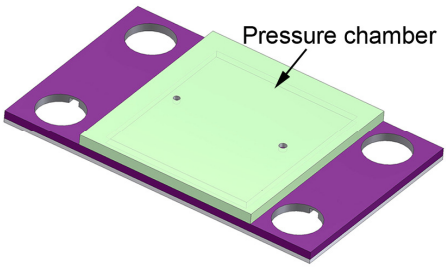
Supplementary Fig. 4: Co-culture of glia with neurons increases the density of dendritic protrusions. (a) Neurons were transfected with GFP at day 5 in culture and imaged at day 8 in culture. Dendritic protrusions were observed along the dendrites. (b) Quantification of the number of dendritic protrusions is shown for neurons co-cultured with glia in vertically-layered and four chamber microfluidic devices. Neurons incubated with externally conditioned glia media in two chamber microfluidic devices are also shown for comparison. Error bars represent S.E.M. for 40-45 dendrites from three independent experiments (* $p < 0.0001$).

Shi et al., Supplementary Figure 1

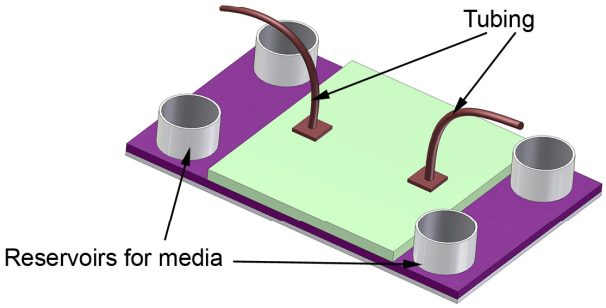
a **PDMS Layer I: Cell culture chambers and valve barriers**



b **PDMS Layer II: Pressure chamber**



c **Reservoir cylinders and tubing attached to the device**



d **Final assembled device**

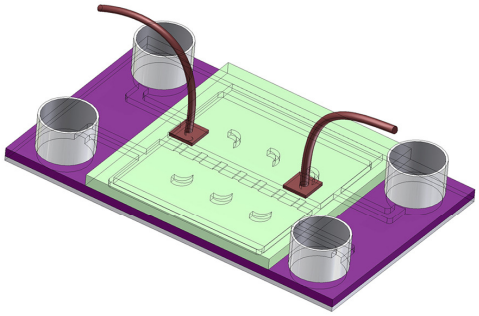
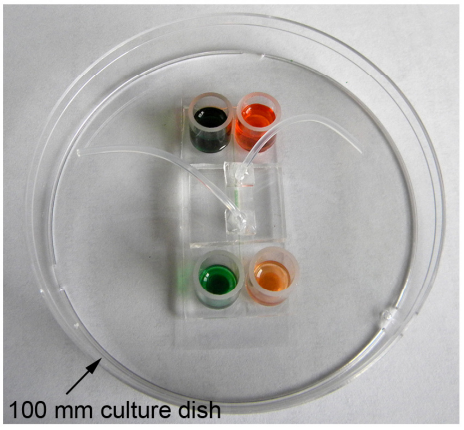
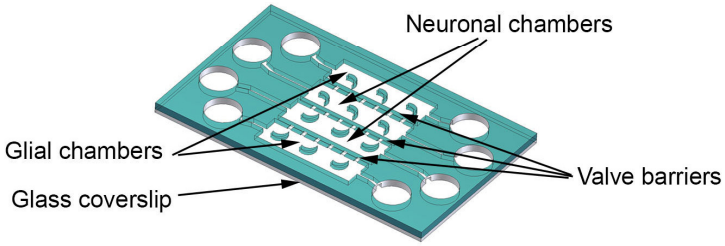


Image of the vertically-layered device

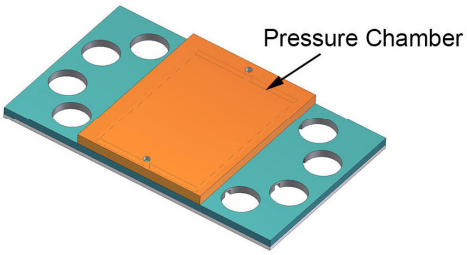


Shi et al., Supplementary Figure 2

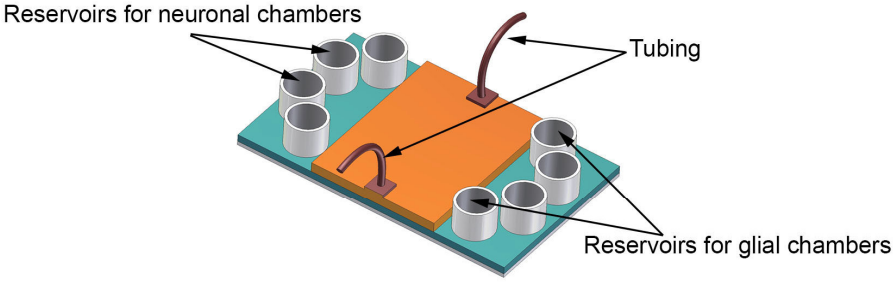
a **PDMS Layer I: Cell culture chambers and valve barriers**



b **PDMS Layer II: Pressure chamber**



c **Reservoir cylinders and tubing attached to the device**



d **Final assembled device**

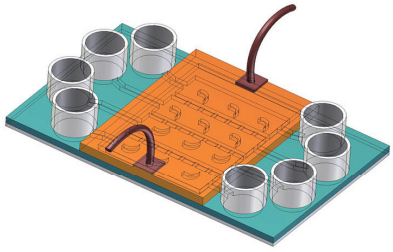
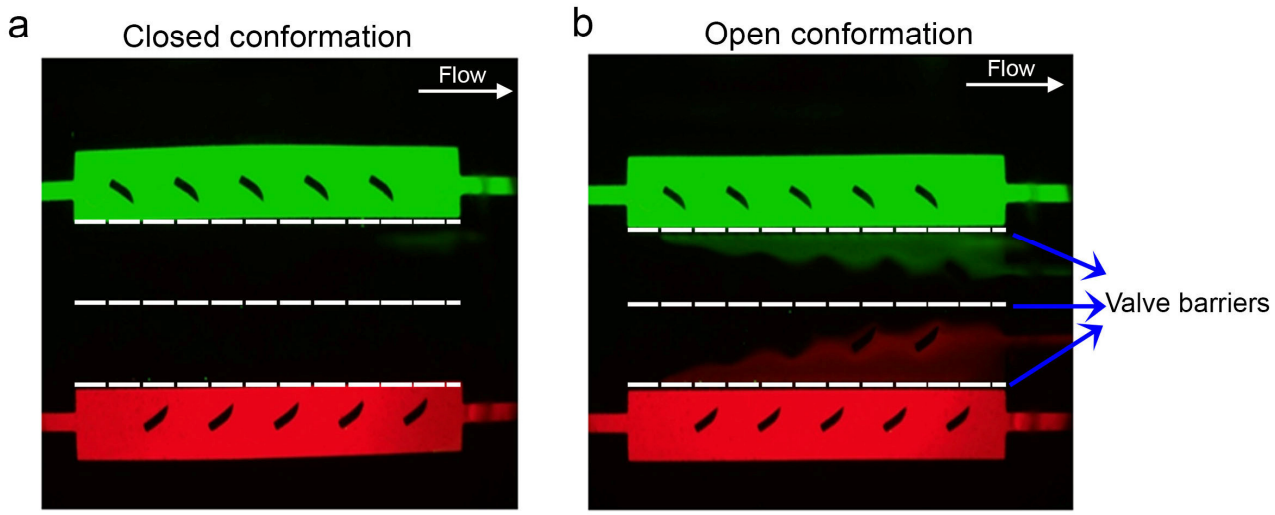


Image of the four chamber device

Shi et al., Supplementary Figure 3



Shi et al., Supplementary Figure 4