Supplementary Information

Biocompatible multi-address 3D cell assembly in microfluidic devices using spatially programmable gel formation

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1. Fabrication procedure of the microfluidic device with sidewall electrodes

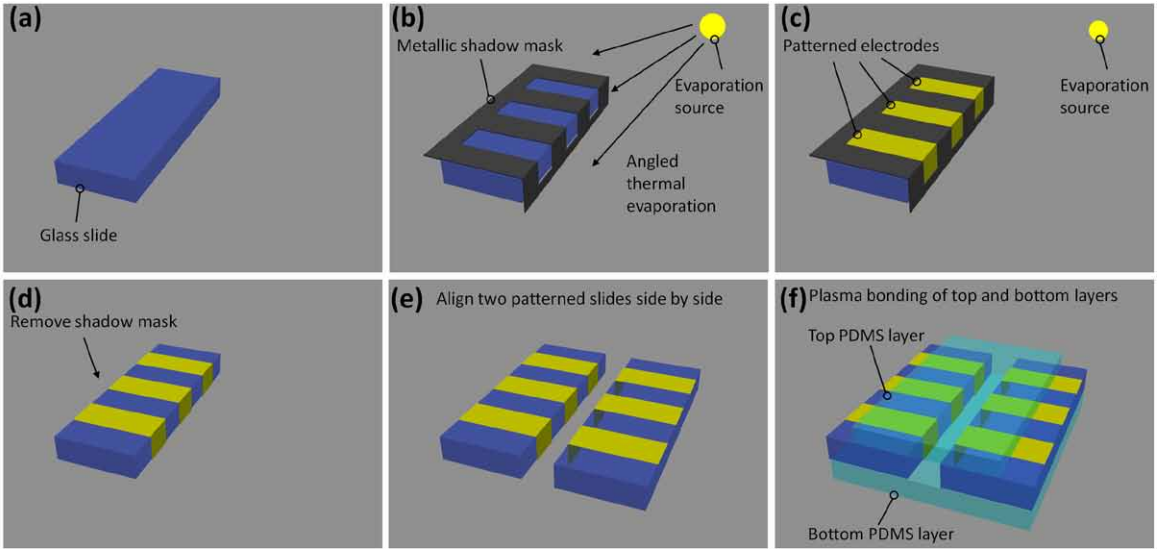

Figure S1. Schematic diagrams illustrating the fabrication procedure of transparent microfluidic device with built-in sidewall electrodes. (a) A glass slide was cleaned with piranha solution and DI water. (b) A bent metallic shadow mask with parallel slits pattern was placed on the glass slide. (c) Multiple parallel electrodes were defined by angled thermal evaporation of Cr and Au. (d) Patterned electrodes on the top and the side of the slide after removal of the shadow mask. (e) Two patterned slides were aligned side by side with a separation of 1 mm. (f) Two pieces of thin PDMS were permanently bonded to the glass slides via oxygen plasma bonding to form the ceiling and the floor of the channel.

The fabrication procedure of the microfluidic device with sidewall electrodes can be described as follows: First, glass slides were soaked in piranha solution (H_2SO_4: H_2O_2 = 3:1) for 10 min followed by thorough rinsing with DI water (Fig. S1a). Second, a bent (90 degree) metallic shadow mask with a parallel slit (width: 1 mm) pattern was place

onto the glass slide (Fig. S1b). Multiple parallel electrodes were defined by angled thermal evaporation of chromium (Cr) (20 nm) and gold (Au) (100 nm) onto the top and the side of the glass slide (Fig. S1c). Both the electrode width and the separation between the electrodes are 1 mm (Fig. S1d). Two glass slides with such patterned electrodes were placed side by side with a separation of ~1 mm (Fig. S1e). They were permanently bonded to two thin layers of cured PDMS (Sylgard 184 Silicone Elastomer Kit, Dow Corning) with oxygen plasma treatment (pressure: 450 mT, forward power: 20W, Oxygen flow rate: 20 sccm O_2, plasma treatment time: 30 seconds) to form the ceiling and the bottom of the channel (Fig. S1f). The channel height is 1 mm. The active sidewall electrode areas in the fluidic channel are 1 mm × 1mm. The microscope objective was located right above the device and was focused on the anode surface in the channel. The optical micrographs were obtained with transmitted light coming from the bottom through the transparent PDMS layers. PTFE tubing was then inserted into the channel to define the inlet and outlet of the channel. The connections between tubing and channel were sealed with PDMS gel, cured instantly at 150 ºC. The flow of solution was controlled by a syringe mounted on a syringe pump. All the electrodepositions were performed using a DC power supply (Keithley 2400 sourcemeter) at controlled constant current densities. In our setup, the deposition solution was in direct contact with the anode and cathode which are connected with the "+" and "–" terminals of a sourcemeter.

2. Fabrication procedure of 2D chip with 5×5 array of electrodes

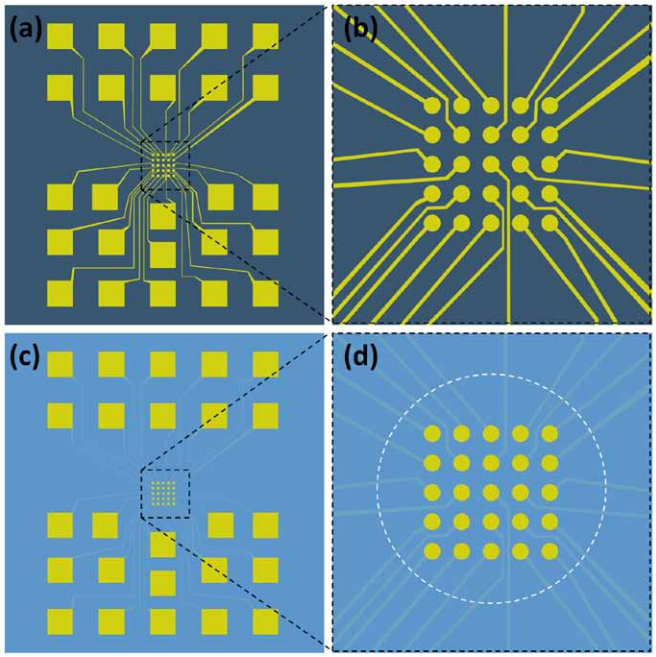

Figure S2. Schematic diagrams illustrating the fabrication procedure of the 2D chip with a 5×5 array of electrodes. (a) Top view of the chip after circular electrode, contact leads, and contact pads are defined with thermal evaporation of metal (Cr and Au), photolithography, and selective etching of metal. (b) A magnified top view of the circular electrode array at the center of the chip. (c) Top view of the chip after passivation of contact leads with photolithography, e-beam evaporation of SiO_2, and selective liftoff off of SiO_2. (d) A magnified top view of the circular electrode array at the center of the chip with passivated contact leads. The white dashed circle indicates the region where deposition solution is in contact with the chip.

The fabrication procedure of the 5×5 circular electrode array can be can be described as follows: First, a 20 nm thick adhesion layer of chromium and a 100 nm thick layer of gold were thermally evaporated onto a glass slide. Photolithography and subsequent etching of Cr and Au were performed to define the 5×5 circular electrode array connected to square contact pads through thin contact leads. The diameter of the circular electrode is 230 μm and the contact pad is 2 mm×2 mm. Figs. S2 a and b show the schematic top view of the chip and the magnified top view of the patterned array of electrodes with contact leads. Second, additional photolithography, e-beam evaporation of 100 nm of SiO_2, and liftoff were performed to passivate the contact leads and leave the circular electrodes and square contact pads exposed. Figs. S2 c and d show the schematic top

view of the passivated chip and the magnified top view of the patterned array of electrodes with passivated contact leads. The white dashed circle indicates the edge of the reservoir in which the deposition solution is housed. A platinum wire immersed in the deposition solution was used as the cathode. Each contact pad corresponds exclusively to one circular electrode. During the electrodeposition, since the contact leads are passivated, the calcium alginate hydrogel can only be deposited on the bare circular electrode area in response to an anodic electrical signal applied on the contact pads. In other words, selective cell immobilization with calcium alginate can be electroaddressed in this array design.