Epilepsy-on-a-chip System for Antiepileptic Drug Discovery

Supplementary Information

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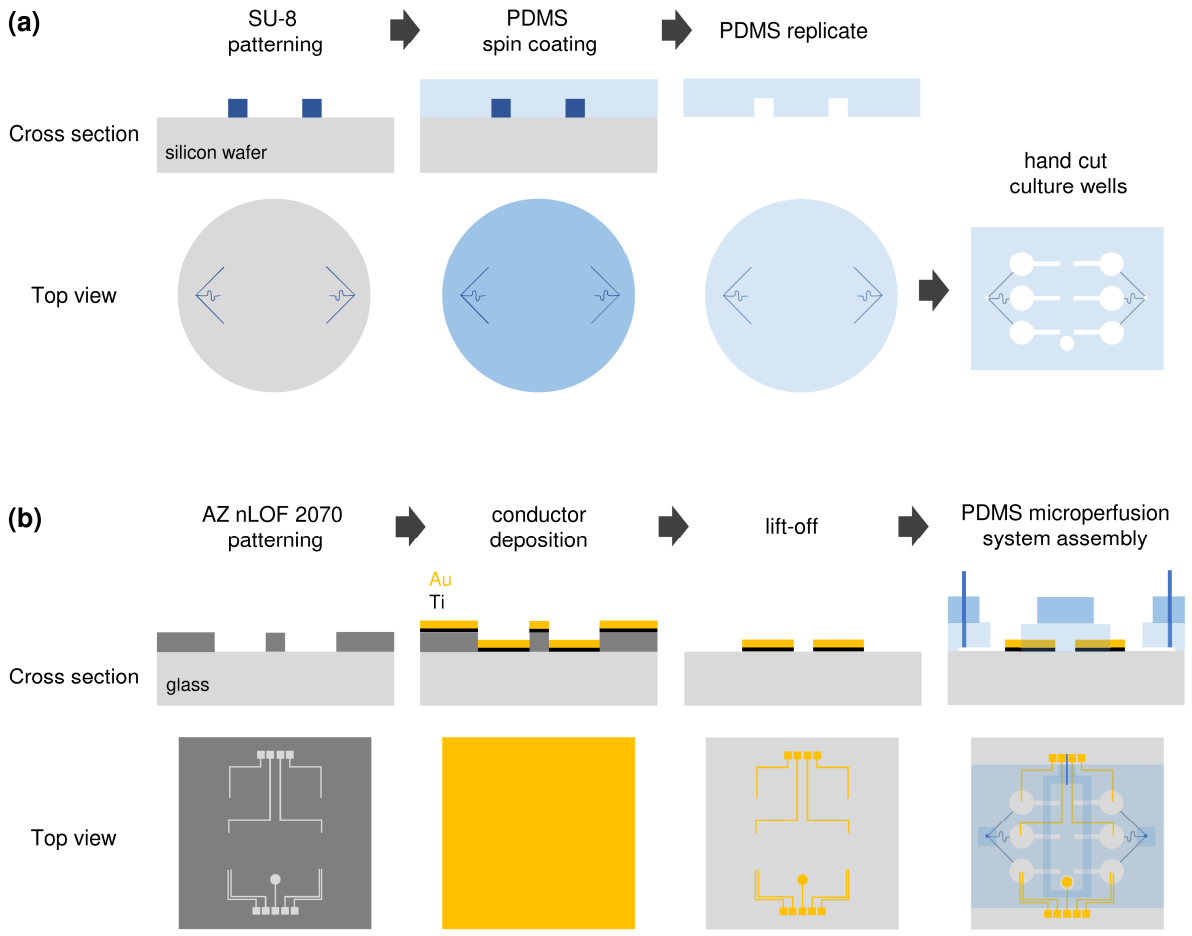

Fig. S1. MEA fabrication and assembly of the microfluidic perfusion compartments. (a) Steps of microfluidic film fabrication: microchannel pattern (20 µm height, 50 µm width) was defined via SU-8 (MicroChem) photolithography on a silicon wafer. PDMS film (100 µm thickness) with imprinted microchannels was replicated from the SU-8 mold. Culture wells (6 mm diameter) and the open channels were cut in the PDMS membrane. (b) Steps of MEA chip fabrication: The electrodes pattern was defined photolithographically with negative lift-off process using AZ nLOF 2070 (MicroChem). Titanium (50 nm) and gold (200 nm) were deposited with e-beam evaporator to make the electrodes. At last, PDMS compartments were attached to the MEA chip through oxygen plasma bonding.

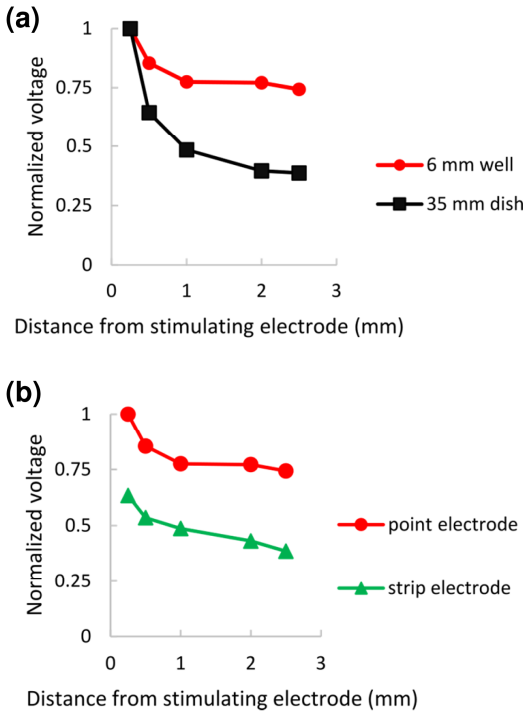

Fig. S2. Dependence of recorded potential on chamber and electrode geometry. (a) Normalized voltage recorded by tungsten microelectrodes (51 µm diameter uninsulated region) in a 6 mm diameter µflow-MEA well and a 35 mm dish, plotted vs. distance from the stimulating electrode. (b) Normalized voltage recorded in a 6 mm diameter µflow-MEA well by a tungsten microelectrode (point electrode) and a µflow-MEA strip electrode, plotted vs. distance from the stimulating electrode.

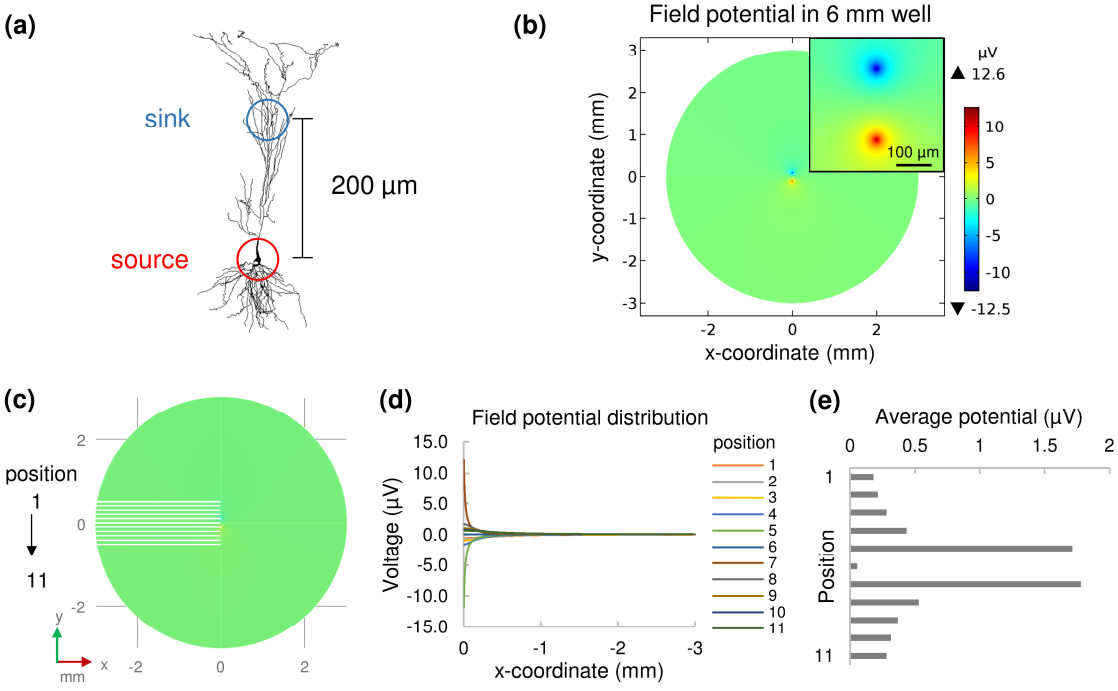

Fig. S3. Field potential of multiunit activity detected by µflow-MEA strip electrodes. (a) Schematic of current dipole model of multiunit activity. (b) Simulated field potential in mini well. (c) Electrodes placed in vicinity of current dipole (position 1 to 11 refers to y = 0.5 mm to y = -0.5 mm, with 0.1 mm interval). (d) Field potential distribution along each electrode. (e) Average potential that is detected by each electrode.

TABLE SI
INHIBITOR-CONCENTRATION PAIRS AND STAGE I SCREEN RESULTS

Inhibitor name	Concentration	$p_{lactate}$	p_{LDH}
VEGF RTK Inhibitor II	5 μM	**0.005**	**0.013**
PDGF RTK Inhibitor III	2 μM	**0.007**	**0.001**
AG 1295 (PDGFR inhibitor)	5 μM	0.084	**0.020**
GTP-14564 (c-fms, c-kit, Flt3 inhibitor)	5 μM	**<0.001**	0.078
EGFR/ErbB-2 Inhibitor	2 μM	**<0.001**	0.309
Flt-3 Inhibitor III	1 μM	**0.006**	0.772
EGFR/ErbB-2/ErbB-4 Inhibitor	2 μM	0.271	0.350
Met Kinase Inhibitor	0.5 μM	0.051	0.400
Flt-3 Inhibitor	2 μM	0.271	0.289
VEGFR2 Kinase Inhibitor II	0.5 μM	0.619	**0.003**
cFMS RTK Inhibitor	2 μM	0.271	**0.037**
VEGF RTK Inhibitor IV	0.3 μM	**0.036**	**<0.001**

p value were calculated relative to vehicle-treated controls from the same pup. Positive hits were highlighted in bold.

TABLE SII
STAGE II SCREEN RESULTS

Inhibitor name	normalized cumulative time seizing	normalized cumulative number of seizures	$p_{time\ seizing/day}$	$p_{number\ of\ seizure/day}$	$n_{cutures}$
cFMS RTK Inhibitor	0.31	0.39	**0.0024**	**0.0050**	5
Aurora Kinase Inhibitor II	0.69	0.75	0.6049	0.5460	6
EGFR/ErbB-2 Inhibitor	0.42	0.46	**0.0090**	**0.0106**	6
Flt-3 Inhibitor III	1.18	1.46	0.5933	0.8220	3
EGFR/ErbB-2/ErbB-4 Inhibitor	1.10	1.17	0.9993	0.8514	3
Met Kinase Inhibitor	1.14	1.02	0.8514	0.9713	3
Flt-3 Inhibitor	1.49	1.28	0.4603	0.6585	3
VEGFR2 Kinase Inhibitor II	1.01	1.05	0.1800	0.4603	3
VEGF RTK Inhibitor IV	0.37	0.44	0.2975	0.2975	3
PDGF RTK Inhibitor III	0.80	1.16	0.4373	0.6101	3
GTP-14564 (c-fms, c-kit, Flt3 inhibitor)	0.91	0.67	0.9699	0.5933	3
VEGF RTK Inhibitor II	0.71	0.97	0.5933	0.9699	3
AG 1295 (PDGFR inhibitor)	1.28	1.19	0.1069	0.0698	3

p value were calculated relative to vehicle-treated controls from the same pup. Positive hits were highlighted in bold.