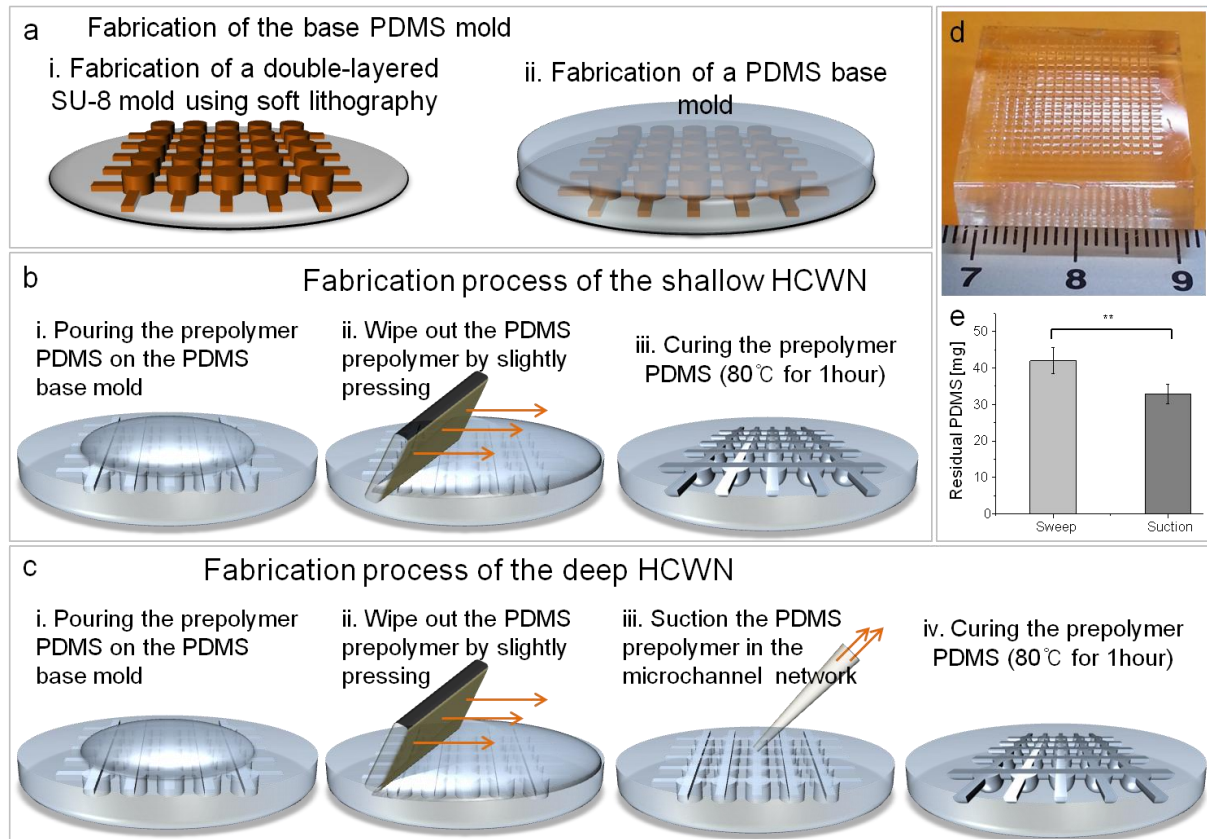Supplementary Figures

Fig. S1. Fabrication process and dimensions of shallow and deep HCWN plates. (a) Fabrication of the PDMS base mold. For base channel fabrication, PDMS prepolymer is poured onto a two-layered SU-8 mold and then cured (i-ii). (b) Process for fabricating shallow HCWN plates. (i) After detaching the PDMS base mold, the PDMS prepolymer is poured onto the channel. (ii) After degassing, the PDMS prepolymer is swept out by gently pressing the soft PDMS base mold using a glass slide (76 x 52 x 1.2 mm). Surface tension creates PDMS meniscuses corresponding to nascent cylindrical microwells and shallow hemicylindrical channels. (iii) The PDMS prepolymer is cured at 80°C for 1 hour. (c) Process for fabricating deep HCWN plates. After performing steps (i) and (ii) as for the shallow system, the residual PDMS prepolymer was removed by suctioning (iii), resulting in the formation of deep hemicylindrical channels with concave microwells through surface tension. (iv) After formation of deep HCWN system features, the PDMS plate was cured. (d) Picture of the deep HCWN (e) The residual PDMS prepolymer after sweeping (b) and suction (c). Statistical analysis was performed using student's t-test. Data shown are mean values (%)±standard error with N=6 (individual connection rate measurements from four regions) (**$P < 0.001$).
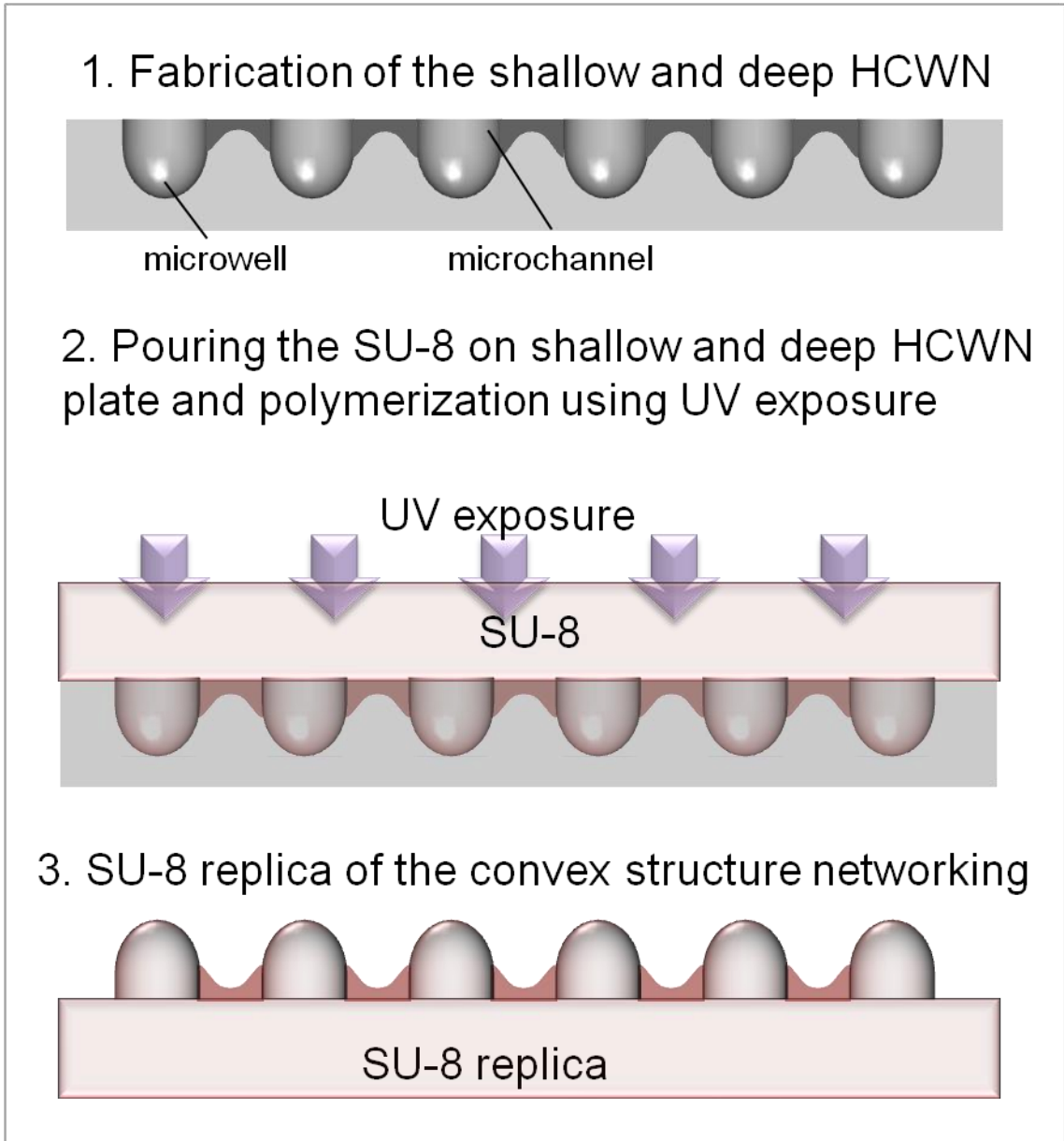
Fig. S2. Schematic diagram of the SU-8 replica fabrication process for shallow and deep HCWN plates. The following changes to figure text are suggested: 1. Fabrication of shallow and deep HCWN plates. 2. Pouring SU-8 n shall and deep HCWN plates3. Su-8 replica of the convex structure network.
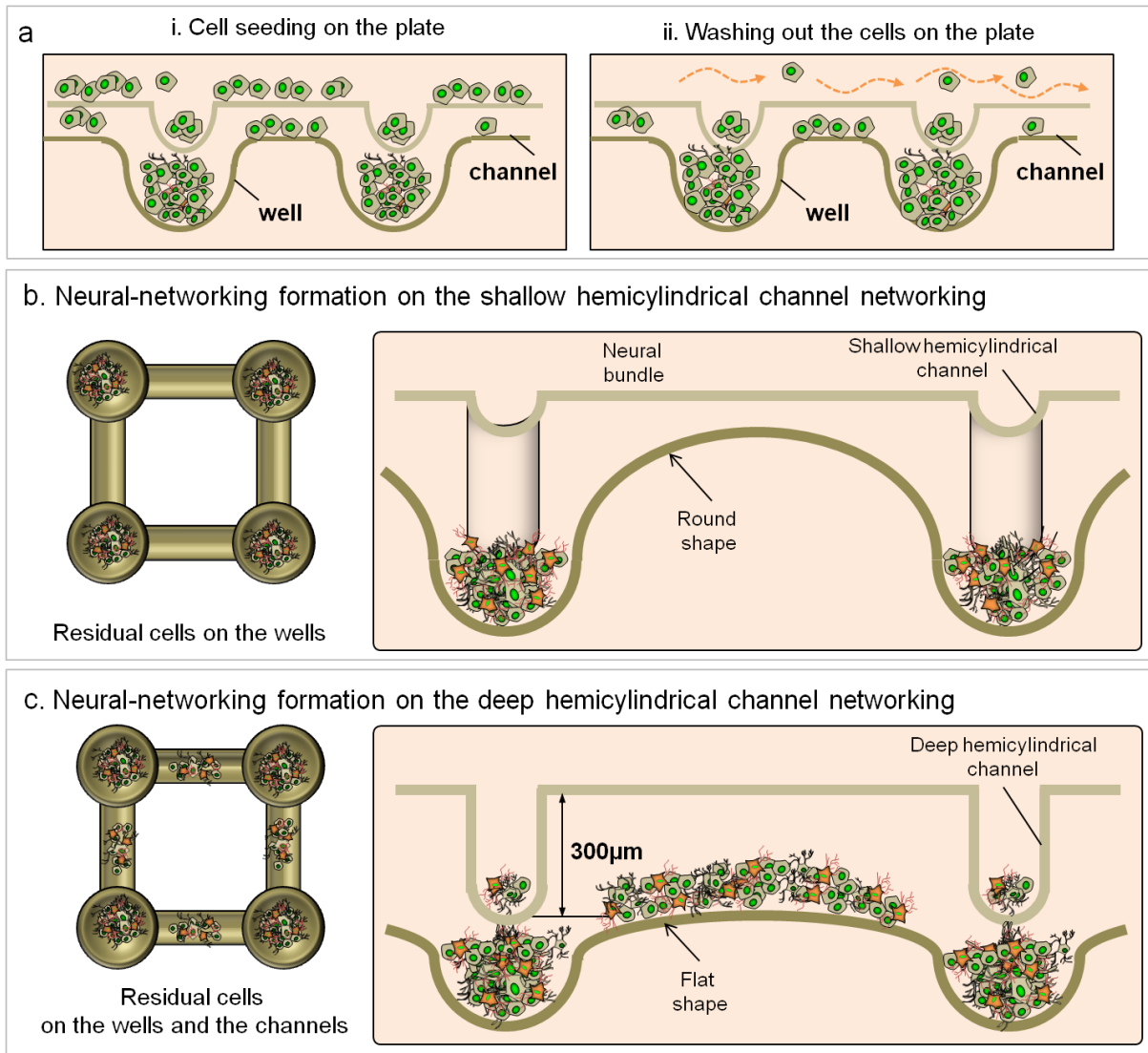
a

i. Cell seeding on the plate

ii. Washing out the cells on the plate

channel

well

channel

well

b. Neural-networking formation on the shallow hemicylindrical channel networking

Residual cells on the wells

Neural bundle

Shallow hemicylindrical channel

Round shape

c. Neural-networking formation on the deep hemicylindrical channel networking

Residual cells on the wells and the channels

Deep hemicylindrical channel

300μm

Flat shape

Fig. S3. Cell seeding on shallow and deep HCWN plates. (a) A 1-ml cell suspension (2.0×10^9 cells/ml) was directly seeded onto shallow and deep HCWN plates with repeated gentle pipetting. Ten minutes after seeding, neural cells had settled within concave microwells and hemicylindrical channel networks (i); culture medium was then gently applied to remove cells that did not settle (ii). (b, c) Settled cells after the wash-out process in the shallow HCWN system (b) and deep HCWN system (c).

a Single neurite connection

b Multi & bundle neurite connection

Connection rate (%)

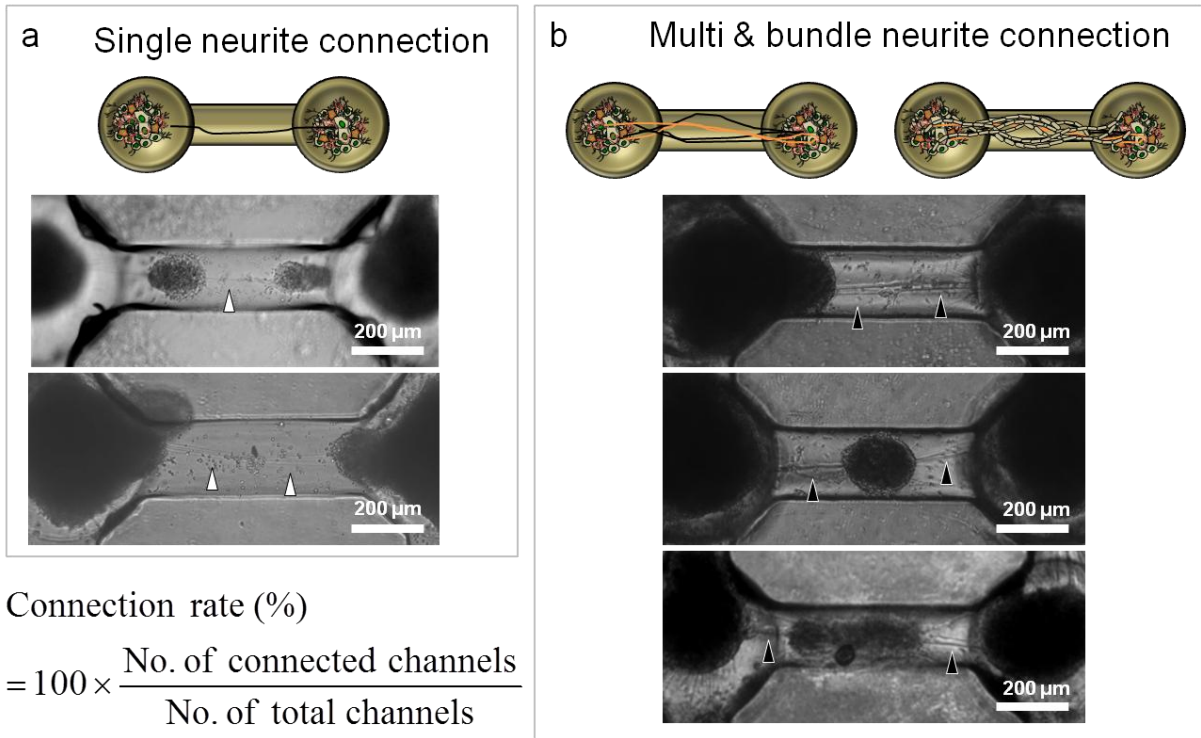$$= 100 \times \frac{\text{No. of connected channels}}{\text{No. of total channels}}$$

Fig. S4. Definition of neurite connection. (a) Single neurite connection through the channel. (b) Multi-connection and bundled neurite networks through the channel.
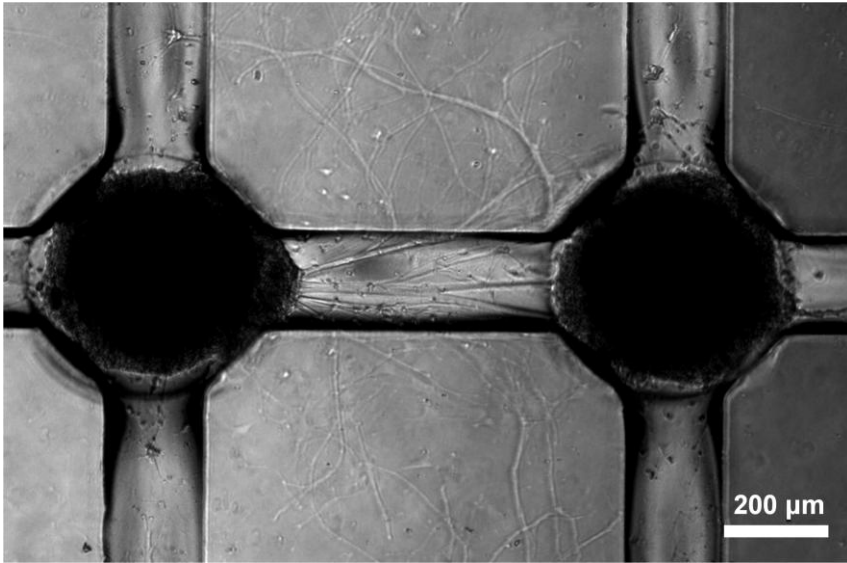
Fig. S5. Neurites growing outside the channel in the shallow HCWN system.