Supplementary data

Hydrogel Skin-covered Neurons Self-assembled with Gustatory Cells for Selective Taste Stimulation

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Figure S1. Schedule summary of experimental procedure.
Figure S2. (A) Photograph of the agarose gel layer, (B) Location of investigated area, (C) Thickness measurement of agarose layer, and (D) average thickness of triplet measurements of agarose gel layer in Neuron-2 and Neuron-3.
Figure S3. Diffusion test of denatonium benzoate through agarose gel using Franz cell. (A) A Franz cell with 0.5 mL of denatonium benzoate 1 mM in HBSS in the donor chamber and 5 mL of HBSS in the receptor chamber, separated by 5-μm-thick and 1-cm²-area agarose gel on a Whatman filter paper substrate. (B, C, and D) UV-absorption spectra detected at 210 nm by HPLC of the diffused denatonium in receptor chamber after 1-8 minutes with agarose and after 1 min without agarose. (E) The weights of diffused denatonium after 1-8 min and calculated diffusion rate. (F) Percentage of diffused denatonium compared to the original adding amount.
Figure S4. (A) Possible routes of calcium signal transmission in the gustatory-neuronal co-culture without and with the agarose gel layer. (B) Map of receiving and giving signals for each cell type in the gustatory-neuronal co-culture without the agarose gel layer. The red crosses indicate the routes that can be prevented by the agarose gel layer.
Figure S5. Optical microscopic images of gustatory-neuronal co-culture without (A) and with (B) the agarose gel layer. Notice the optical reflection of light due to the agarose gel layer in (B). Scale bar for (A) and (B) is 25 µm. SEM images of agarose morphology (C) and SEM image of co-culture sample with agarose layer on top (D). The dotted circles in (D) indicate neuronal cells under the agarose gel layer.
Figure S6. Calcium imaging of gustatory only, treated with 1 mM denatonium benzoate. First frames of 5 min recording with the location of selected cells marked with numbers (A), fluorescence intensity spectra (B), and captured images (C) of the selected cells.
Figure S7. Data analysis from calcium signal spectra of gustatory only. Number of signal peak per cells (A), starting time-point of first signal of a cell (B), duration of signaling of a cell (C), average value of full width at half maximum of signal peaks of a cell (D), periodicity of signaling (E), and signal peak amplitude of a cell (F).
Figure S8. Data analysis from calcium signal spectra of gustatory when culture without neuron (G only), with neuron (G in NG), and with neuron and agarose (G in NGA). Number of signal peak per cells (A), starting time-point of first signal of a cell (B), duration of signaling of a cell (C), average value of full width at half maximum of signal peaks of a cell (D), periodicity of signaling (E), and signal peak amplitude of a cell (F).