Supplementary Information

for

A multiscale, vertical-flow perfusion system with integrated

porous microchambers for upgrading multicellular spheroid

culture

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Movie S1: Real-time movie showing the flow behaviors of 1.1 μ m particles through the porous microchambers at different locations in the device. Particle behaviors at the chamber bottom for 5 s are shown. The input flow rate of the particle suspension was 10 μ L/min, which was set to clearly visualize the particle movements.



Figure S1. Scanning electron microscopic (SEM) images of the surface of the porous microchambers when the centrifugation time was varied from 1, 15, and 60 min.



Figure S2. Count of surface pores from the SEM images of randomly selected 10 porous microchambers. Each small yellow dot indicates the count number of the surface pore.



Figure S3. Scanning electron microscopic (SEM) images of the NaCl particles ($\phi = 30-60 \mu m$) used as a porogen to prepare the porous microchambers.



Figure S4. Visualization of flow profiles using 1.1 μ m particles in the non-porous chamber with the horizontal flow. (a) Design of the microchannel and the depth (z) position of observation. (b) Particle behavior at different z positions. The movements of single particles in an interval of 5 s were analyzed and averaged. Vectors of movements of representative 10 particles are shown as white arrows. (c) Schematic image showing the flow profile in the cross-section of the chamber. We confirmed that the stagnant region was formed in the area from the ~1/3 location from the surface to the bottom of the chamber.



Figure S5. HE staining of the thin sections of the spheroids for the horizontal flow and vertical flow conditions.