## Supplementary Information

for

## A multiscale, vertical-flow perfusion system with integrated

## porous microchambers for upgrading multicellular spheroid

culture

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## **List of Supplementary Information**

**Figures S1**: Scanning electron microscopic observation of the surface of the porous microchambers at centrifugation times of 1, 15, and 60 minutes.

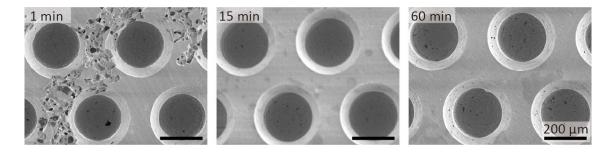
**Figures S2**: Count of surface pores from the SEM images of randomly selected 10 porous microchambers.

Figures S3: Scanning electron microscopic observation of the NaCl particles utilized.

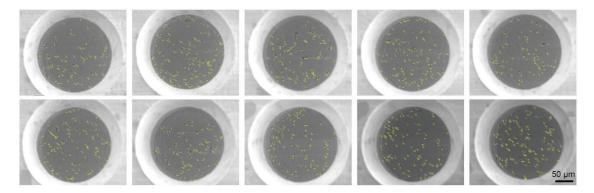
Figures S4: Visualization of flow profiles using 1.1  $\mu$ m particles in the non-porous chamber with the horizontal flow.

**Figures S5**: HE staining of the thin sections of the spheroids for the horizontal flow and vertical flow conditions.

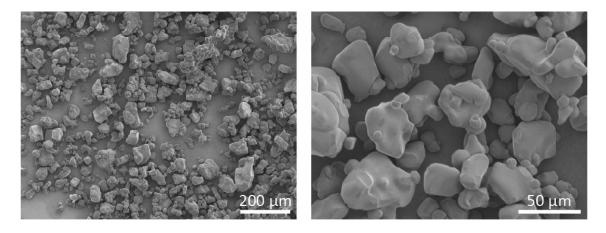
**Movie S1**: Real-time movie showing the flow behaviors of 1.1  $\mu$ m particles through the porous microchambers at different locations in the device. Particle behaviors at the chamber bottom for 5 s are shown. The input flow rate of the particle suspension was 10  $\mu$ L/min, which was set to clearly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



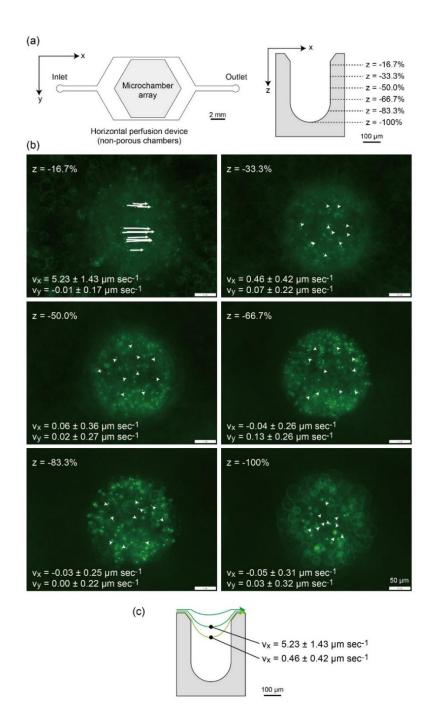
**Figure S1.** Scanning electron microscopic (SEM) images of the surface of the porous microchambers when the centrifugation time was varied from 1, 15, and 60 min.



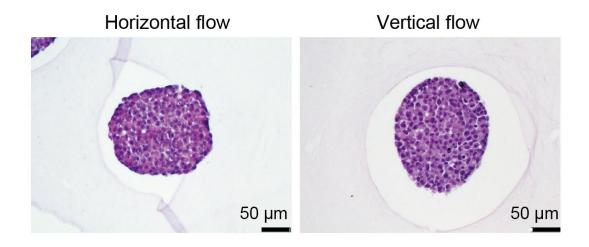
**Figure S2.** Count of surface pores from the SEM images of randomly selected 10 porous microchambers. Each small yellow dot indicates the count number of the surface pore.



**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  $\mu$ 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.