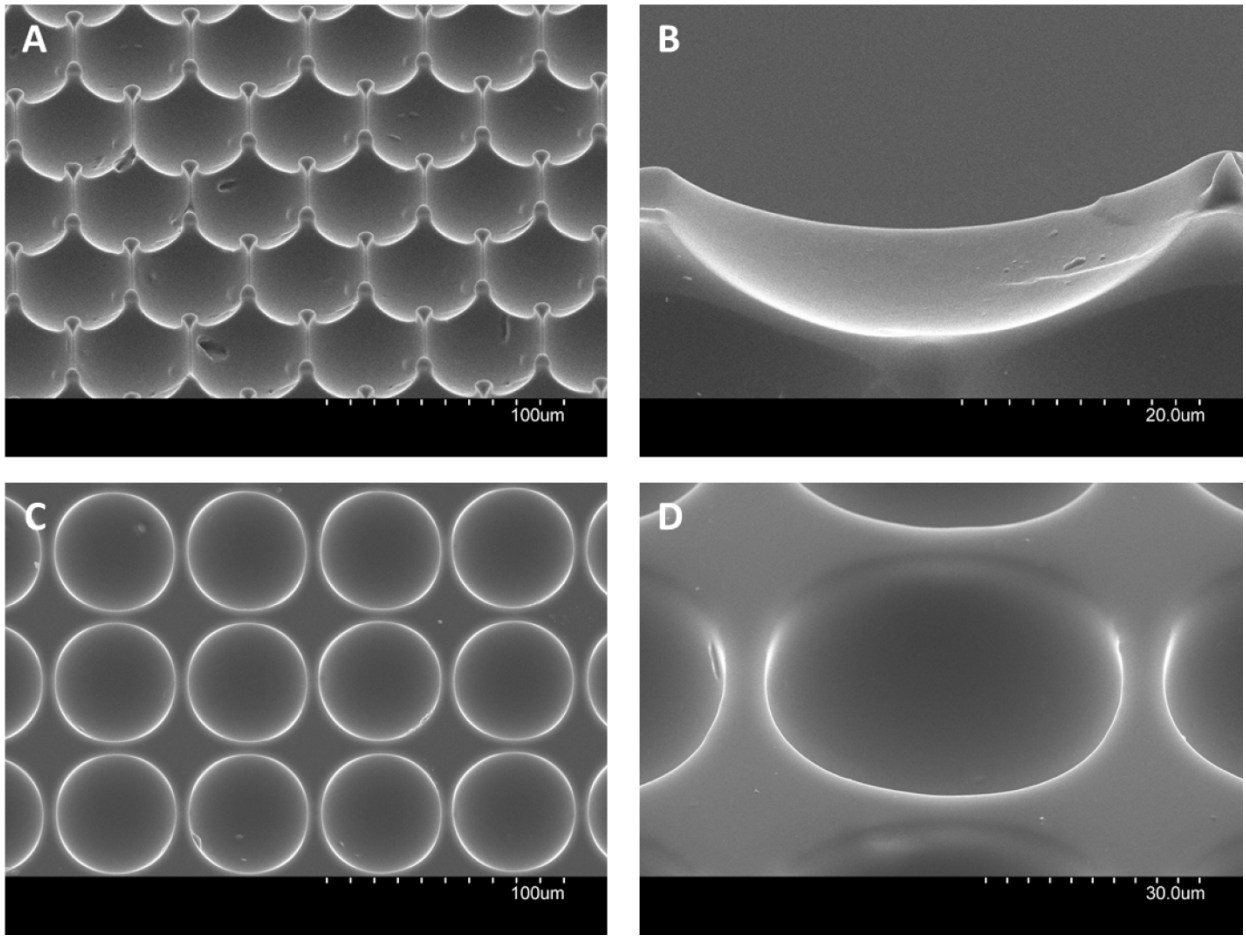
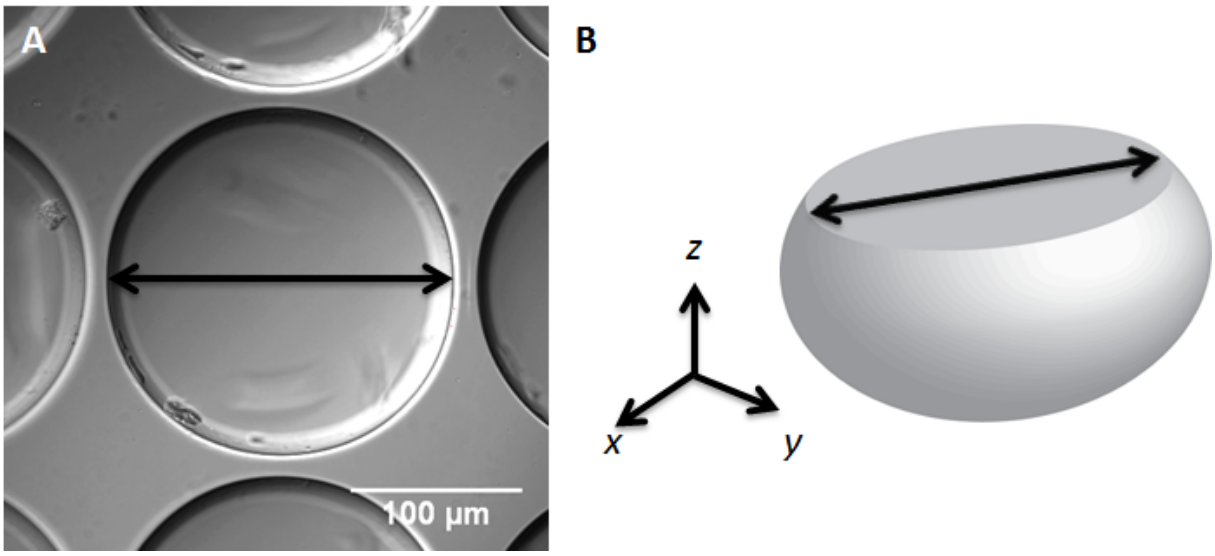
Electronic Supplementary Material (ESI) for Biomaterials Science.

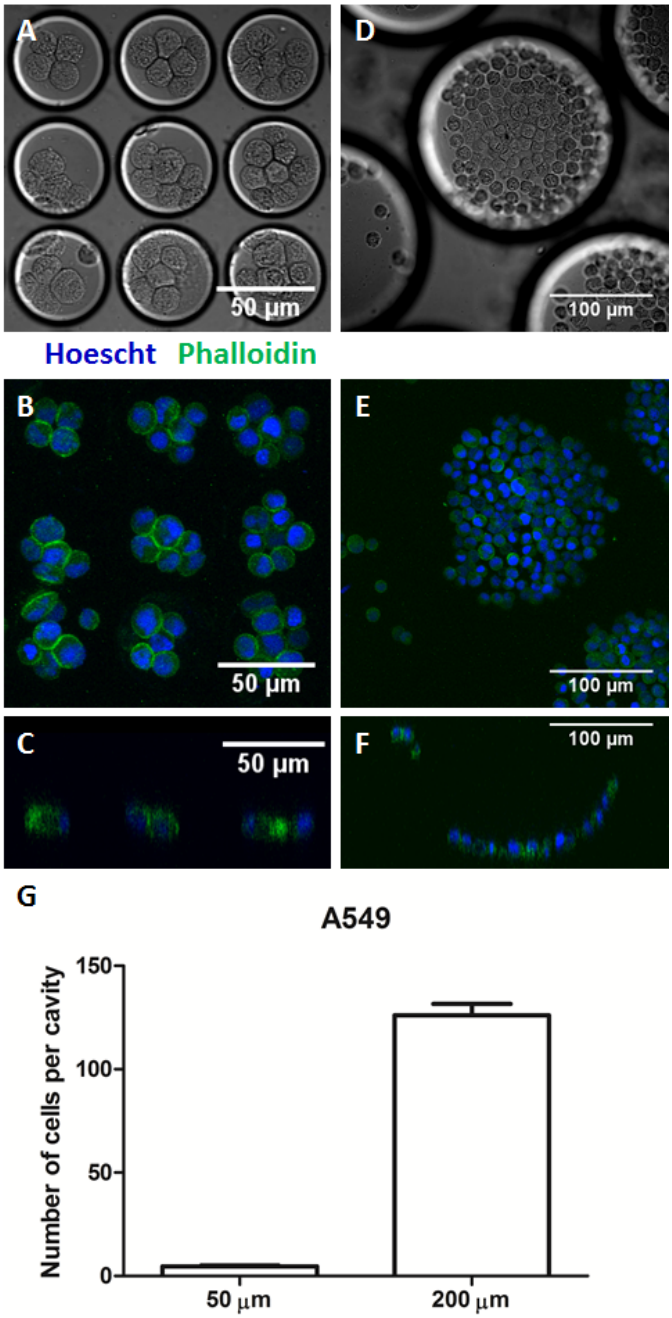
Supplementary Information



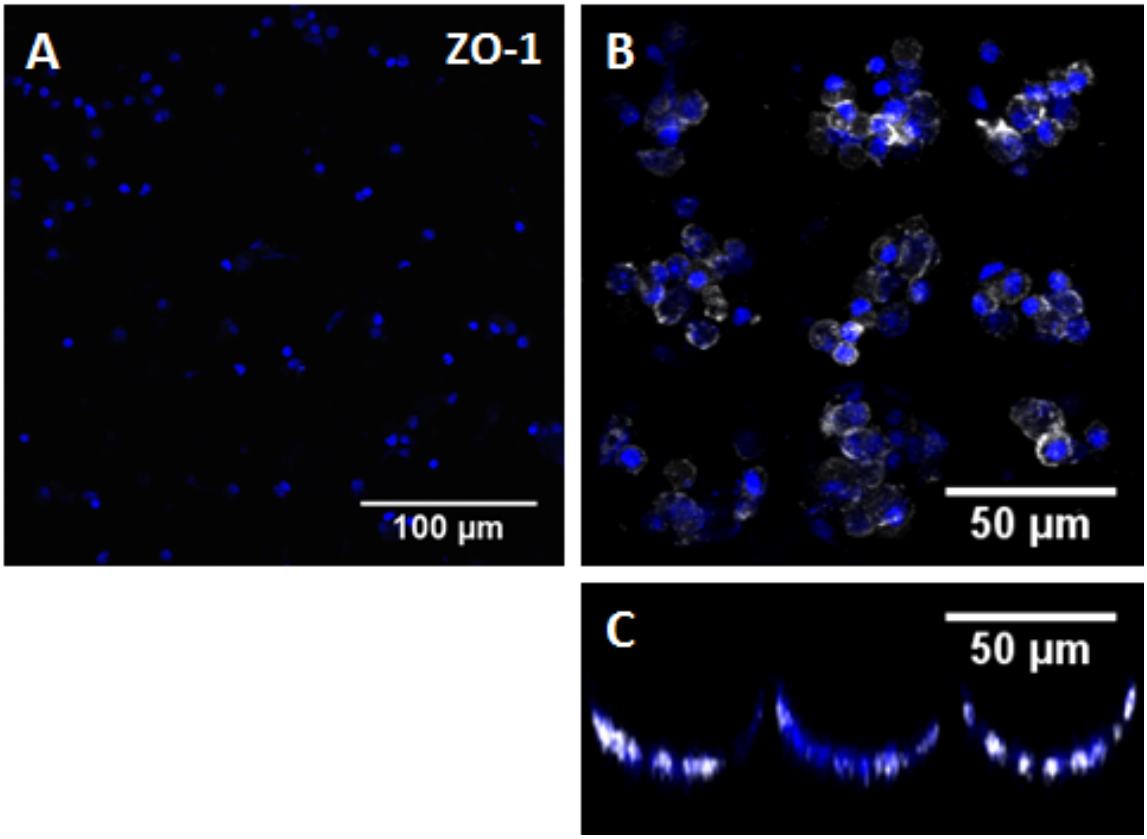
SI Figure 1 – Optimization of cavity substrate manufacturing process. A – SEM image of 50 µm diameter cavities in close-packed configuration. B – SEM image of individual 50 µm diameter cavity in close-packed configuration, depicting shallow curvature. C – SEM images of 50 µm diameter cavities in grid-packing configuration. D – SEM image of individual 50 µm diameter cavity in grid configuration.



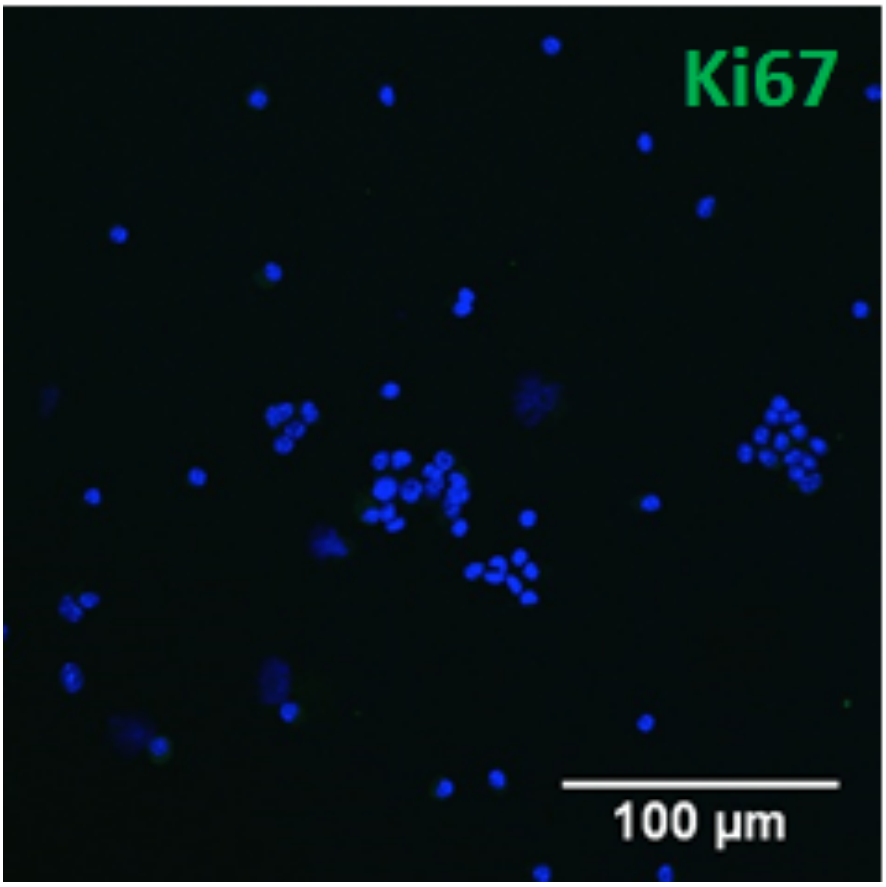
SI Figure 2 – Measurement of cavity diameter. A – Example of phase contrast image used for cavity measurement. Diameters were measured at the focal plane of the cavity opening (double-headed arrow). B – Schematic of cavity diameter measurement.



SI Figure 3 – Optimization of cell seeding to achieve reproducible monolayer cultures within the cavities. A – DIC image of A549 cells cultured in 50 µm diameter cavities. **B** – Corresponding confocal microscopy image of (A). Nuclei stained with Hoescht (blue) and actin stained with phalloidin (green). **C** – Corresponding confocal side profile of (A). **D –** DIC image of A549 cells cultured in 200 µm diameter cavities. **E** – Corresponding confocal microscopy image of (D). Nuclei stained with Hoescht (blue) and actin stained with phalloidin (green). **F** – Corresponding confocal side profile of (D). **G** – Graph showing the number of cells per cavity for 50 µm and 200 µm diameter cavities (*n*= 10). Error bars are standard error of the mean.



SI Figure 4 – AECs express tight junctions in cavities but not flat substrates. A – Confocal microscopy image of AECs stained with Hoescht for nuclei (blue) and ZO-1 (white) on flat substrates at day 1. **B** – Confocal microscopy image of AECs stained with Hoescht for nuclei (blue) and ZO-1 (white) on substrates containing 50 µm diameter cavities at day 1. **C** – Representative confocal side profile of ZO-1 staining for cavity-culture AECs.



SI Figure 5 – AECs are not proliferative at the time of isolation. Confocal microscopy image of AECs stained with Hoescht for nuclei (blue) and ki67 (green) on flat substrates at day 0.