

Supplementary Information

Compartmentalized 3D Tissue Culture Arrays under Controlled Microfluidic Delivery

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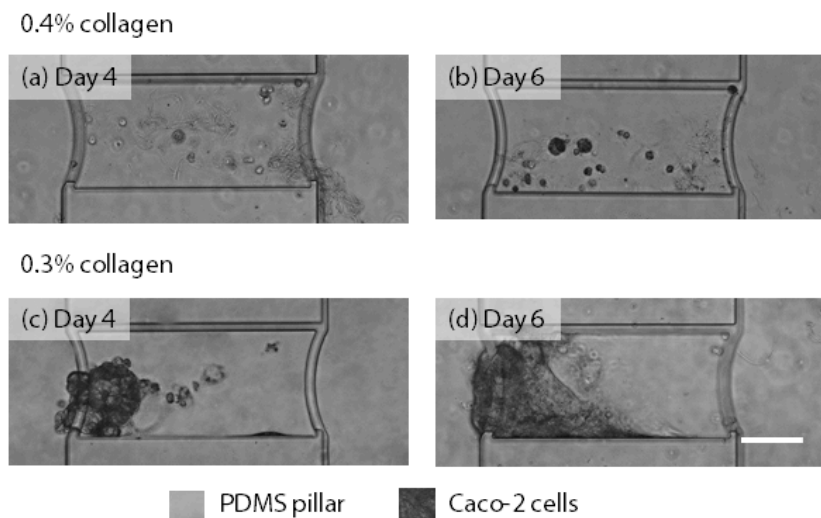


Figure S1. Top-view phase contrast microscopy images of Caco-2 cells cultured in **(a-b)** 0.4% **(c-d)** and 0.3% collagen concentrations under $300 \mu\text{l h}^{-1}$ flow rate. Scale bar is $125 \mu\text{m}$.

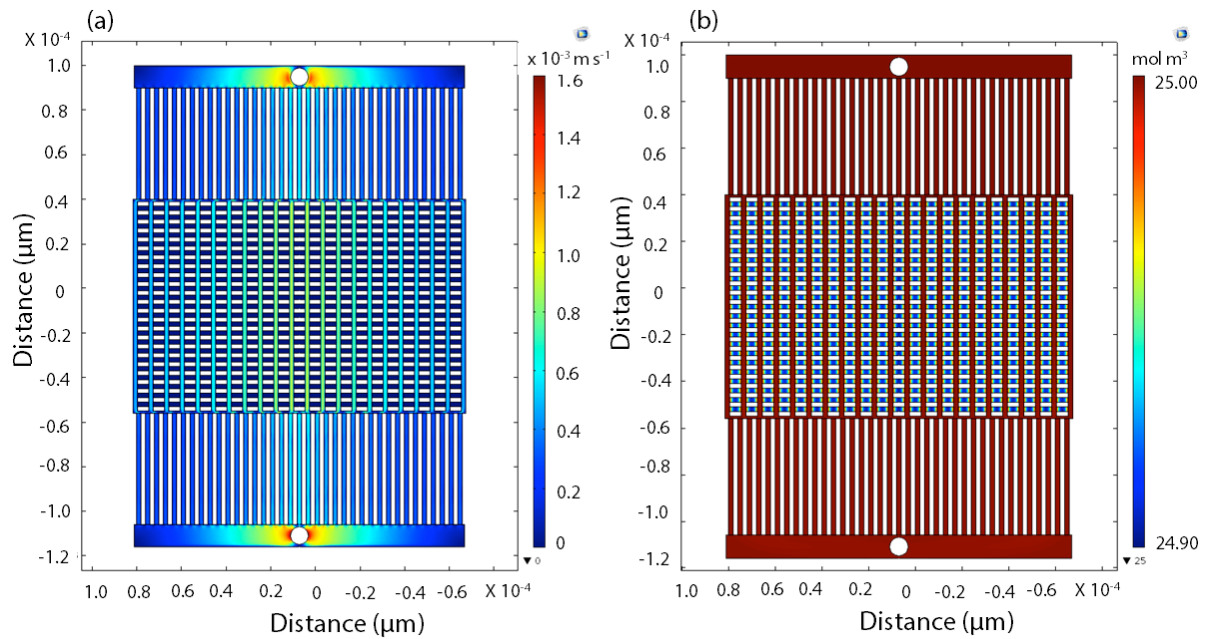


Figure S2. (a) Laminar flow simulation in the microchip. The flow velocity is the highest in the center of the rectangular chamber and gradually decreases towards the sides. (b) Predicted concentration distribution of glucose throughout the microchip assuming a glucose consumption in the compartments. The scale bar presents the same value from top to bottom. Please note from the scale bar that the glucose concentration is practically uniform.

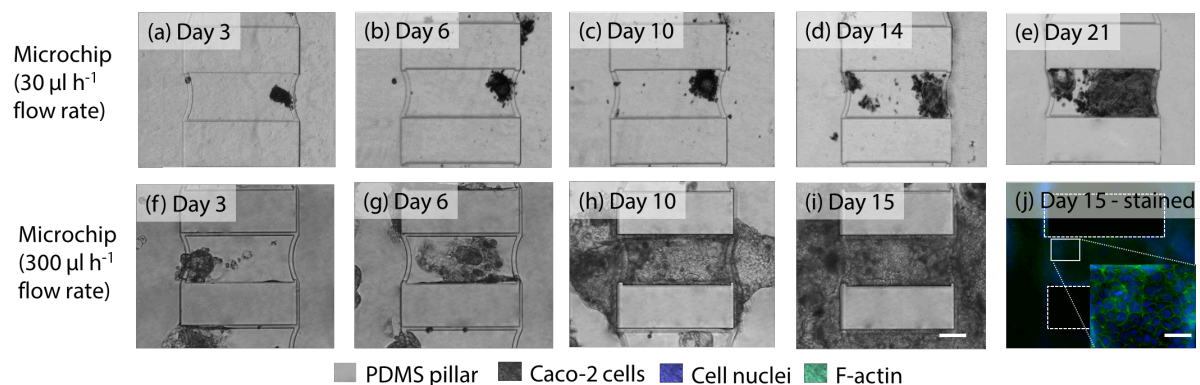


Figure S3. Top-view phase contrast images of long-term Caco-2 cultures in the microchip. The results are shown for the microchip perfused under (a-e) $30 \mu\text{l h}^{-1}$, and (f-j) $300 \mu\text{l h}^{-1}$ flow rates. In the fluorescence images (bottom line, day 15) staining was applied: blue colors present cell nuclei (NucBlue), and green colors present cytoskeleton (F-actin). Scale bar for the images is $125 \mu\text{m}$. The scale bar in the close-up image is $40 \mu\text{m}$.

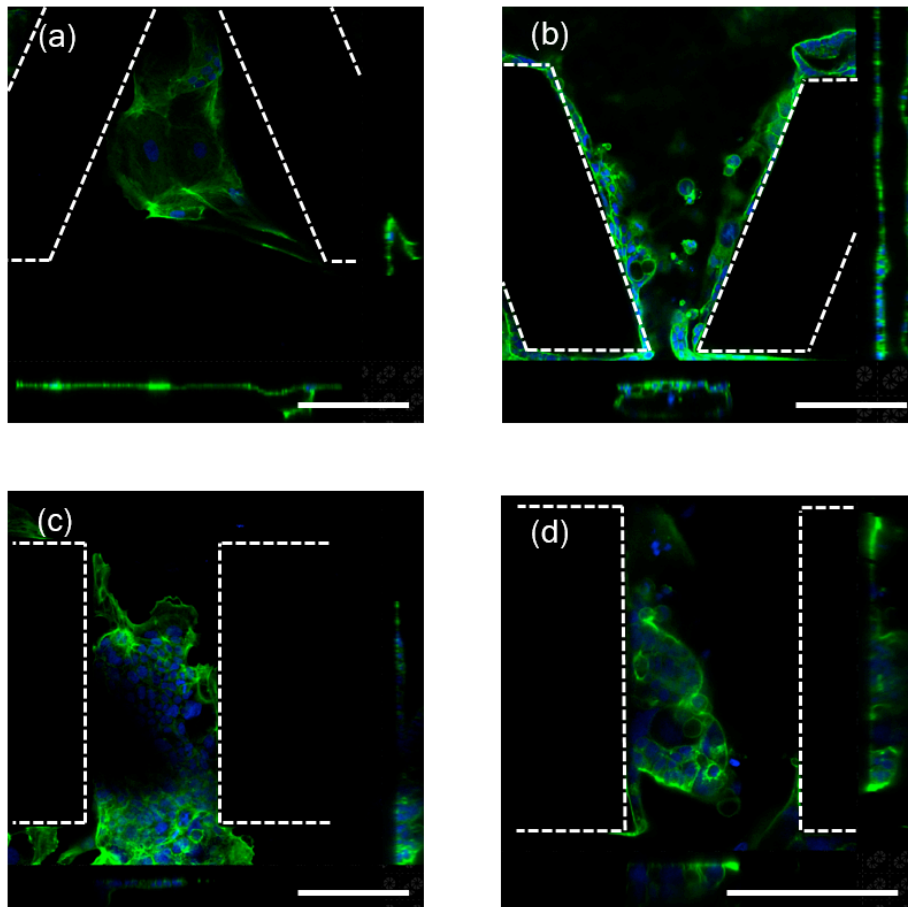


Fig. S4. Confocal fluorescence microscopy images of Caco-2 cells. Top-down and orthogonal views are portrayed in each image. **(a, b)** Triangular compartments and **(c, d)** rectangular compartments of Caco-2 cells cultured under $300 \mu\text{l h}^{-1}$ flow rate of culture medium. DAPI and GFP represent cell nuclei and F-actin filaments, respectively. Cells form tubes and irregular 3D folds. Scale bars are $200 \mu\text{m}$.

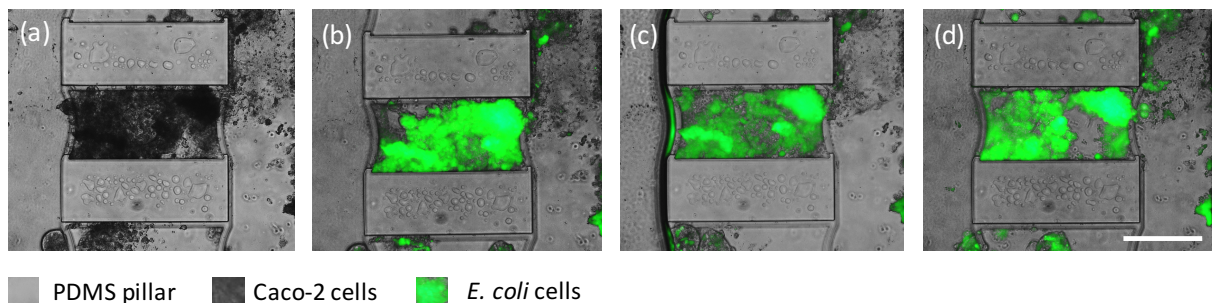


Figure S5. Overlay of phase contrast and fluorescent microscopy images of a GFP-expressing *E. coli* and Caco-2 co-culture followed by a chloramphenicol treatment **(a)** before incubation with GFP-expressing *E. coli*. **(b)** after a 1.5 h incubation of GFP-expressing *E. coli*. **(c)** after 30 min chloramphenicol treatment **(d)** after 36 h chloramphenicol treatment. Scale bar is $250 \mu\text{m}$.

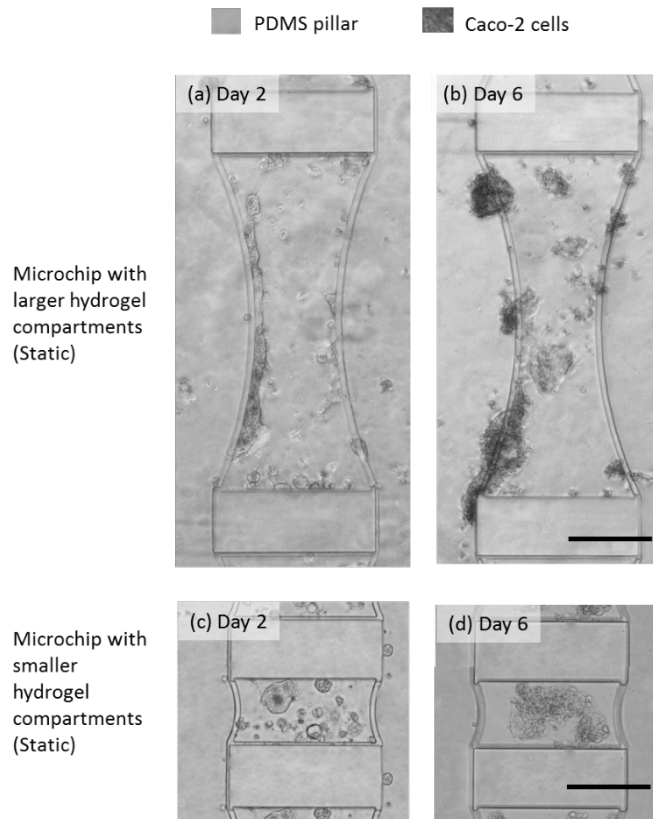


Figure S6. Top-view phase contrast microscopy images of Caco-2 cells cultured under static conditions in compartments with various sizes including (a, b) larger and (c, d) smaller compartment designs. Scale bars are 125 μm .

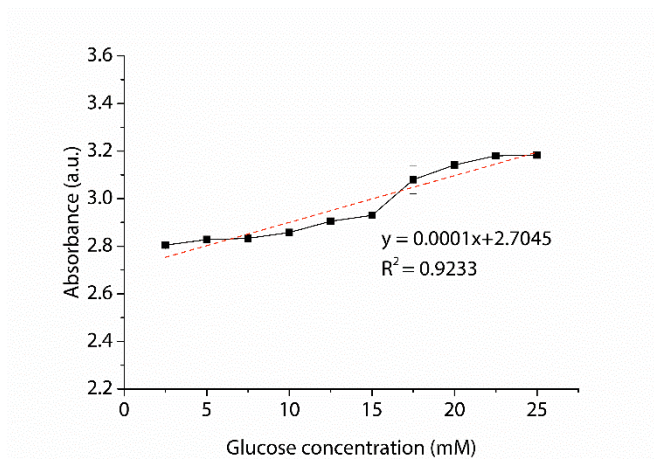


Figure S7. Calibration curve used in glucose concentration measurements.

Movie S1. Rotating confocal image of Caco-2 cells grown in a rectangular shaped compartment on day 8. Cells formed tube structures with 3D folds and podia inside. In the fluorescence images (bottom line, day 15) staining was applied: blue colors present cell nuclei (NucBlue), and green colors present cytoskeleton (F-actin).