Electronic Supplementary Material (ESI) for Lab on a Chip.


# Supporting Information

# Horizontal and vertical microchamber platforms for evaluation of the paracellular permeability of an epithelial cell monolayer

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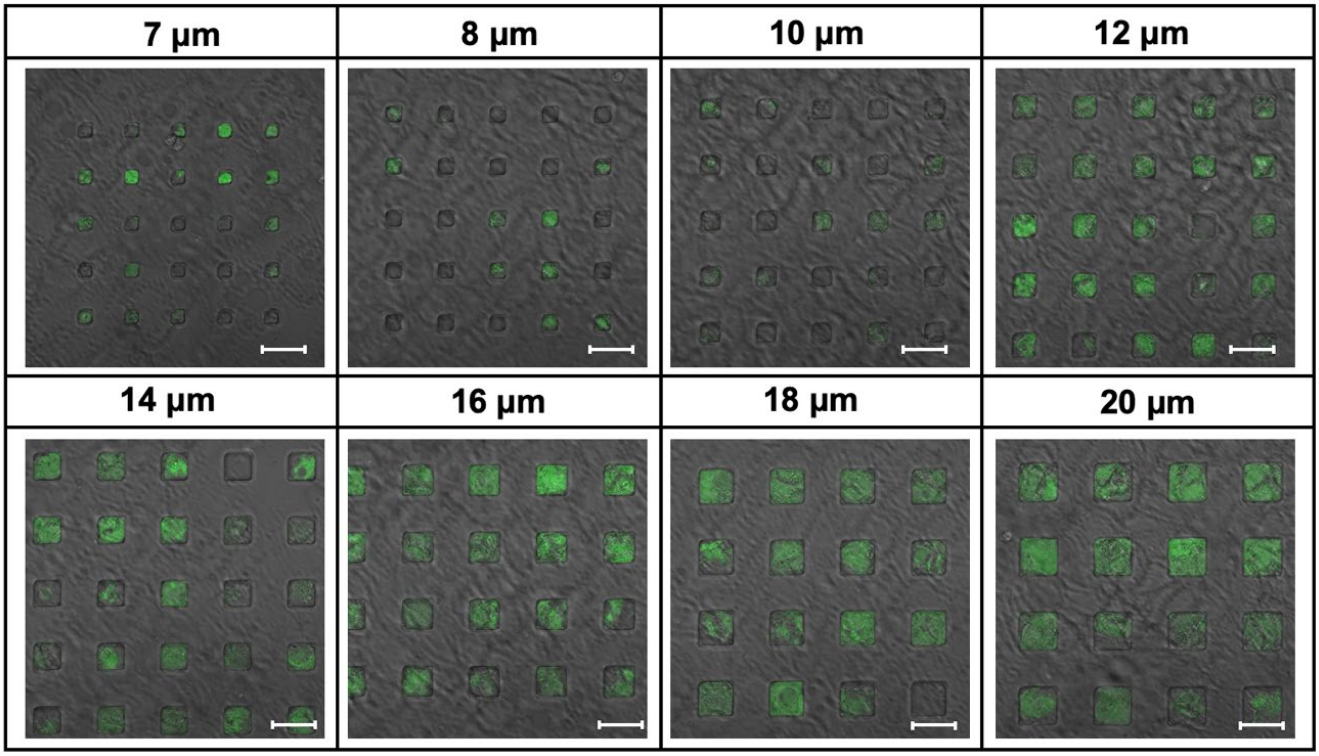


Fig. S1 Caco-2 penetration to the inside of microchambers for different chamber sizes. Size of sides with square chambers are indicated on top of each figure, while the depth of microchambers is fixed to 10 μm. Focal plane of the confocal microscope was set to the middle of microchambers. Green fluorescence show the cytosol of Caco-2, penetrated into the microchamber space, which was stained by calcein-AM. Scale bars = 20 μm.

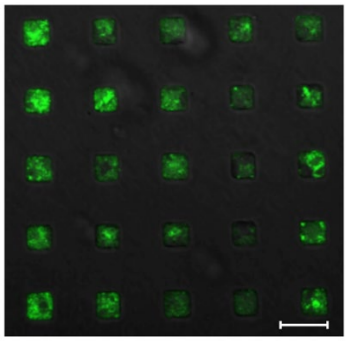

Fig. S2 An example image of MDCK cells adhesion to the inside of the chamber (Size of chamber: 10 μm per side and 10 μm in depth). Here, MDCK cells stably expressing claudin 4-GFP were established by transfecting the wildtype MDCK cells with CLDN4-GFP gene (OriGene, MD, USA) via electroporation (ELEPO21, Neppa Gene, Japan). Device was coated with fibronectin solution (50 μg/ml), and MDCK cells were cultured for 3 days. MDCK cells penetrated into microchambers more often compared to Caco-2. Green: GFP- tagged claudin 4. Scale Bar = 20 μm.

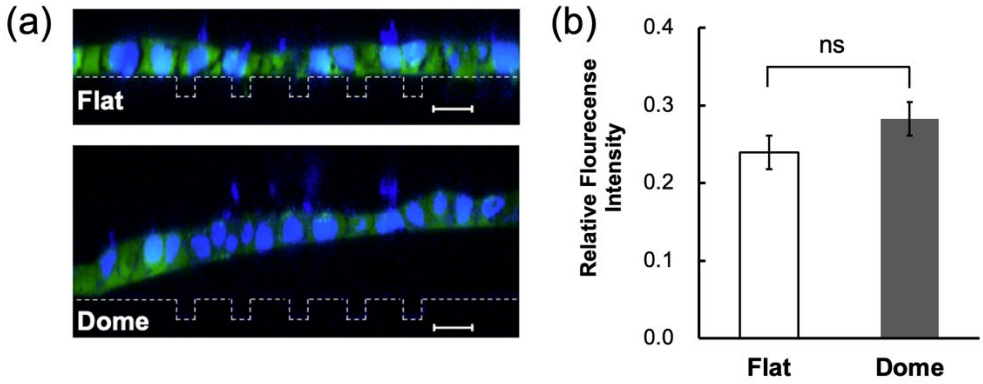


Fig. S3 (a) Reconstructed images of the flat and dome-formed areas of the Caco-2 monolayer. Blue: Hoechst, Green: Calcein-AM. Scale bar = 20 μm (b) Comparison of fluorescence intensity distributions in microchambers beneath flat and dome regions, obtained at 80 min after the addition of f-dextran solution.

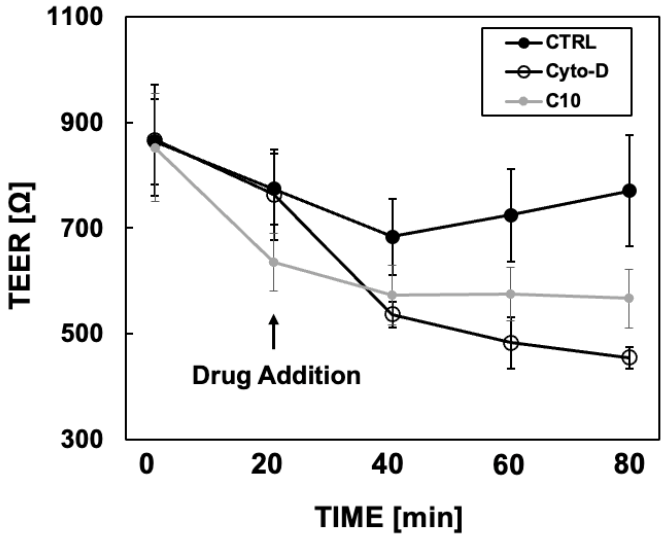


Fig. S4 Time variation of the TEER measurement showing the effect of C10 and Cyto-D. Although decreasing trends can be seen with drug administrations, large variations hinders quantitative comparisons.

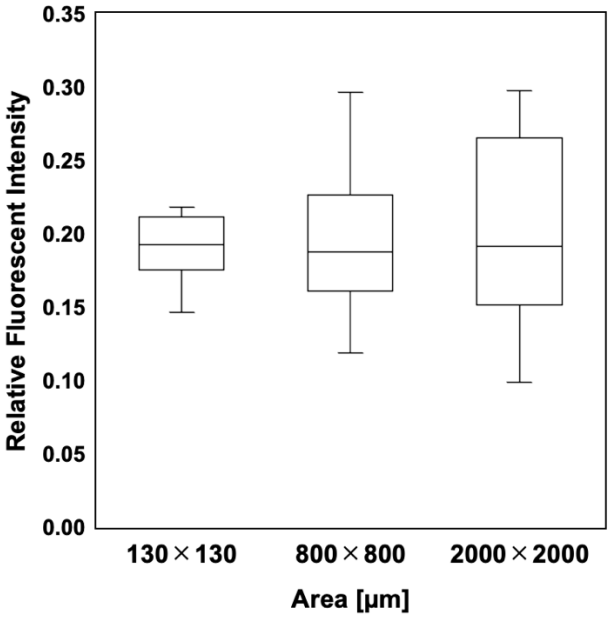


Fig. S5 Fluorescence intensity distributions in microchambers over different spatial ranges. A total of 16 microchambers were sampled in each range. 5×5 microchambers (one unit) are present in 130 × 130 $\mu m^2$ area, which can be obtained in a single scan with x40 objective. In 800 × 800 $\mu m^2$ and 2000 × 2000 $\mu m^2$ areas, 4 × 4 and 7×7 units are present, respectively, from which 16 points (one point from each unit or from every odd unit) are sampled. Variation in intensity values become larger in wider areas.