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

Supplementary information

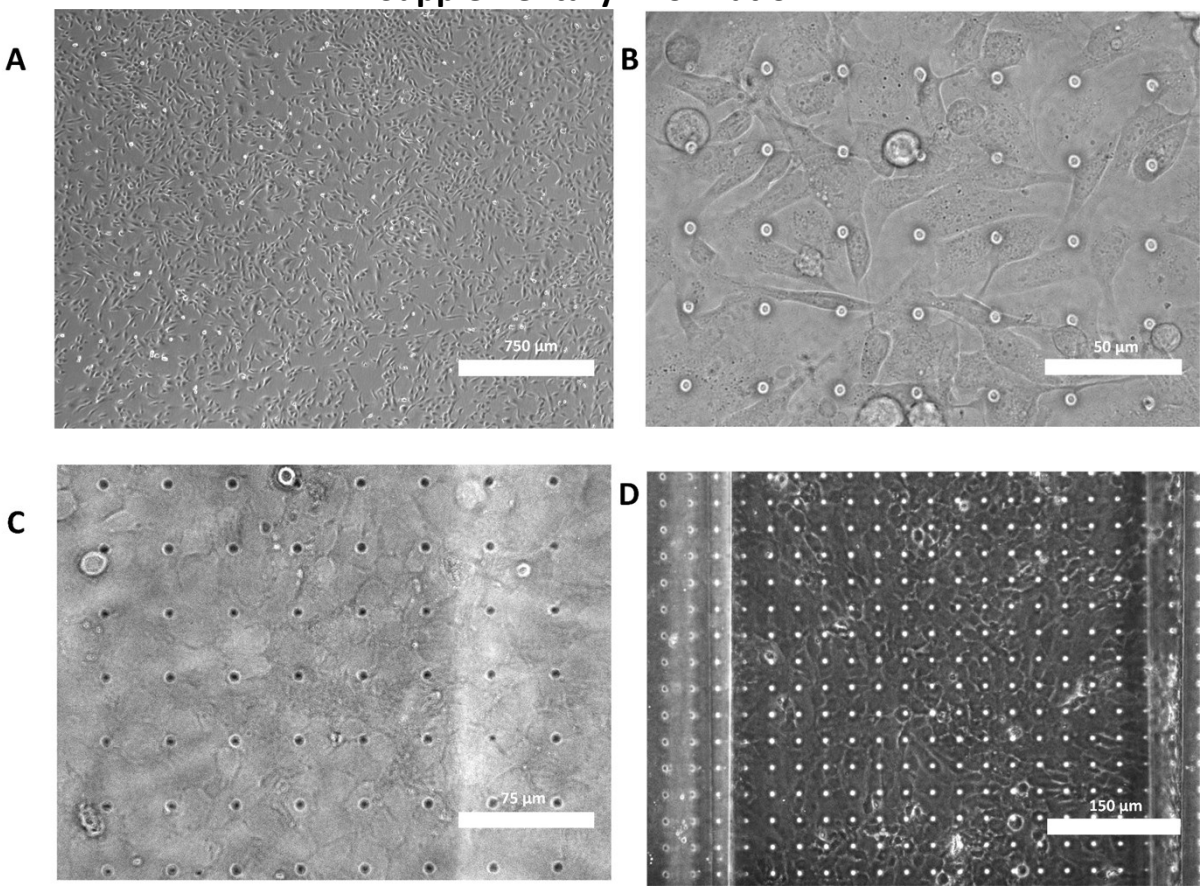

Figure 1: Phase-contrast images of hCMEC/D3 cell culture: (A) hCMEC/D3 in the culture flask. (B) hCMEC/D3 cells after 1h of incubation in the multiplexed chip. The cells adhered to the PDMS membrane. (C) & (D) hCMEC/D3 after being in culture for 5 days (with different magnification).

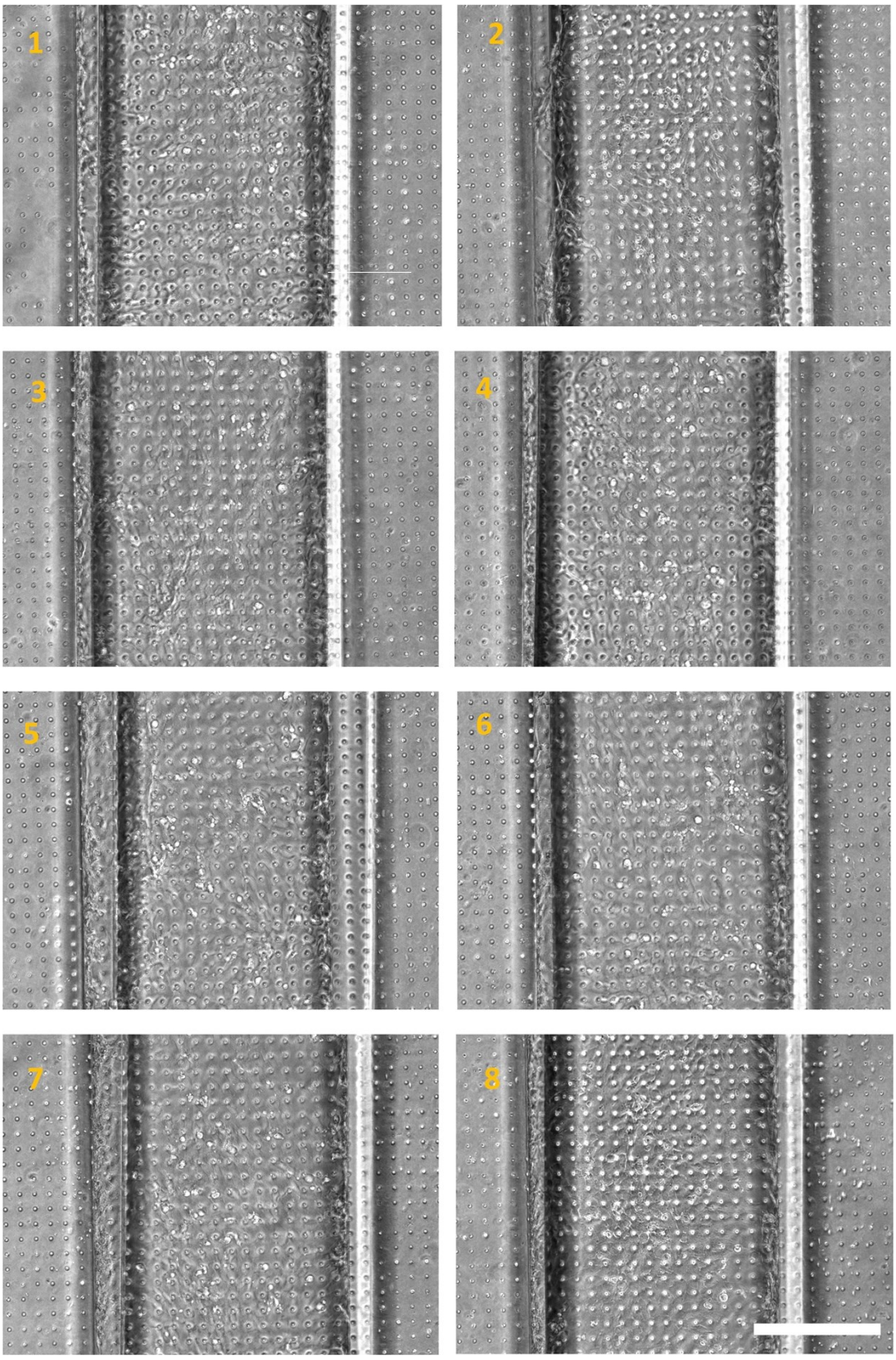

Figure 2: Phase contrast images of 8 channels lined with hCMEC/D3 (scale bar 250 µm).Transparent and thin PDMS membrane allowed visualization of the cells inside the chip.

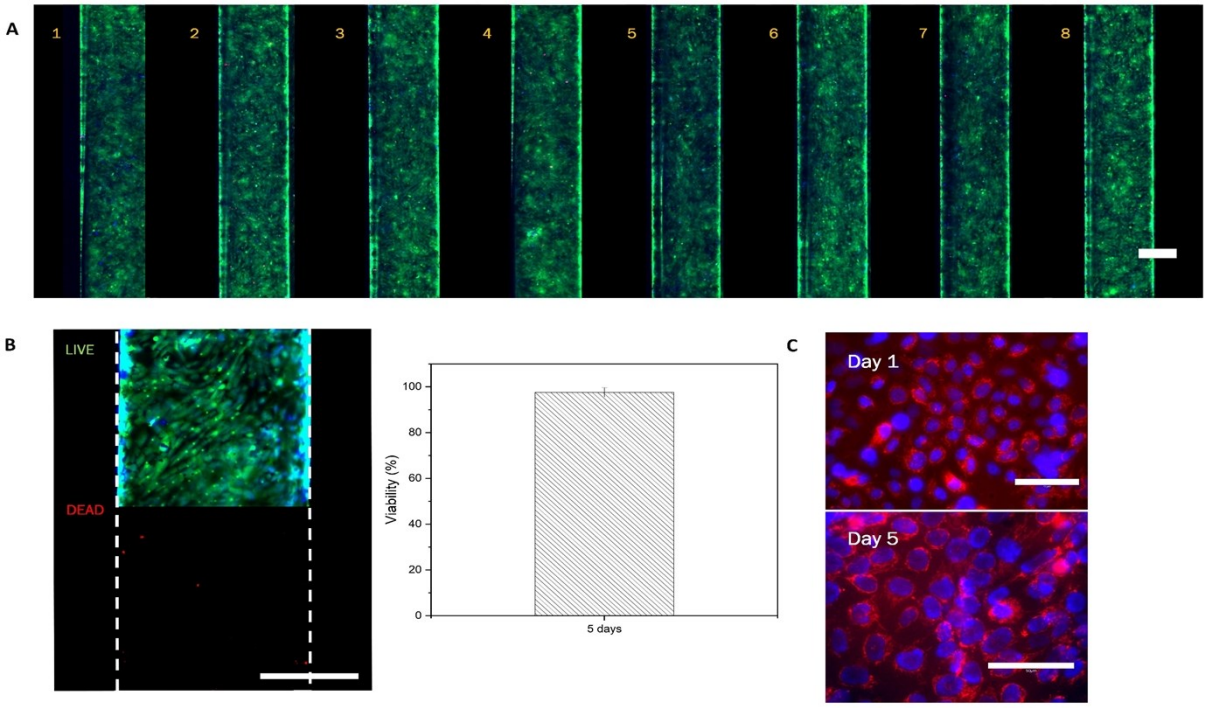

Figure 3: Cell viability and mitochondrial activity of hCMEC/D3 culture. (A) Live-dead staining after 5 days of culture in all 8 (scale bar 750 µm) channels and (B) magnified image (scale bar 250 µm)with quantified cell viability (97.5±2%, n=8). (C) Mitochondrial staining at day 1 (top image, scale bar 75 µm) and day 5 (bottom, scale bar 50 µm). The mitochondrial activity remained unchanged during the cell culture indicating healthy monolayer.

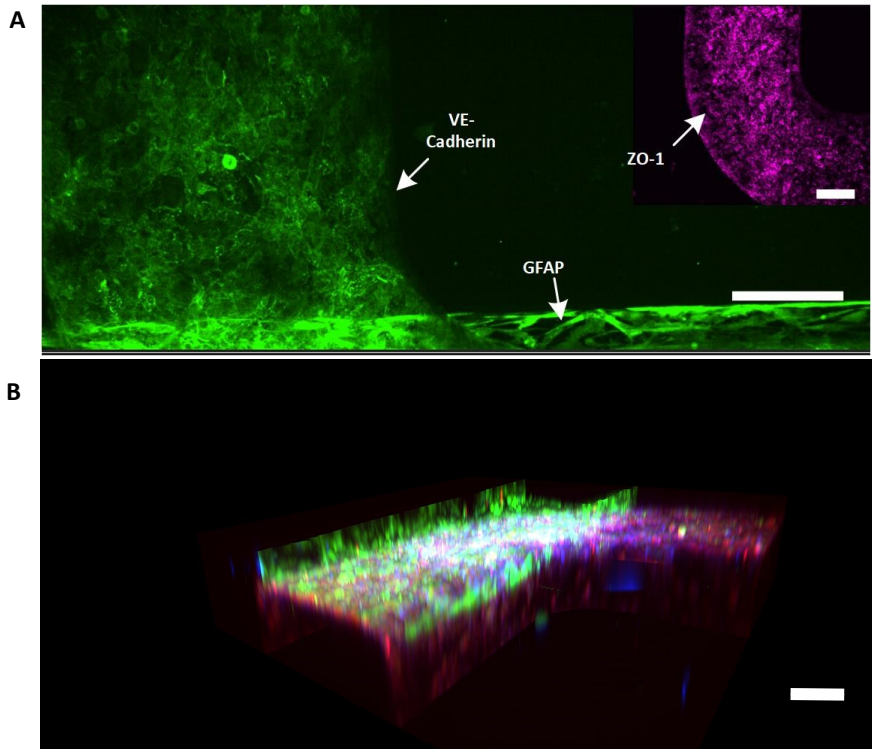

Figure 4: Protein expression in hCMEC/D3 and HAc. (A) Immunofluorescence staining of VE-cadherin and ZO-1 in hCMEC/D3 and GFAP in HAc. Although the same color was used for staining VE-cadherin and GFAP, it was possible to distinguish both cell types by the characteristic morphology. (B) A confocal image revealed HAc and hCMEC/D3 cells growing on the opposite sides of the membrane and hCMEC/D3 growth on the walls of the channels (during the seeding process, cells can attach to the coated walls before completely sedimenting on the membrane). The scale bar is 150 µm.

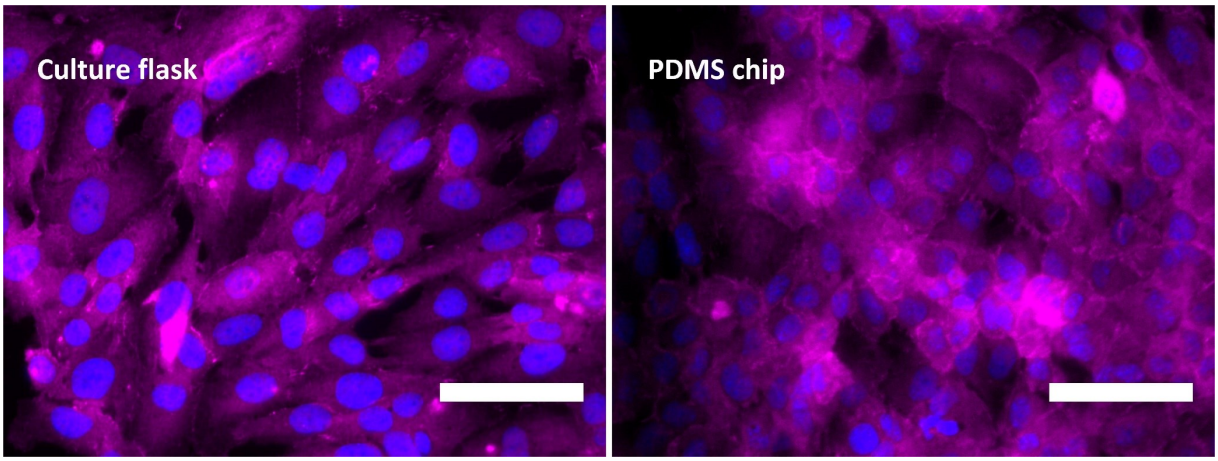

Figure 5: hCMEC/D3 morphology on different culture substrate. The cells were cultured to confluency in the culture flask coated with collagen-I (left) and in the microfluidic chip also coated with collagen-I (right). Immunostaining of ZO-1 and Nuclei revealed spindle-like morphology on the polystyrene substrate while on PDMS, cells had cobblestone morphology. Scale bar is 75 µm.

Video "Reconstruction": Reconstruction of the channels covered with hCMEC/D3 (cyan) cells and HAc (green). https://drive.google.com/file/d/1p3cOtA_noPH0QI8nnlg_jgeYlQ7V_PI3/view?usp=sharing