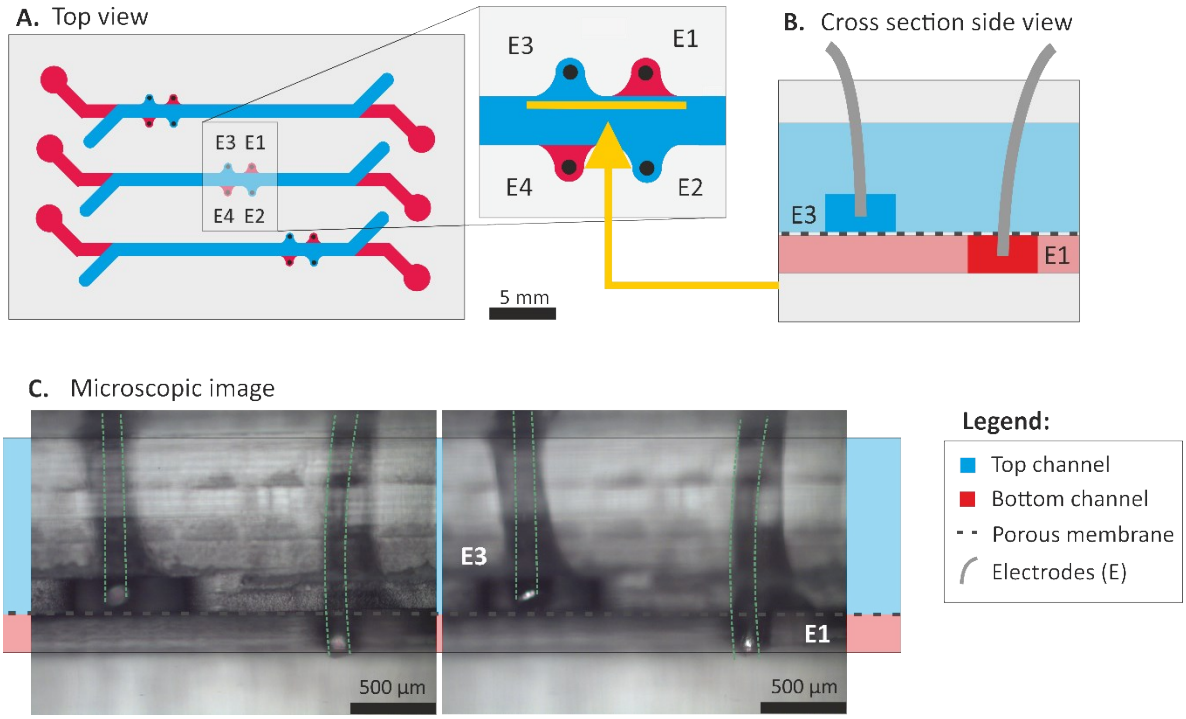
Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPLEMENTARY INFORMATION

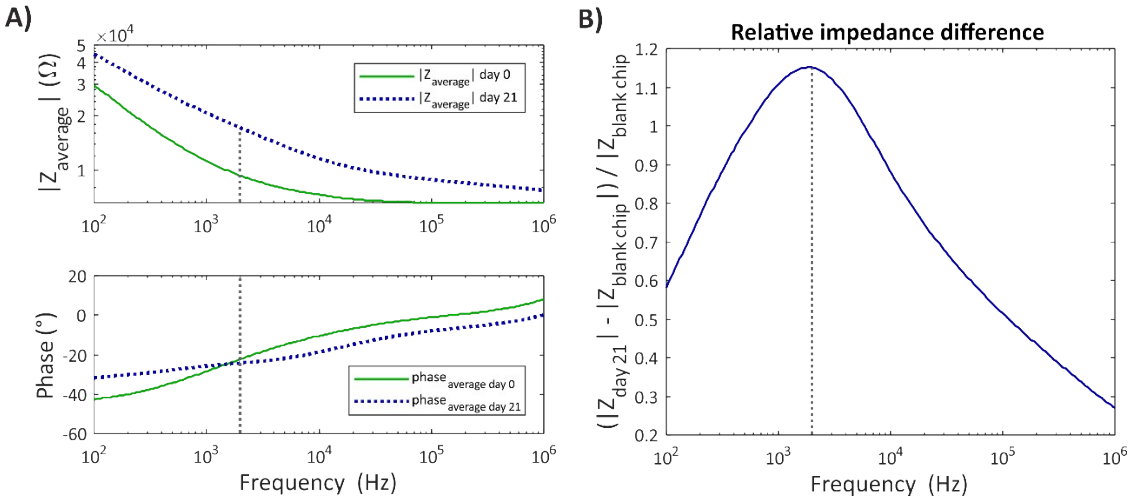
S.1 Electrode integration



Supplementary Fig. 1: A) Schematic top view of the chip with electrode configuration. The yellow arrow indicates the site of the cross-sectional view shown in B. B) Schematic cross-section of the chip. C) Microscopic images of the cross-section of a fabricated PDMS chip with the inserted platinum electrodes in the electrode wells. Two different focal planes are shown (left and right). The electrodes, channels, and membrane are indicated.

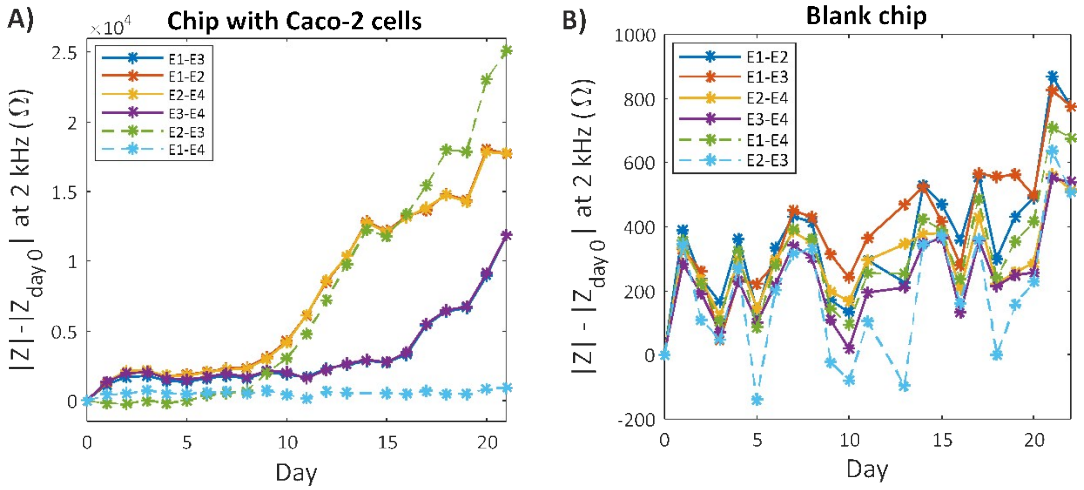
S.2 Gut-on-chip

Determining readout frequency:



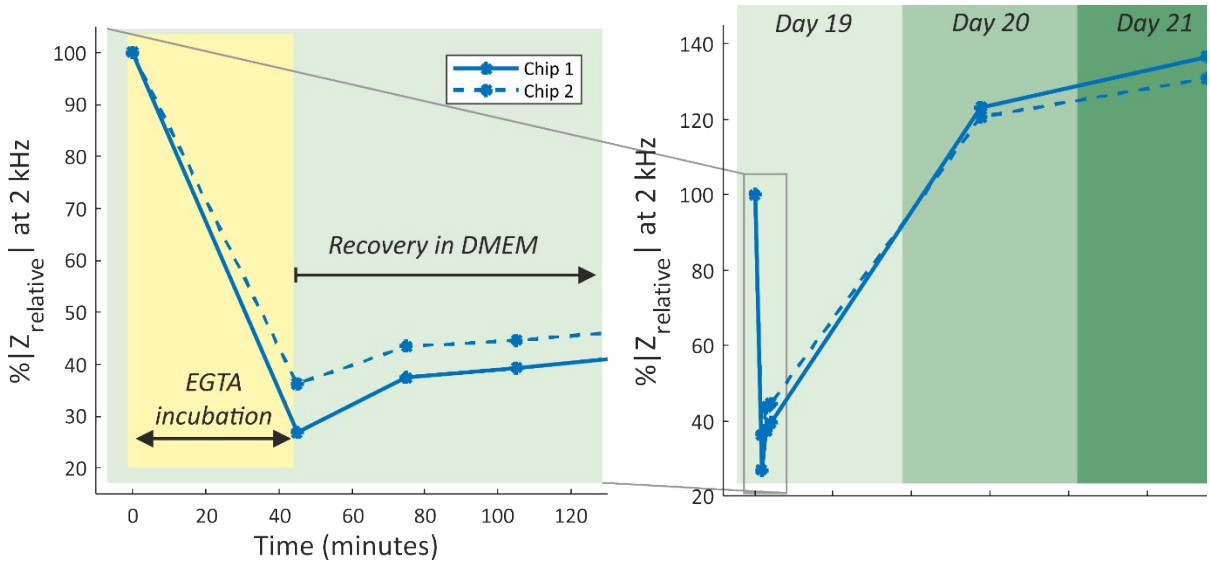
Supplementary Fig. 2: Gut-on-chip.
A) The averages of the magnitudes and phases of the four measurements through the membrane are averaged and plotted for a blank chip, day 0 (solid, green line), and a chip with Caco-2 cells at day 21 (dotted, blue line). B) The relative impedance difference between day 21 and day 0 with respect to day 0 is plotted over frequency, to determine the readout frequency. The biggest relative impedance difference was observed at approximately 2 kHz, indicated by the dashed vertical line.

Impedance measurements per electrode pair for 1 chip:



Supplementary Fig. 3: Gut-on-chip.
A) Impedance measurements in a chip with Caco-2 cells during cell culture. The measured impedance at readout frequency 2 kHz for each electrode pair is shown over time. The solid lines correspond to the four measurements through the PDMS membrane with cells. The dotted lines correspond to the measurements without the PDMS membrane in between. The electrodes stated in the legend correspond to the electrode indications in Figure 1. The first measurement (day 0, $|Z_0|$), before cell seeding, was subtracted from all subsequent measurements per electrode pair ($|Z|$). B) Impedance measurements of the PDMS membrane in a blank chip (filled with DMEM culture medium). The measured impedance at 2 kHz for each electrode pair is shown over time. The first measurement (day 0) was subtracted from all subsequent measurements per electrode pair. Please note the different y-axis scales.

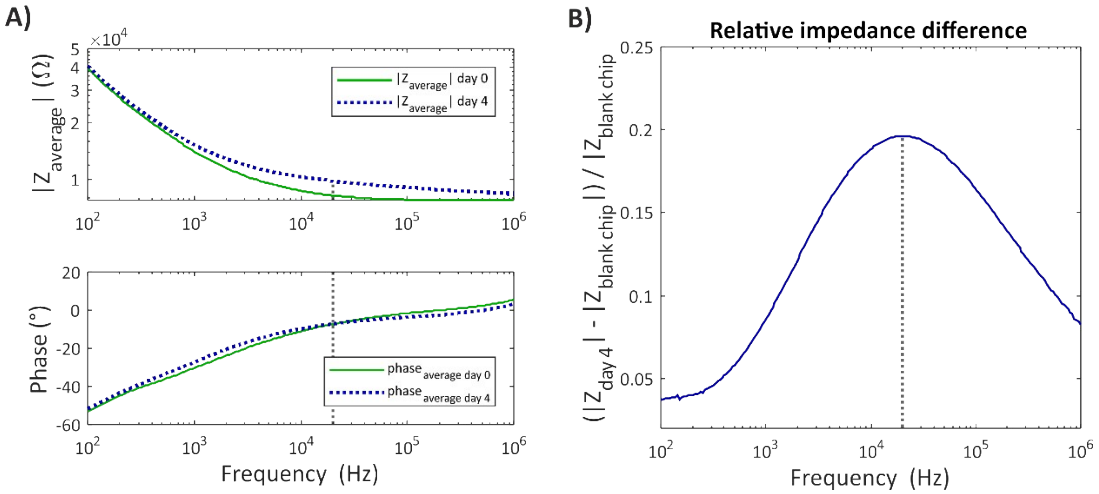
S.3 EGTA treatment



Supplementary Fig. 4: Response of the Caco-2 barrier function to the treatment of EGTA incubation. The relative impedance at 2 kHz of the four electrode pairs measuring through membrane and cell layer over time in two chips with Caco-2 cells (P30) is shown. All impedances were calculated as percentages with respect to the relative impedance of day 19 (n=2). A) Close-up of day 19, with the decreased impedance after 45 minutes of EGTA incubation, with subsequent increase of the impedance in response to incubation in DMEM (30 minutes and 60 minutes after incubation). B) A recovery of the impedance to more than 100% is observed overnight.

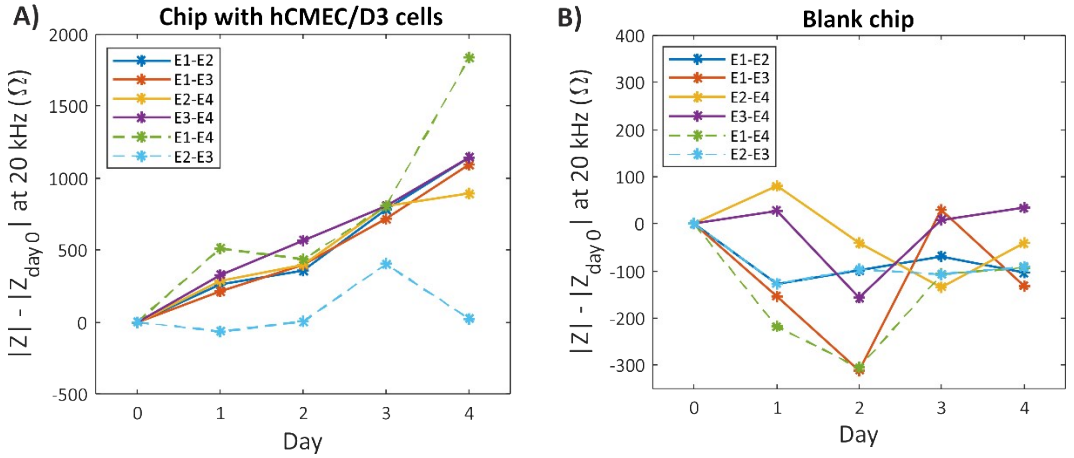
S.4 BBB-on-chip

Determining readout frequency:



Supplementary Fig. 5: BBB-on-chip.
A) The averages of the magnitudes and phases of the four measurements through the membrane are averaged and plotted for a blank chip, day 0 (solid, green line), and a chip with hCMEC/D3 cells at day 4 (dotted, blue line). B) The relative impedance difference between day 4 and day 0 with respect to day 0 is plotted over frequency, to determine the readout frequency. The biggest relative impedance difference was observed at approximately 20 kHz, indicated by the dashed vertical line.

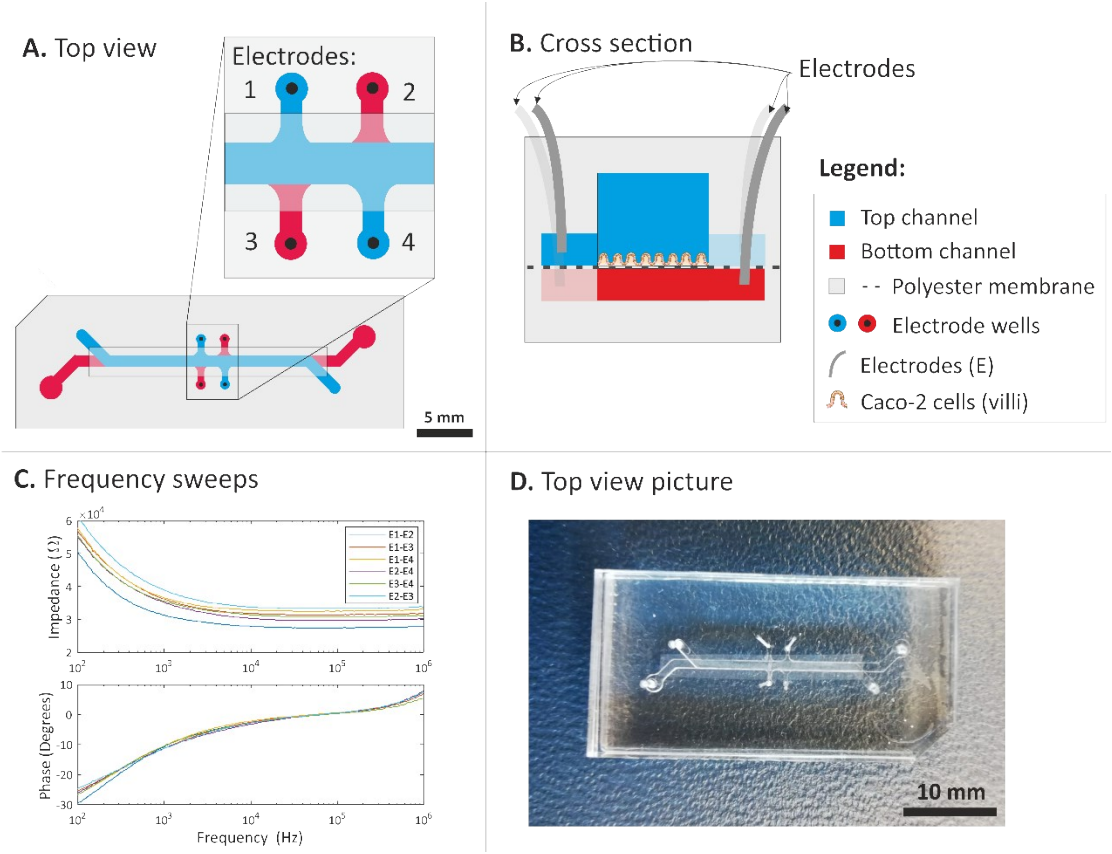
Impedance measurements per electrode pair for 1 chip:



Supplementary Fig. 6: BBB-on-chip.
A) Impedance measurements in a chip with hCMEC/D3 cells during cell culture. The measured impedance at readout frequency 20 kHz for each electrode pair is shown over time. The solid lines correspond to the four measurements through the PDMS membrane with cells. The dotted lines correspond to the measurements without the PDMS membrane in between. The electrodes stated in the legend correspond to the electrode indications in Figure 1. The first measurement (day 0, $|Z_0|$), before cell seeding, was subtracted from all subsequent measurements per electrode pair ($|Z|$). B) Impedance measurements of the PDMS membrane in a blank chip (filled with EGM culture medium). The measured impedance at 20 kHz for each electrode pair is shown over time. The first measurement (day 0) was subtracted from all subsequent measurements per electrode pair. Please note the different y-axis scales.

S.5 Towards a completely cleanroom-free fabrication method

To obtain a completely cleanroom-free fabricated TEER chip, the thin PDMS membrane can be replaced with a polyester or polycarbonate membrane. These commercially available membranes can be glued between the two PDMS layers, using a protocol reported before[1]. To obtain this chip, another PMMA mold was designed and fabricated (Supplementary Figure 5). The electrode wells in this design are placed further from the main channel, as the polyester membrane (GVS life sciences, polyester track-etched (PETE) membrane, pore size 8 µm, thickness 10 – 20 µm) should be cut narrower than the distance between the electrode wells (E1-E3). This is important for a proper binding between the two PDMS layers, and to ensure that there is no leakage current between the electrode wells during impedance measurements, such that all current passes through the membrane with cells. Supplementary Figure 5C shows the frequency sweeps from all the electrode combinations, which is similar to the frequency sweeps in empty chips with a PDMS membrane. With this proposed method, the entire organ-on-chip with integrated electrodes is completely cleanroom-free.



Supplementary Fig. 7: towards a completely cleanroom-free fabrication method A) Schematic top view of the chip with electrode wells further deviated from the main channel to allow the integration of a polyester membrane. B) Schematic cross-section of the chip. C) Frequency sweeps of all electrode combinations in an empty chip with the polyester membrane, showing a similar behavior as an empty chip with a thin PDMS membrane (Fig. 3A and 5A). D) Top view of an assembled chip with the polyester membrane.

References

1 M. W. van der Helm, M. Odijk, J.-P. Frimat, A. D. van der Meer, J. C. T. Eijkel, A. van den Berg and L. I. Segerink, *Biosens. Bioelectron.*, 2016, **85**, 924–929.