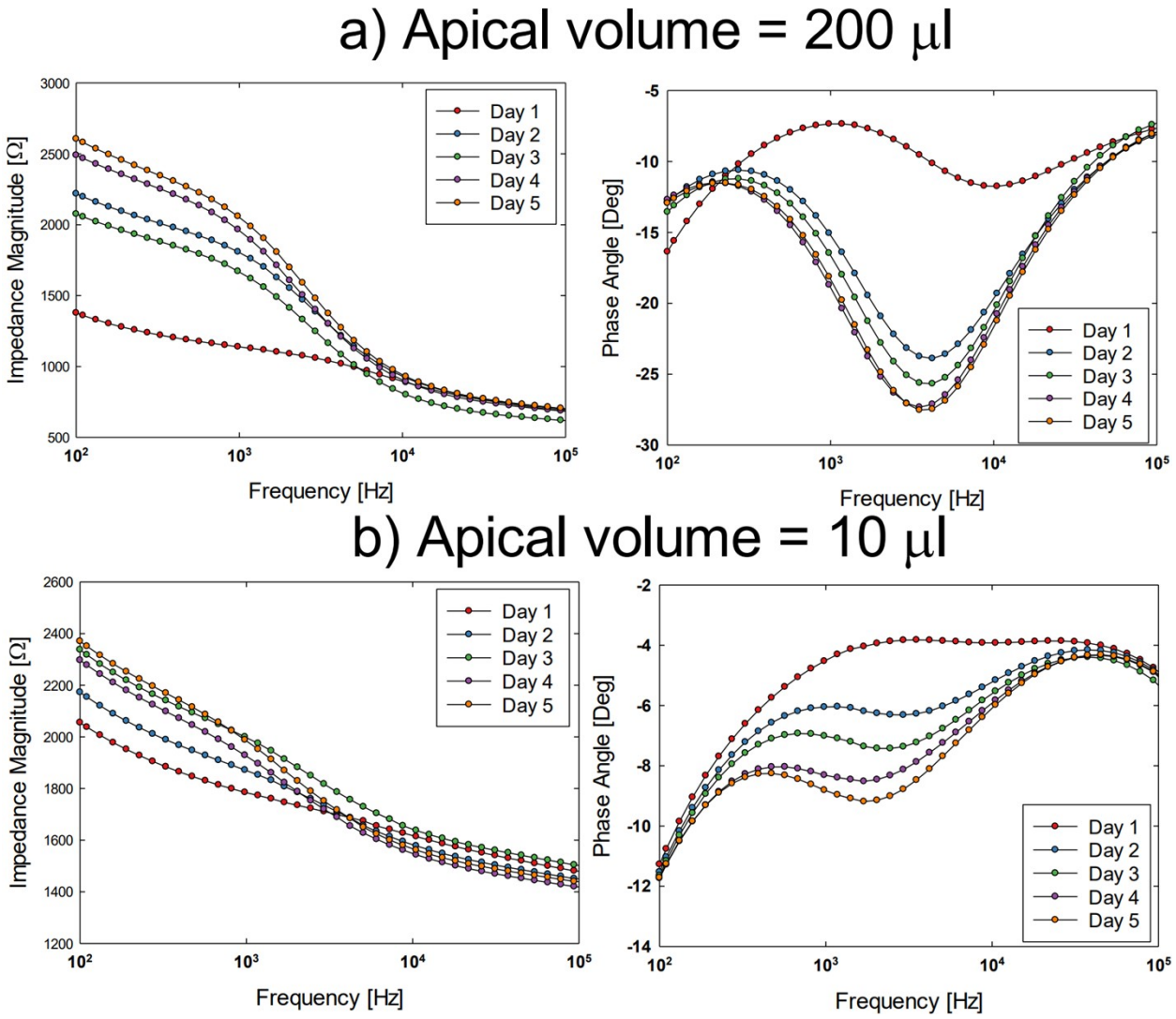
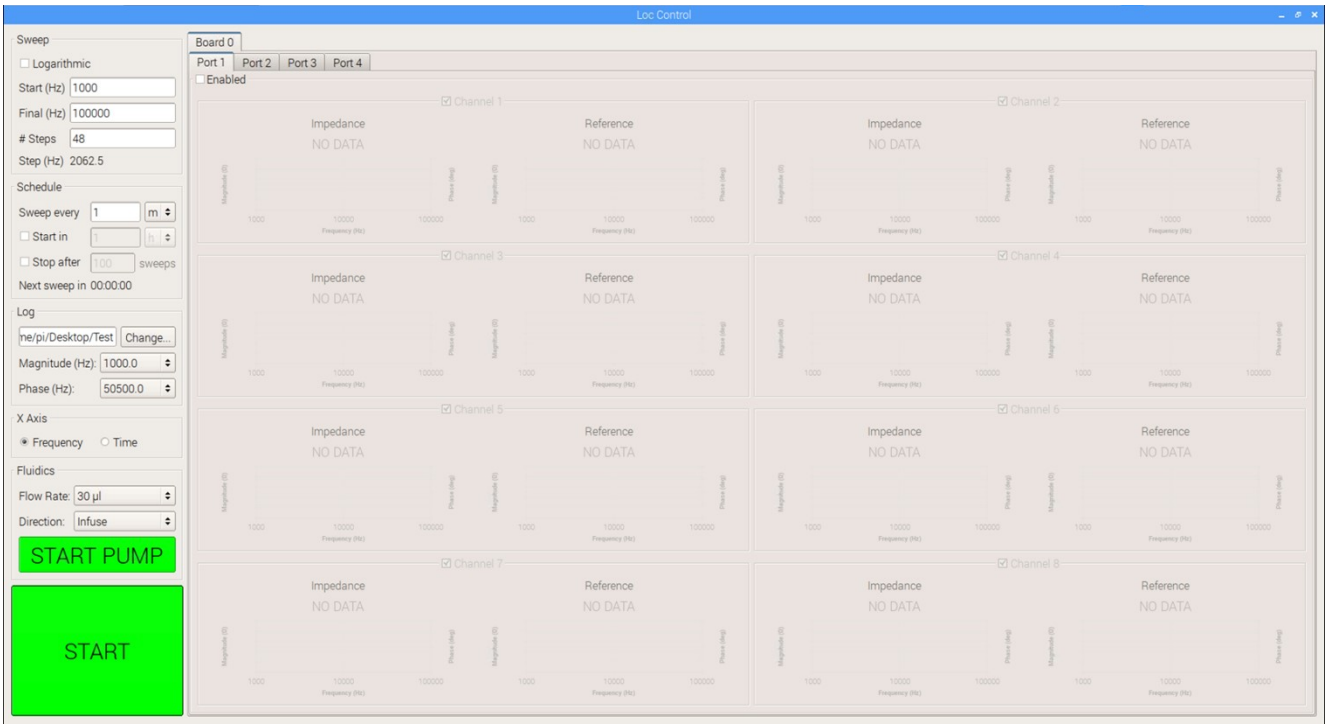
Electronic Supplementary Material (ESI) for Lab on a Chip.



ESI Figure 1: Assessment of impedance spectroscopy measurements using coplanar electrodes beneath the cell culture with different apical volumes. 16HBE14o- cells were cultured in Transwell supports for 5 days. Daily impedance spectra were obtained during apical medium replacement, with 200 µL or 10 µL of MEM medium. Data are from n=2 experiments in duplicate. Although the change in impedance magnitude and phase angle are less with 10 µL it demonstrates changes in barrier function can be measured but with reduced sensitivity. From [1].



ESI Figure 2: Interface used to control the homemade impedance analyser. The user determines the start and end frequencies for the EIS spectra, and the number of points in the range. Data is displayed in real-time as it is collected, either as individual spectra or the complex impedance at a specific frequency. The syringe pumps (controlled by an Arduino microcontroller) are also programmed through this interface.

Detailed Equation List:

Reference Electrode Equations

Electrode CPE:

$$Z_{CPEM} = \frac{1}{Q_M} \times (j\omega)^{-\alpha_M}$$

Cell Media Resistance:

$$Z_{Cell\ Medium} = R_{Cell\ Medium} = R_M$$

Final equation:

$$Z_{Reference\ Electrode} = Z_{CPEM} + R_M$$

Cell Electrode Equations

Electrode CPE:

$$Z_{CPEC} = \frac{1}{Q_C} \times (j\omega)^{-\alpha_C}$$

Basolateral Cell Media Resistance:

$$R_B = R_M \times \frac{Cell\ Constant_{Media\ Electrodes}}{Cell\ Constant_{Basolateral\ Compartment}}$$

Apical Cell Media Resistance:

$$R_A = R_B \times \frac{Cell\ Constant_{Basolateral\ Compartment}}{Cell\ Constant_{Apical\ Compartment}}$$

Cell Barrier Impedance:

$$Z_{CB} = 2 \times \frac{R_{CB}}{j\omega C_{CB} R_{CB} + 1}$$

Since electrodes are in a cis configuration, current flows through the epithelial cell barrier twice. It is assumed that the electrical field is uniformly applied to the entire porous membrane support (0.2 cm^2), as seen in the COMSOL simulation (figure 1).

Final equation:

$$Z_{CellElectrode} = Z_{CPEC} + \left[\left(2 \times \frac{R_{CB}}{j\omega C_{CB} R_{CB} + 1} + R_A\right)^{-1} + \frac{1}{R_B}\right]^{-1}$$

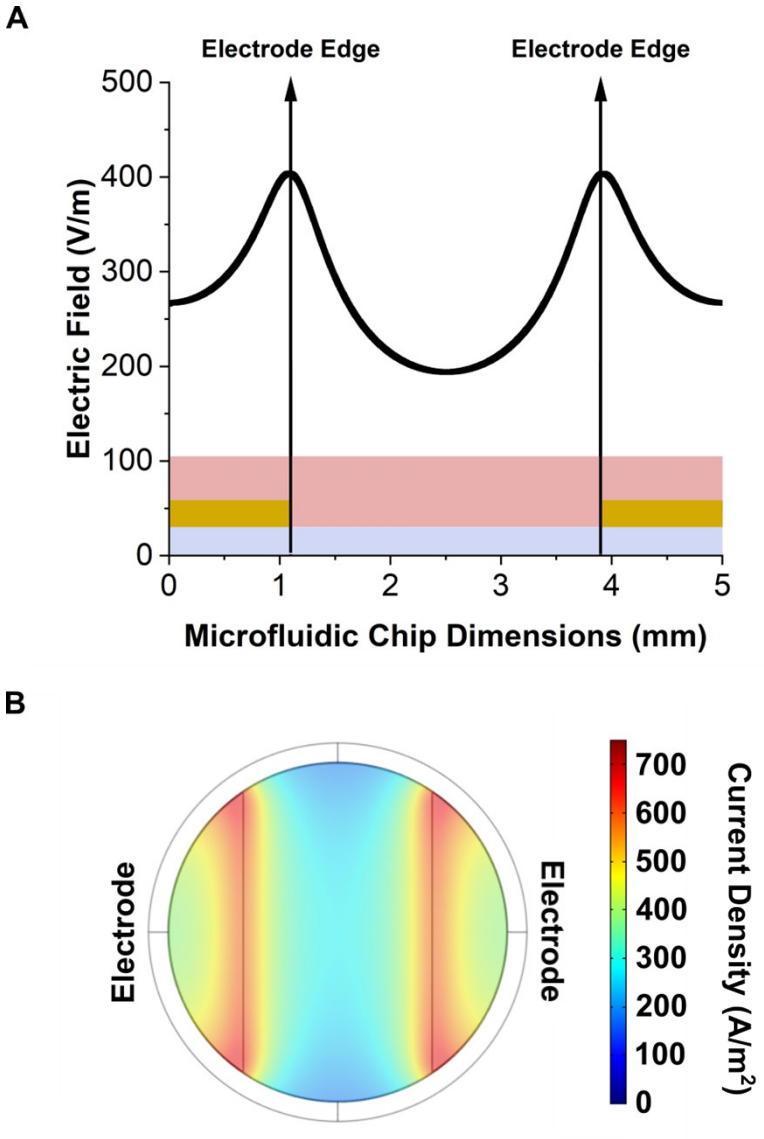
ESI Table 1: Variables used in the electrical circuit model and their respective upper and lower boundaries.

Media Electrode		**Cell Electrode**	
Variable	Boundary	Variable	Boundary
CPE Exponent (α_M)	0 – 1	CPE Exponent (α_C)	α_M ± 5%
CPE Magnitude (Q_M)	10^{-6} – 10^{-3}	CPE Magnitude (Q_C)	Q_M × 2.3 ±5%
Cell Media Resistance (R_M)	0 – 10^3 Ω	Basolateral Resistance (R_B)	R_B
		Apical Resistance (R_A)	R_A ± 5%
		Cell Barrier Resistance (R_{CB})	0 – 10^4 Ω
		Cell Barrier Capacitance (C_{CB})	10^{-9}- 10^{-7} F

Geometrical Cell Constants:

The geometric cell constants (*k*) are defined by electrode geometry, which in turn governs the electric field distribution in the compartments with the electrodes. Values of k for all compartments (media electrodes, cell electrode apical and basolateral compartments) were obtained from finite element simulations using COMSOL. The electric currents module was utilised. Each electrode compartment geometry was simulated separately. Electrodes and surrounding electrolyte conductivity (σ) were set to 1 S/m and a potential difference of 1 V was applied between electrodes. The geometrical cell constant (k) for each compartment can be calculated from:

$$R = \frac{1}{\sigma \times k}$$

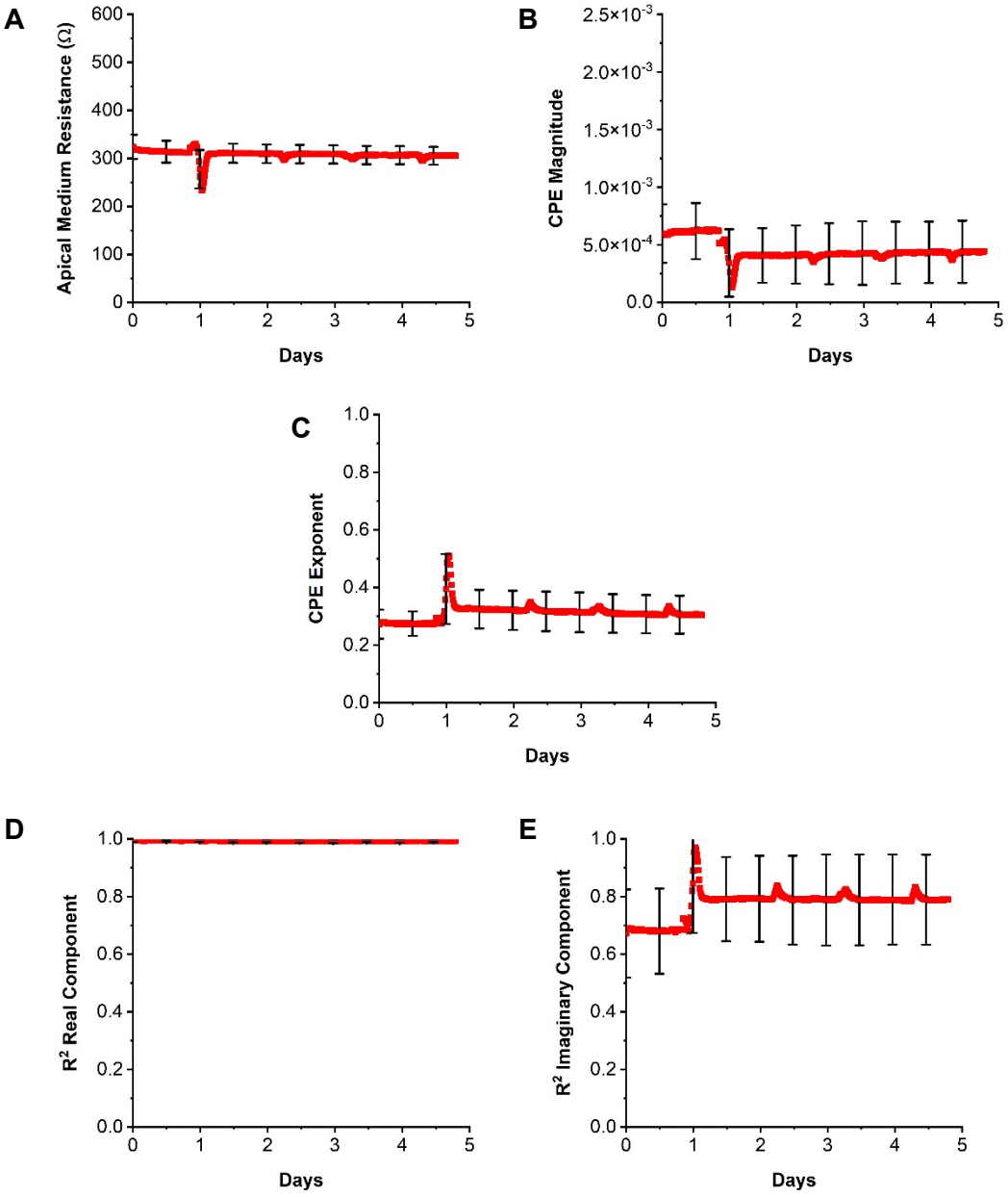


ESI Figure 3: (A) COMSOL simulation of electric field magnitude across the porous membrane. (B) Current density across the porous membrane surface.

The ratio in current density between basolateral and apical compartments (above the electrodes) is defined by the ratio of the respective geometrical cell constant.

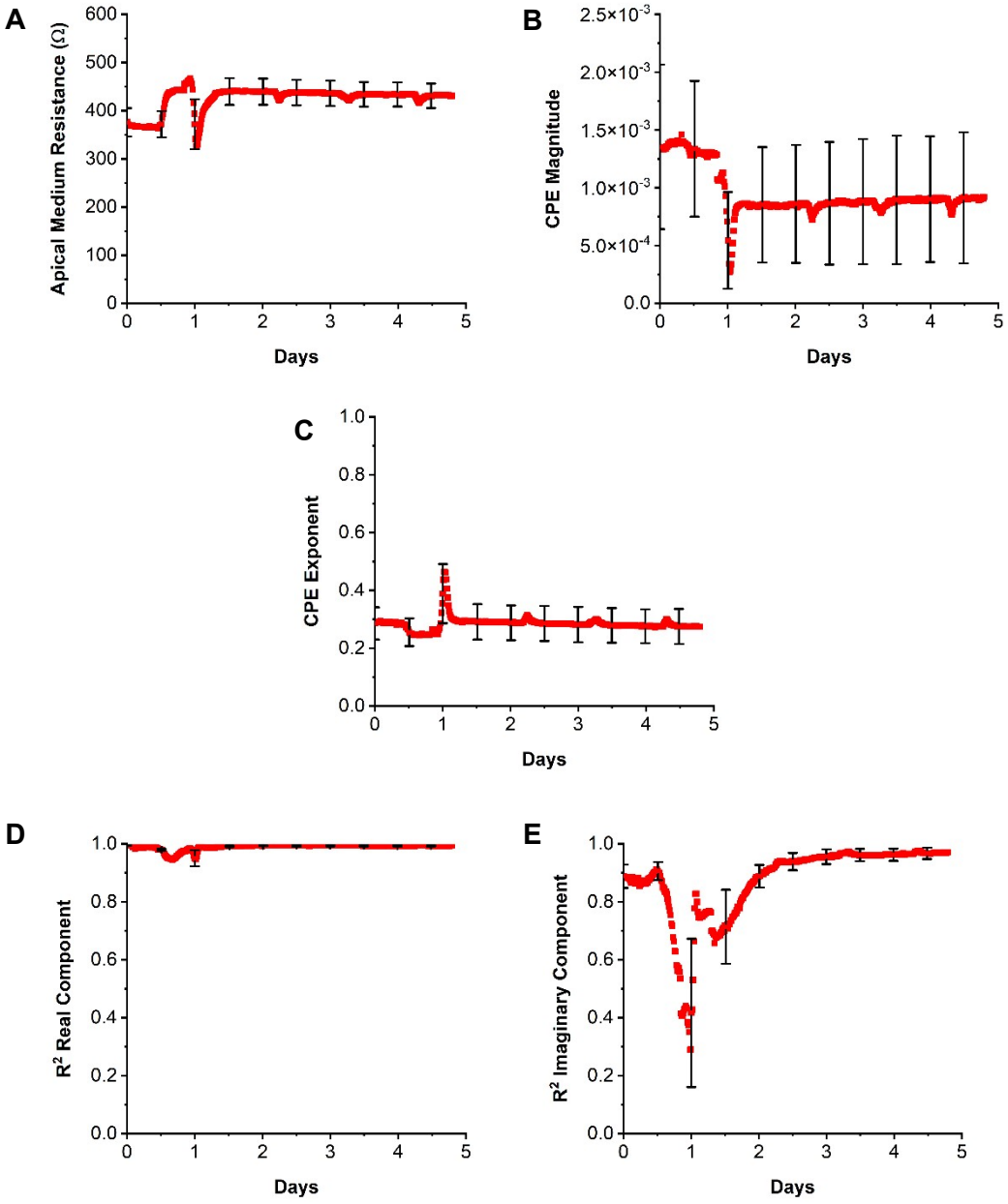
Caco-2 Growth

Media Electrode Parameters



ESI Figure 4: Calculated parameters for the cell medium resistance (A), media electrodes CPE magnitude (B) and exponent (C) during an experiment. R-squared was calculated for each EIS measurement, both real (D) and imaginary (E) components. Red line represents the average of 8 microfluidic chips operating in parallel and the error bars are the standard deviation between chips from data point recorded every 12 hours. The step change seen at day 1 is due to initiation of basolateral flow in the system.

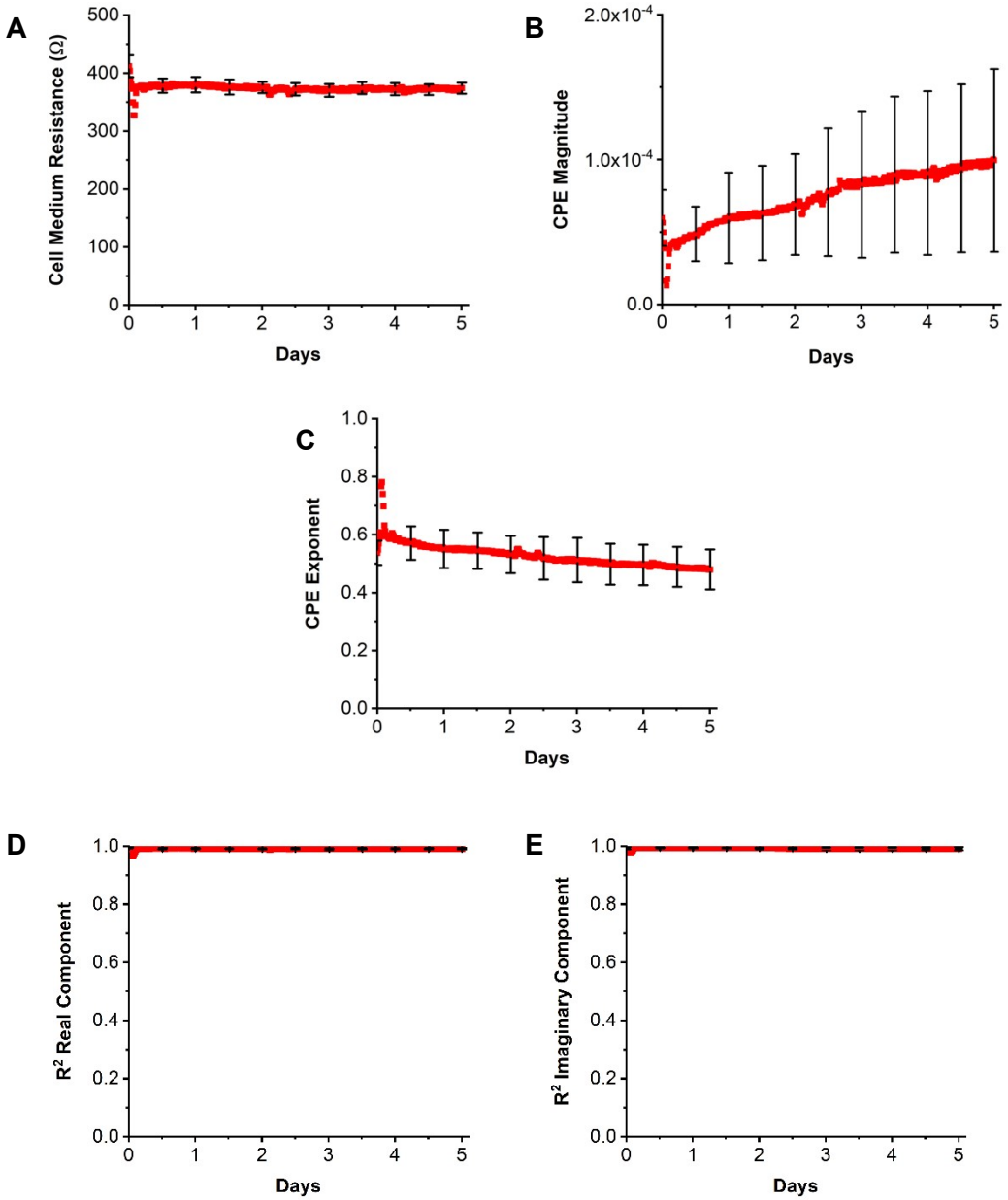
Cell Electrode Parameters



ESI Figure 5: Calculated parameters for the apical medium resistance (A) and media electrodes CPE magnitude (B) and exponent (C). R-squared was calculated for each EIS measurement, both real (D) and imaginary (E) components. Red line is the average for 8 microfluidic chips running in parallel. The error bars are the standard deviation plotted at 12-hour intervals. The step change seen at day 1 is due to initiation of basolateral flow in the system.

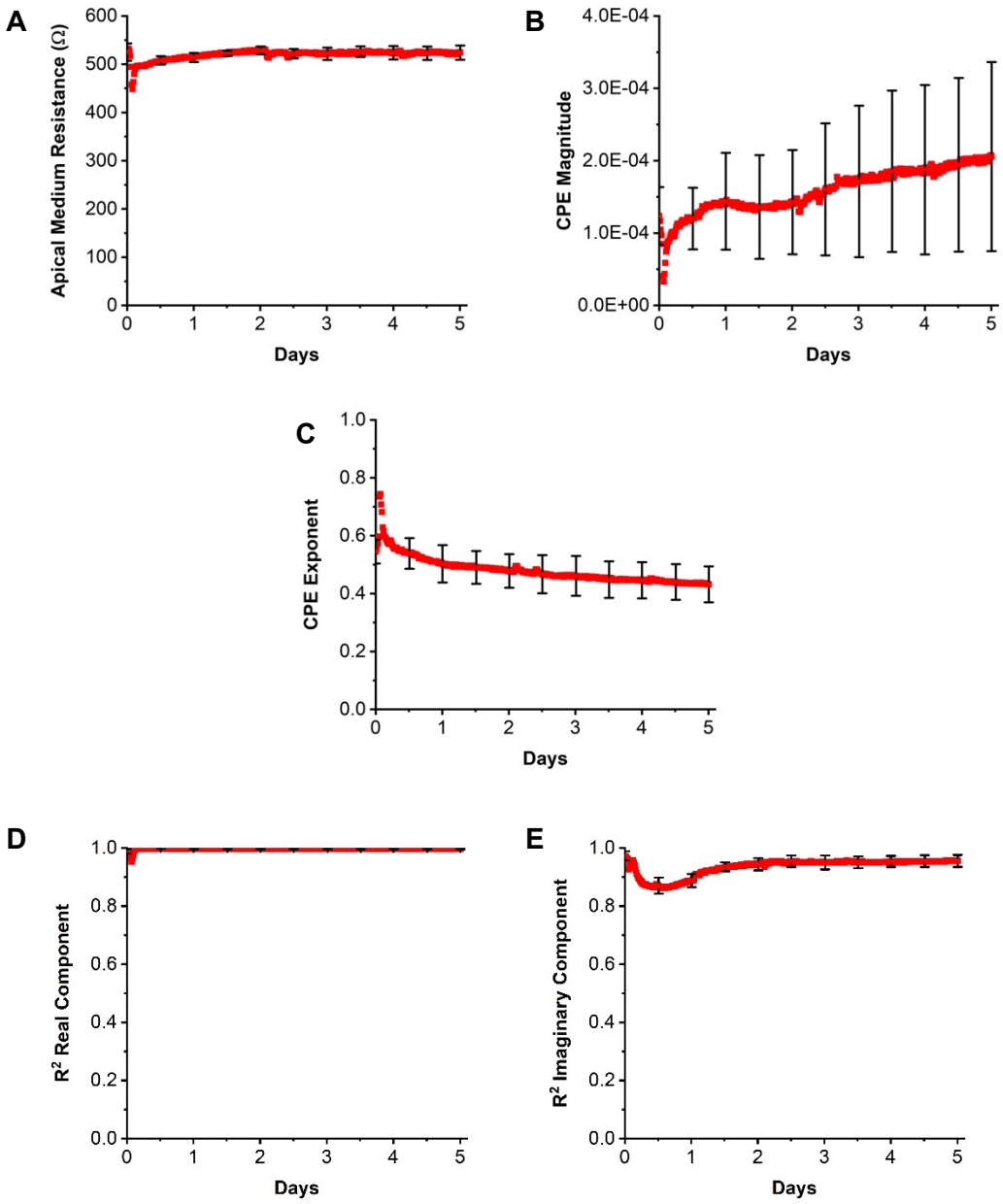
16HBE14o- Growth

Media Electrode Parameters



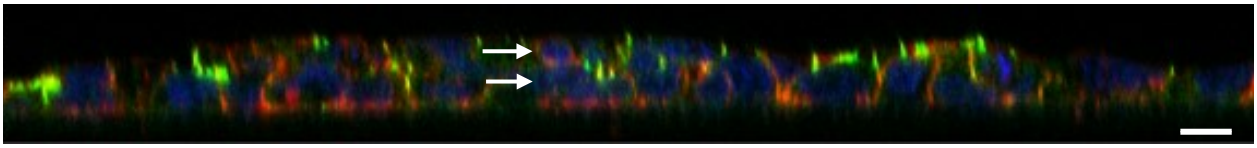
ESI Figure 6: Calculated parameters for the cell medium resistance (A), media electrodes CPE magnitude (B) and exponent (C) during an experiment. R-squared was calculated for each EIS measurement, both real (D) and imaginary (E) components. Red line represents the average of 8 microfluidic chips operating in parallel and the error bars are the standard deviation between chips from data point recorded every 12 hours.

Cell Electrode Parameters



ESI Figure 7: Calculated parameters for the apical medium resistance (A) and media electrodes CPE magnitude (B) and exponent (C). R-squared was calculated for each EIS measurement, both real (D) and imaginary (E) components. Red line is the average for 8 microfluidic chips running in parallel. The error bars are the standard deviation plotted at 12-hour intervals.

(A)



ESI Figure 8: Image showing example of cell stacking of 16HBE14o- cells grown in the microfluidic chip for 5 days. Z projection stack captured using confocal imaging at 63X at wavelengths of 405 nm (DAPI), 561 nm (Actin) and 488 nm (Occludin) (Leica TCS laser scanning microscope). White scale line indicates 10 μm. White arrows indicate a stack of two individual cells.

ESI References:

[1] Reale, Riccardo (2017) Microfluidic airway on-chip. *University of Southampton, Doctoral Thesis*, 169pp