Supplementary information

Title: High throughput transepithelial electrical resistance (TEER) measurements on perfused membrane-free epithelia

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S.1

S1.1 Biocompatibility and robustness test

Electrodes were made from stainless steel 316F. To ensure absence of adverse effects of the TEER electrode board on OrganoPlate grown models, OrganoPlates seeded with Caco-2 and RPTEC cells were cultured with an electrode board inserted into the wells from day 1 to day 7. After one week of culture, viability was evaluated via LDH and phase contrast imaging. No difference in morphology or viability was found between a plate cultured with or without the electrode board.

S1.2 Fitting methodology

The impedance model used for fitting the impedance data can be written as an impedance Z_m (Ω) measured at frequency f (Hz) and evaluated in the following equation:

$$Z_m = \left(\frac{1}{Z_{par}} + \frac{1}{R_{ser} + \frac{R_{teer} \cdot Z_l}{R_{teer} + Z_l}} \right)^{-1} \qquad (1)$$

All elements are complex impedances in Ω. R_{ser} being the microfluidic resistance and R_{teer} the resistance of the cell layer. Z_{par} and Z_l denote capacitances associated with the parasitic capacitance of the system and the cell capacitance respectively, and are described in the following equations:

$$Z_{par} = \frac{1}{2\,\pi\, i\, f\, C_{par}} \quad , \quad Z_l = \frac{1}{2\,\pi\, i\, f\, C_l} \qquad (2)$$

where $i = \sqrt{-1}$

Therefore, Z_m can be considered as a complex-valued function of frequency f *parameterized* by $R_{teer}, R_{ser}, Q_l, Q_{par}$, denoted by $g\,(f_k; R_{teer}, R_{ser}, Q_l, Q_{par})$.

We estimated TEER value R_{teer} from impedances measured at various frequencies $f_1, f_2, \cdots, f_N$. This was done by fitting the measured impedance $\{Z_m^{f_k}\}_{k=1}^{N}$ to the model (1) with (2), where $Z_m^{f_k}$ is the impedance measured at frequency f_k. In short, we applied a gradient descent-based optimization method to minimize the distance described by:

$$J(R_{teer}, R_{ser}, C_l, C_{par}) = \sum_{k=1}^{N} \left| g\,(f_k; R_{teer}, R_{ser}, C_l, C_{par}) - Z_m^{f_k} \right|^2$$

However, the minimization with this approach can be unstable when the influence of TEER on impedance is exceedingly low because $\left|\frac{\partial J}{\partial R_{teer}}\right| \approx 0, \left|\frac{\partial J}{\partial C_l}\right| \approx 0$ when $R_{teer} C_l \ll 1$. We then simplify (1) with (2) and rewrite Z_m as a rational polynomial of $s := i2\pi f$:

$$Z_m = \tilde{g}(s) = \frac{a_1 s + a_0}{b_2 s^2 + b_1 s + 1},$$

where the coefficients a_0, a_1, b_1, and b_2 are defined as

$$a_0 = R_{teer} + R_{ser},\ a_1 = R_{teer} R_{ser} C_l,$$

$$b_1 = (R_{teer} + R_{ser}) C_{par} + R_{teer} C_l,$$

$$b_2 = R_{teer} R_{ser} C_l C_{par}$$

To fully exploit the fact that the model is a form of rational polynomial, we applied the vector fitting method[1,2], which is specially designed for rational polynomials, to estimate the coefficients a_0, a_1, b_1, b_2 from the measured impedances $\{Z_m^{f_k}\}_{k=1}^{N}$. Then, we estimated the parameters $R_{teer}, R_{ser}, C_l, C_{par}$ as:

$$C_{par} = \frac{b_2}{a_1},\ R_{ser} = \frac{a_1^2}{a_1 b_1 - a_0 b_2},$$

$$R_{teer} = a_0 - \frac{a_1^2}{a_1 b_1 - a_0 b_2},\ C_l = \frac{b_1 - a_0 C_{par}}{R_{teer}}$$

1B. Gustavsen and A. Semlyen, "Rational approximation of frequency domain response by vector fitting", IEEE Transactions on Power Delivery, vol. 14, no. 3, pp. 1052–1061 , 1999.

2B. Gustavsen, "Improving the pole relocating properties of vector fitting, IEEE Transactions on Power Delivery, vol. 21, no. 3, 2006

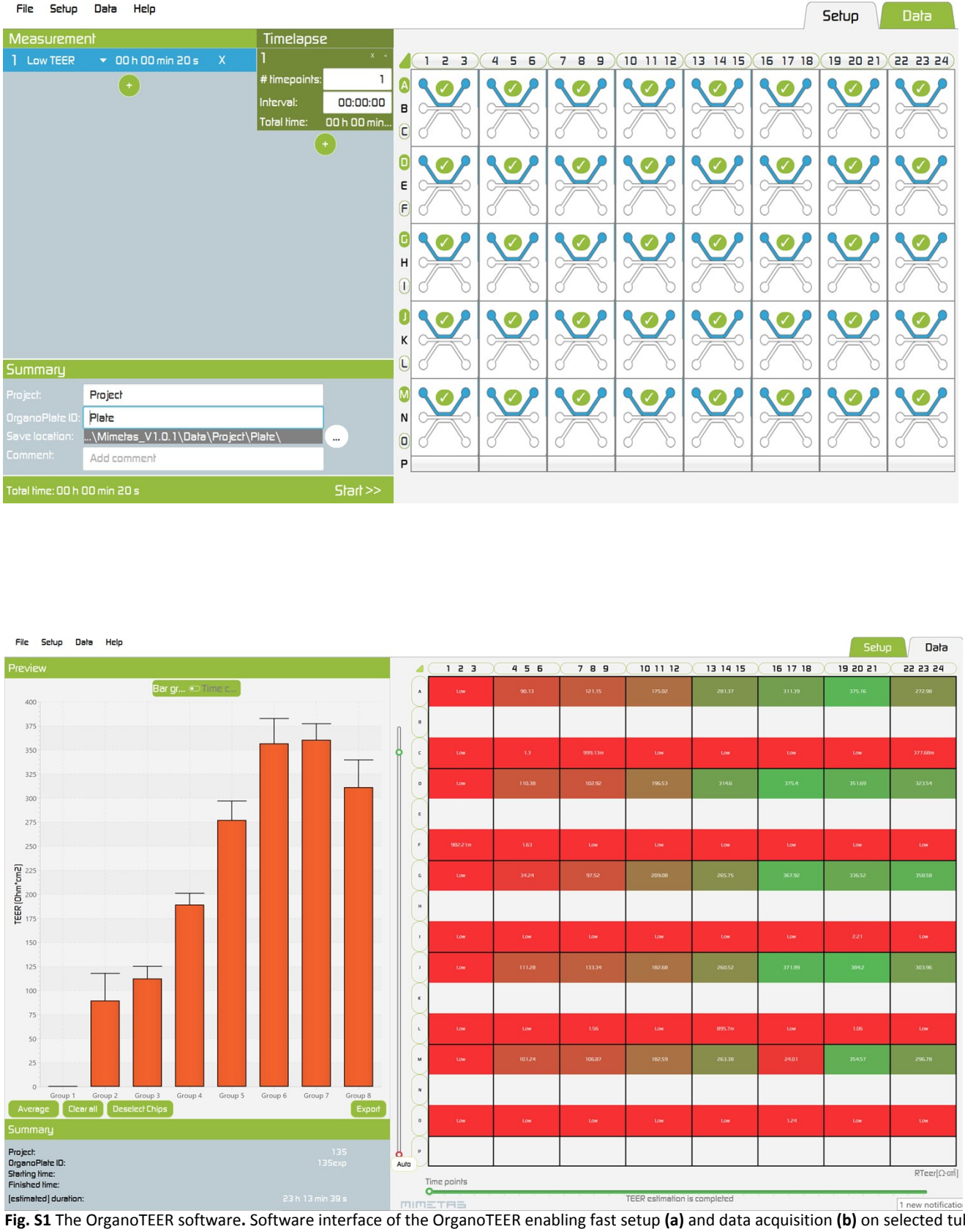


Fig. S1 The OrganoTEER software**.** Software interface of the OrganoTEER enabling fast setup **(a)** and data acquisition **(b)** on selected tubules and time-lapse configuration.

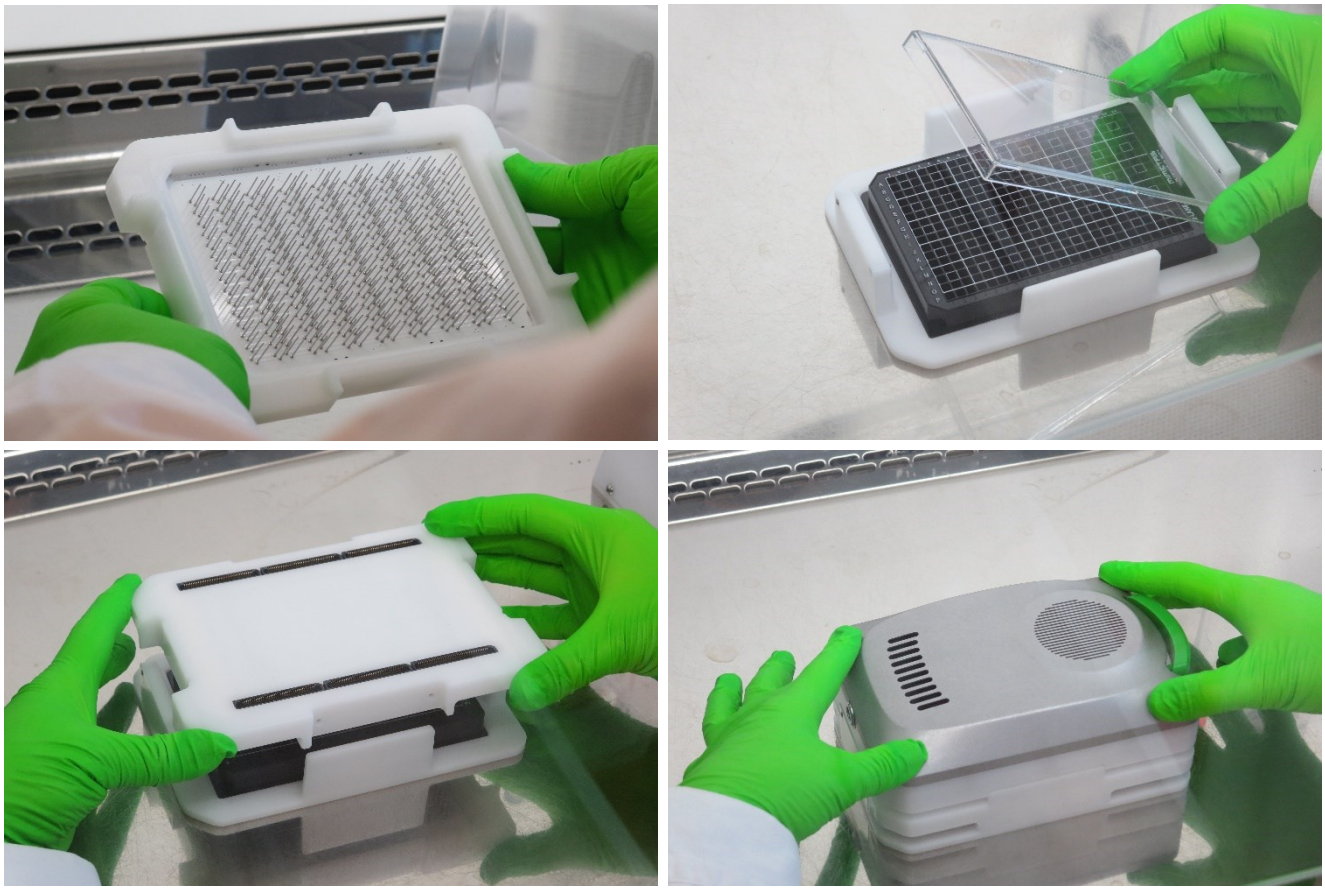
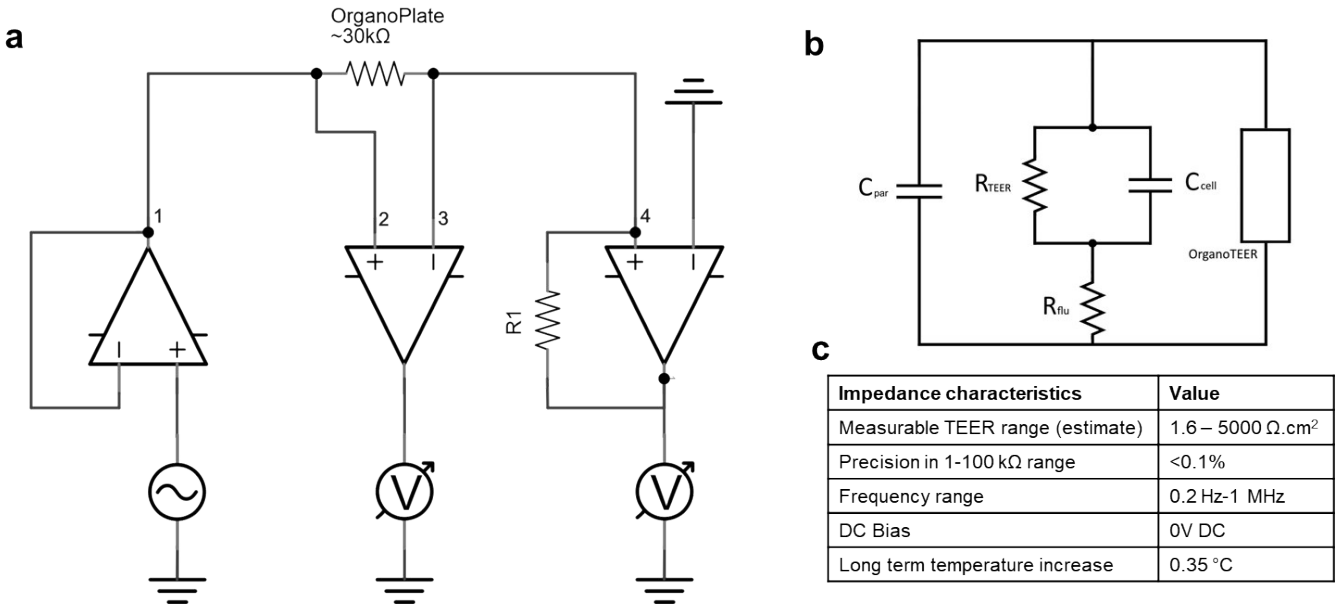


Fig. S2 Pictures of the assembling process of the OrganoTEER. A sterile electrode board **(a)** is inserted into an OrganoPlate **(b)** using a guiding plate holder **(c)**. The measurement unit is then connected on top of the electrode board **(d).**



c

Impedance characteristics	Value
Measurable TEER range (estimate)	1.6 – 5000 Ω.cm^2
Precision in 1-100 kΩ range	<0.1%
Frequency range	0.2 Hz-1 MHz
DC Bias	0V DC
Long term temperature increase	0.35 °C

Fig. S3 Simplified schematic of the AC voltage actuation and sensing as performed in the OrganoTEER **(a)** A Simplified schematic of the AC voltage actuation and sensing as performed in the OrganoTEER (a) A controlled voltage source linked to an amplifier was used to impose a current of constant sinusoidal voltage on port 1. A voltage sensor linked to a transimpedance amplifier measured the current flowing through the Organoplate on port 4. The voltage between port 2 and 3 was further measured to compensate for voltage drop due to parasitics and electrical double layers on the current carrying electrodes. **b).** Simplified model of the OrganoPlate. An OrganoPlate grown tubule can be viewed as an electrical circuit where the cell membrane constitutes an electrical resistance R_{TEER} (mostly correlated to the paracellular pathway) and an electrical capacitance C_{cell} (linked to the transcellular pathway) in series with the microfluidic resistance R_{flu}. The parasitic effects are represented by a parallel capacitance C_{par}. The full methodology is presented in Suppl. Data S.1†. **(c).** Measurement characteristics of the OrganoTEER. Values in Ω can be converted to Ω.cm^2 by multiplication with the estimated meniscus area of 0.0057cm^2.

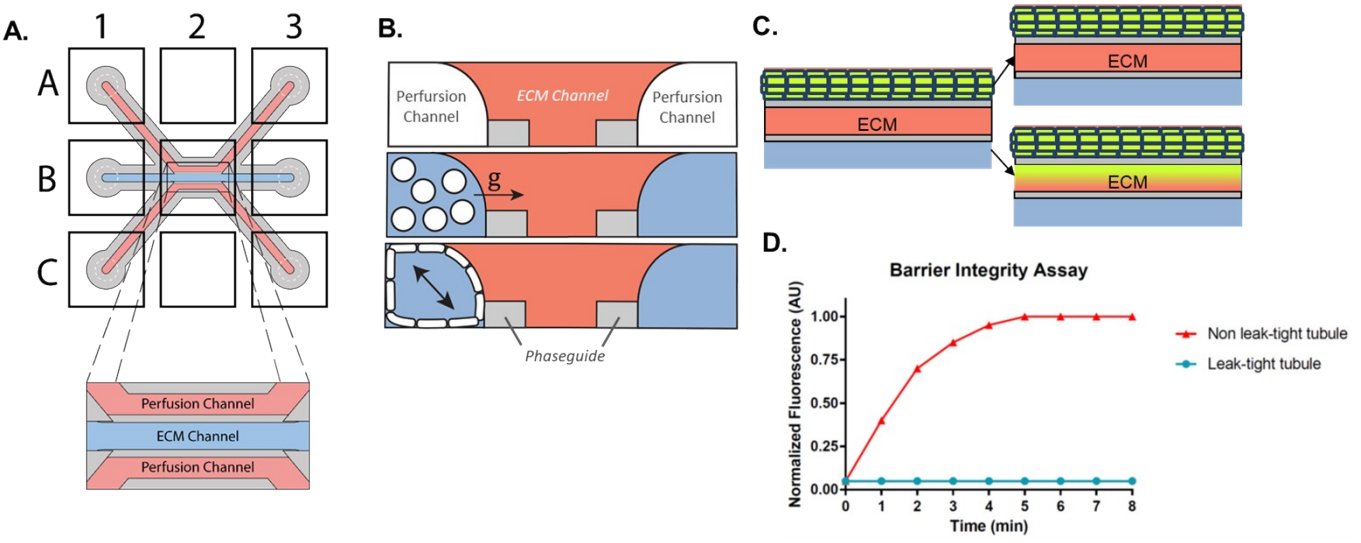


Fig. S4 Seeding of an OrganoPlate and Barrier Integrity assessment **(a)** A single chip of the OrganoPlate 3-lane. Each square corresponds to a well. Six wells hold medium and provide access to three channels that converge below the center well. The outer channels are separated by ECM patterned by using phaseguides, enabling membrane free contact between compartments. **(b)** Schematic cross-section of the microfluidic channels of a 3-lane chip. A tubule can be formed on either side of the chip. ECM is first loaded in the centre compartment. The meniscus formed at the edges of the phaseguides allows sedimentation of endothelial or epithelial cells. Application of gravity assisted flow of media leads to tubule formation within 2-4 days period **(c)** By perfusing a fluorescent compound in an OrganoPlate grown tubule, we can monitor the diffusion (or lack thereof) through the cell barrier into the ECM area. **(d)** Using time-lapse imaging, we can monitor the diffusion rate of a fluorescent dye from the tubule into the ECM compartment. A more permeable tubule will result in a steeper increase of fluorescent levels inside the ECM.