A high throughput TEER measurement device for on-chip tissue barrier assessment

By Finnadvance

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Fig. 1 The AKITA® Plate 96

Introduction

Trans Epithelial/Endothelial Electrical Resistance (TEER) is a measurement technique that has been widely adopted to evaluate tissue barrier function in vitro, typically in organ-on-chip (OOC) models.
This method is a non-invasive, label-free alternative to tracer transportation assays which makes it suitable for non-destructive, real-time assessment of cellular response to biochemical stimuli, enabling high throughput drug screening capability of OOC technology. However, the widely used conventional TEER measurement devices in the market offer single-channel, fixed-frequency impedance measurement through chopstick-like electrodes. As a result, the handheld TEER readings provide low reproducibility, and non-uniform current density, and are extremely time-consuming.

We introduce the AKITA® Lid, a high throughput TEER device that performs fast and reliable measurement coupled with a standardized OOC platform termed AKITA® Plate, which allows the establishment and measurement of up to 24 barrier cultures on one ANSI/SLAS standard plate. With our platform, the Gut-On-Chip and Skin-on-chip models were developed and assessed using TEER.

Methods

AKITA® Plate platform for Gut-on-Chip and Skin-on-Chip model

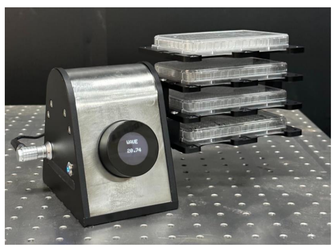


Fig. 2 The AKITA® Wave rocker

- ANSI/SLAS standard footprint
- 24 experiments, scalable up to 96
- Pumpless flow control

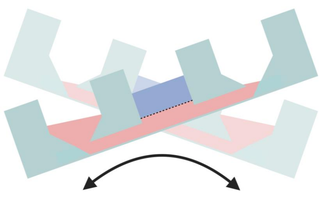


Fig. 3 Gravity-induced flow by AKITA® Wave

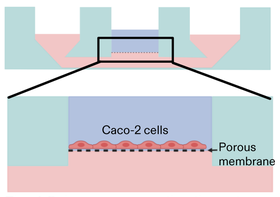
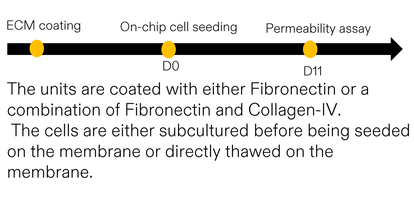


Fig. 4 Scheme representing the gut-on-chip model in AKITA® Plate



The units are coated with either Fibronectin or a combination of Fibronectin and Collagen-IV.
The cells are either subcultured before being seeded on the membrane or directly thawed on the membrane.

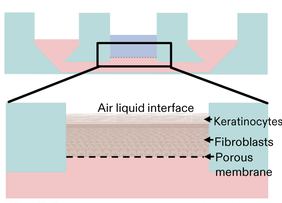
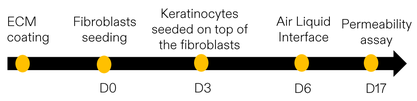


Fig. 5 Scheme representing the skin-on-chip model in AKITA® Plate



Because high amounts of calcium have been shown to increase the maturation of keratinocytes, both high and low concentrations have been tested. We also test the dynamic (flow) and static conditions.

AKITA® Lid

- Tetrapolar electrodes configuration (6 electrodes are used to distribute the electric field across the membrane evenly)
- 100Hz to 200kHz measurement range
- Gold-plated electrodes
- Measurement under 2 minutes for the AKITA® Plate96

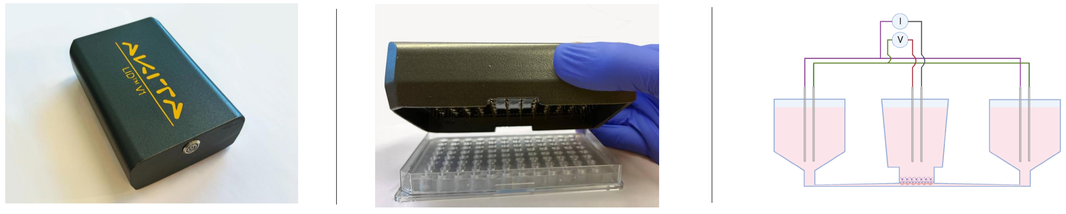


Fig. 6 The AKITA® Lid (left), in operation with the AKITA® Plate96 (middle), a single cell culture unit with the submerged electrodes (right)

Results

1. Assessment of the AKITA® Lid performance

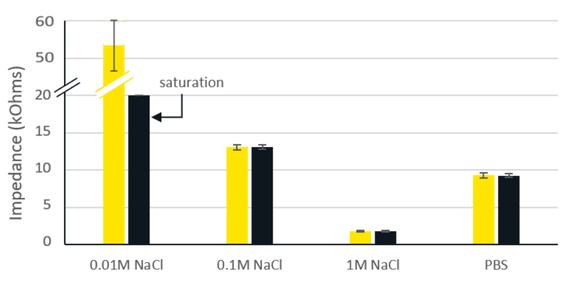
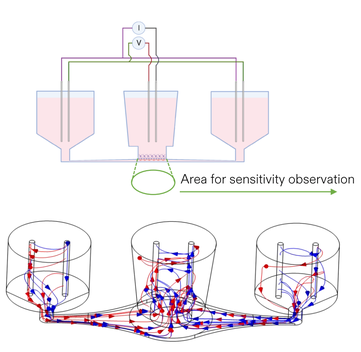


Fig. 7 Impedance measurement made by the AKITA® LID and the EVOM1 (World Precision instruments) for solutions of varying conductivity. N=4

We performed impedance measurements on the AKITA® Plate 96 filled with buffers at different ion concentrations and compared them to the same measurements by EVOM1 (World Precision Instruments). As expected, as the ions concentration increases, the impedance decreases. The EVOM1 and our AKITA® Lid have consistent results, except for the least ion-concentrated buffer: the EVOM1 was saturated at 20 kOhms impedance due to its fixed frequency AC current (12 Hz).

2. Assessment of the sensitivity of the TEER measurement



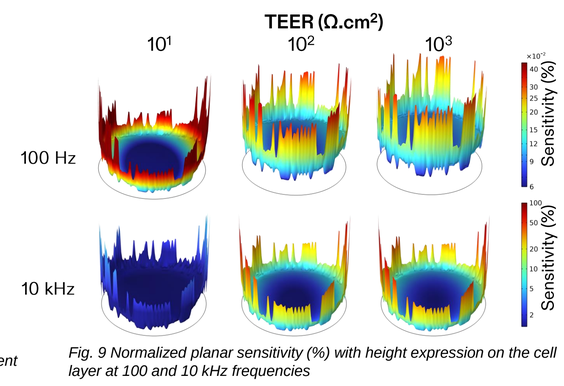


Fig. 8 Current density streamline plot for the normal current (blue colour) and the reciprocal current (red colour).

Fig. 9 Normalized planar sensitivity (%) with height expression on the cell layer at 100 and 10 kHz frequencies

Sensitivity field distribution on the membrane where the cells grow was simulated using COMSOL Multiphysics. Alternative current (AC) of 10µA was injected through the Current Carrying (CC) electrodes and Voltage Sensing (VS) electrodes were set as floating potential.

Sensitivity is directly proportional to the TEER at higher frequency whereas at very low frequency, sensitivity is inversely proportional to TEER.

3. Barrier integrity of the Gut-on-Chip model

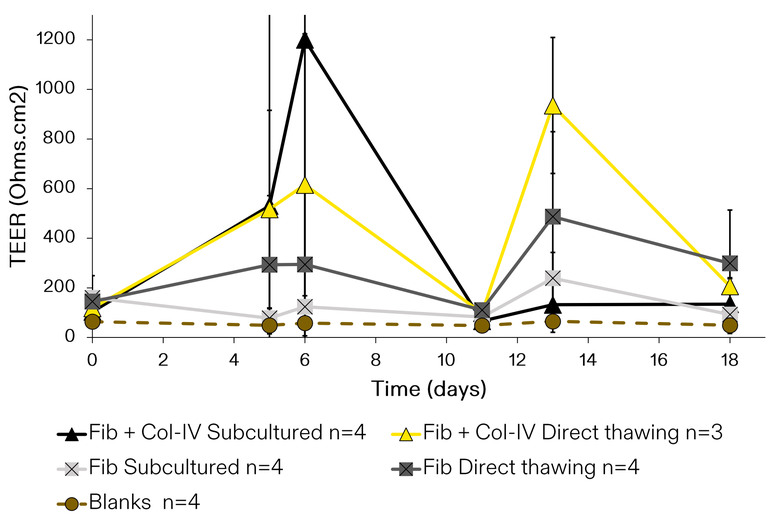


Fig. 10 TEER values for units measured during the model maturation up to 18 days. Cell are cultured either with Fibronectin coating (Fib) or a combination of fibronectin and Collagen-IV (Fib + Col-IV)

All groups of cells show a TEER between 200 and 1200 Ohms.cm2, which is consistent with the values found in the literature [1].

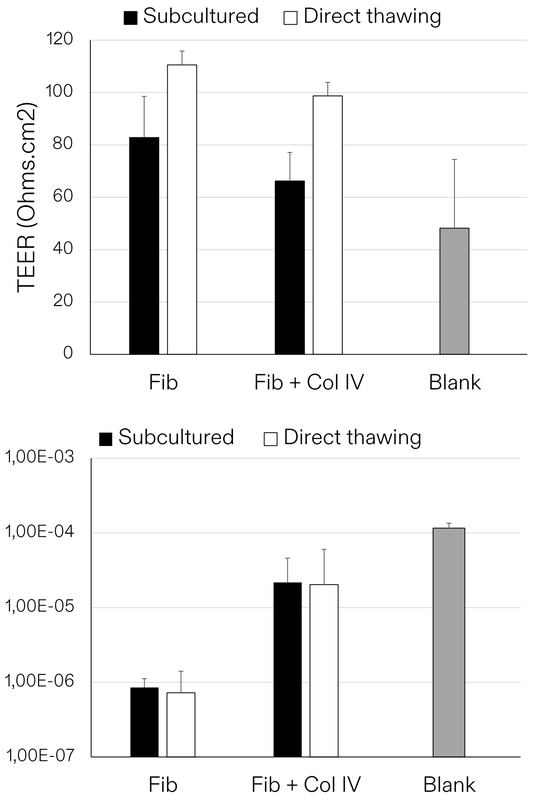
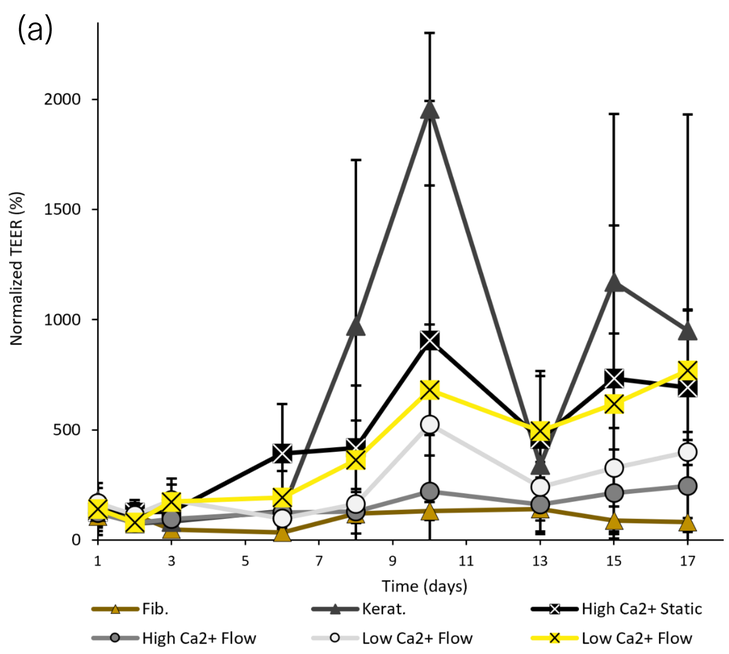
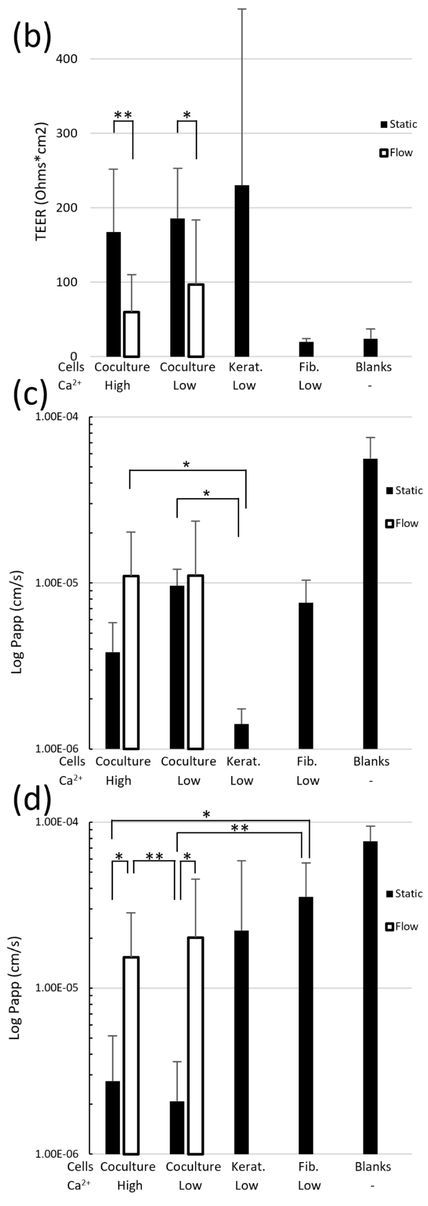


Fig. 11 The TEER values acquired at day 11 (up). Apparent permeability values for the same gut-on-chip units measured with 0,4 kDa dextran (down)

Fibronectin appears to give better TEER results for both subcultured and directly thawed cells, which is consistent with the permeability results: the permeability is lower than 1E-6 cm/s for both subcultured and directly thawed cells grown on fibronectin. The permeability values are similar for cells grown on the same coating material. The AKITA® Lid helps highlighting the differences between those groups.

4. Barrier integrity of the Skin-on-Chip model





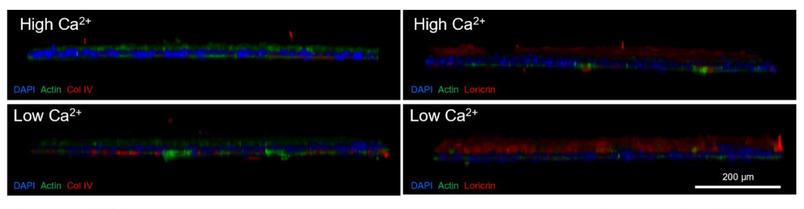


Fig. 12 (a) TEER values for units measured during the model maturation up to 17 days (b) The TEER values acquired at tissue maturation, day 17. (c) Apparent permeability values for the same skin-on-chip units measured with 0,4 kDa Dextran, and 4kDa Dextran (d). (e) Cross-sectional confocal images from stained skin-on-chip models cultured either with high or low Ca2+medium. Stained with DAPI (blue), phalloidin (green),and collagen IV (left panel, red) or loricrin (right panel, red).

References:
[1] Bikle, D. D., Xie, Z. & Tu, C.-L. Calcium regulation of keratinocyte differentiation.Expert RevEndocrinol Metab7, 461–472 (2012).
Srinivasan B, *et al.*, TEER measurement techniques for in vitro barrier model systems. J Lab Autom. ;20(2):107-26 (2015)

Conclusion

We presented a high throughput TEER device, AKITA® Lid, that enables fast and reliable measurements, with a wide range of frequencies. The device ensures the reproducibility of the measurements thanks to the standardized and high throughput multiplexed design of the electrode array. The device was validated by multiple approaches including benchmarking with a commercially available device as well as real measurements on in vitro gut model and a skin model, formed by the AKITA® Plate96. Our results also suggest that the AKITA® Plate and the AKITA® Lid can further be applied to a wide range of tissue barrier models to accelerate in vitro model development in the context of drug testing.

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