Electrical impedance spectroscopy: to evaluate and monitor organotypic development and skin barrier function *in vitro*

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1 Background

For research into skin biology, diseases, and drug or chemical interactions, organotypic 3D human epidermal equivalents (HEEs) are frequently used. Studies heavily rely on end-points analysis for which HEEs are harvested to study cellular responses. Non-intrusive methods that enable longitudinal analysis by repetitive measurements can minimize batch effects, increase study reproducibility and maximize experimental throughput. Here we used a novel 12-well format Electrical Impedance Spectroscopy (EIS) device, customized to fit with a 24-transwell cell culture system to replace conventional static and labor-intensive transepidermal electrical resistance (TEER) analysis using voltohmmeters.





A) The Locsense Artemis, a novel impedance measuring setup, provides a method for measuring EIS spectra optimized for simultaneous, multi-well measurements of 3D HEEs in 24-wells plate setup.
B) Close up of lid showing electrodes. C) Lid of the setup allows for easy cleaning of probes with ethanol. D) Schematic overview of measuring setup, resulting in both impedance as well as phase data.
E) Schematic overview of 3D HEE culture. Stimulations were added from day 5 of air exposure onwards.
EIS could be measured daily for HEEs from air exposure onwards.



A) FLG, AHR, TFAP2A, and CLDN1 were knocked out using CRISPR/Cas9 in immortalized N/TERT-2G keratinocytes, resulting in lowered EIS. **B)** Impedance at 10³ Hz was used for statistics (one-way ANOVA with Tukey multiple testing correction, *** p<0.0002, **** p<0.0001). **C)** Morphology confirms terminal differentiation defects in knock out cell lines.

3 EIS increases over time during HEE formation



5 Induction of differentiation restores EIS values



A) Atopic dermatitis associated Th2 cytokines were added to HEEs, causing impedance decline. Therapeutic AHR agonists (AHR1–5) or AHR-blocking ligands (MOCK1–2) were added as well to assess



EIS was measured during HEE development to determine the formation of the skin barrier. **A)** Impedance indicates an increase in barrier functionality from day 1–10 of HEE development, after which impedance is declining on days 12-14. **B)** EIS spectra correlate to epidermal thickness $(10^2-10^3$ Hz range), differentiation protein expression (see **D**), and **C)** stratum corneum thickness $(10^4-10^5$ Hz range). * p<0.05 **D)** Morphology of HEEs show increasing epidermal stratification over time. At day 12– 14, increase in stratum corneum and loss of stratum granulosum and epidermal thickness is observed, possibly explaining the declining electronic impedance. rescue of barrier defects. **B)** Some AHR ligands bind and activate the AHR pathway, whilst others only bind without activation. **C)** Morphological analysis confirms that Th2 cytokine treatment partially impairs epidermal differentiation, explaining the impedance results. MOCK1–2 treatment does not rescue this, whereas AHR activation by AHR1–5 treatment does (only MOCK1 and AHR1 are shown).

6 Conclusion and perspectives

- Impedance spectroscopy is a powerful tool to monitor epidermal barrier formation in HEEs without damaging construct, hampering epidermal development, and thus the need to harvest the culture.
- Impedance at low frequencies (100-2000 Hz) correlate to epidermal thickness and differentiation, impedance at high frequencies (>10.000 Hz) correlate to *stratum corneum* thickness.
- Deliberate impairment of the epidermal barrier through CRISPR/Cas9–induced genetic engineering or Th2 cytokine–induced inflammatory conditions, results in diminished impedance spectra.
- Th2 cytokine—induced inflammation can be rescued by AHR-activating ligands, effectively restoring diminished impedance spectra. AHR-blocking ligands show no such therapeutic properties.

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