

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

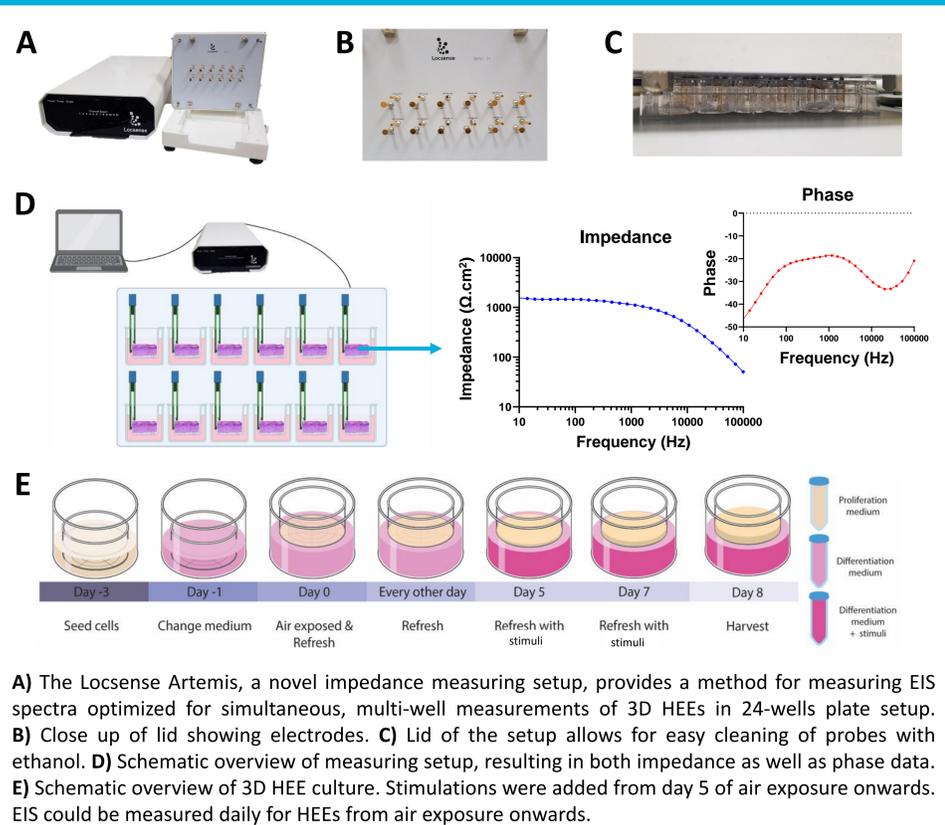
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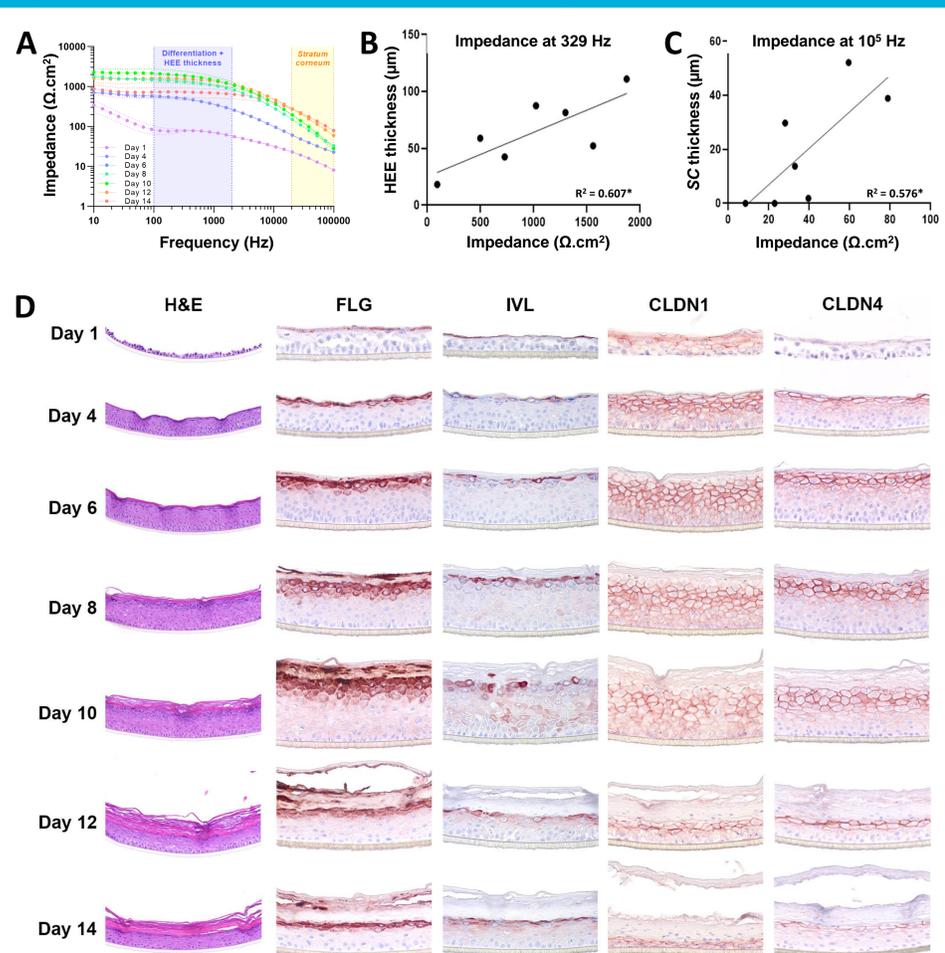
1 | Background

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.

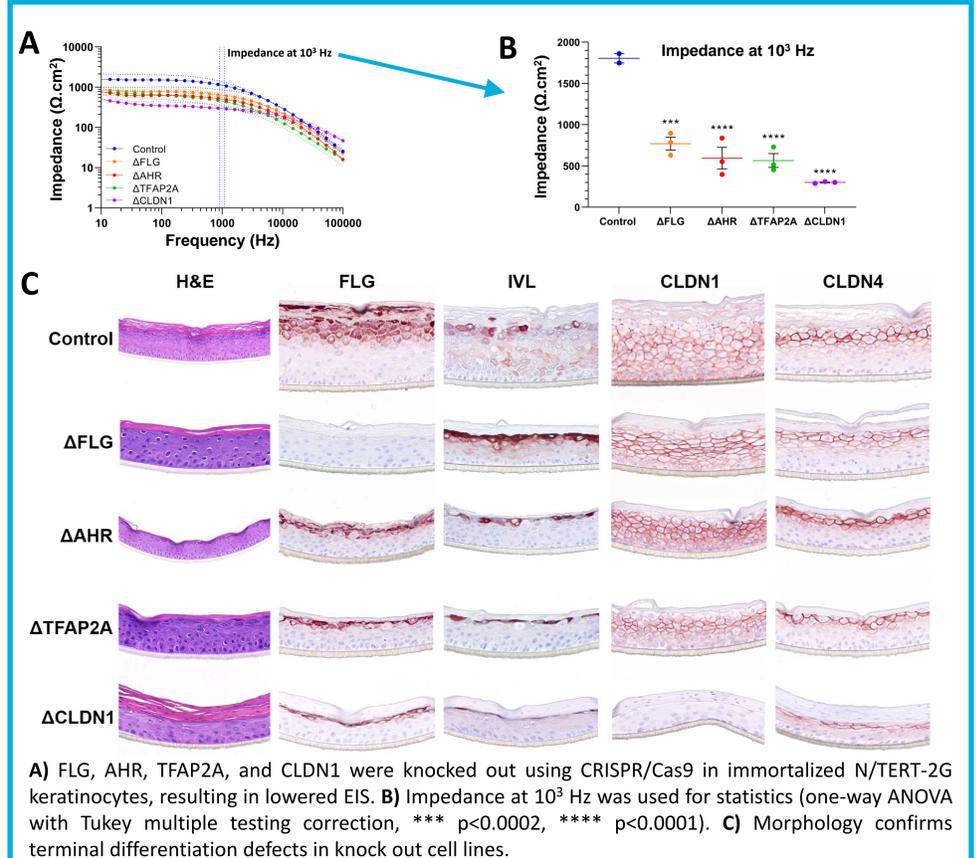
2 | Electrical impedance spectroscopy (EIS)



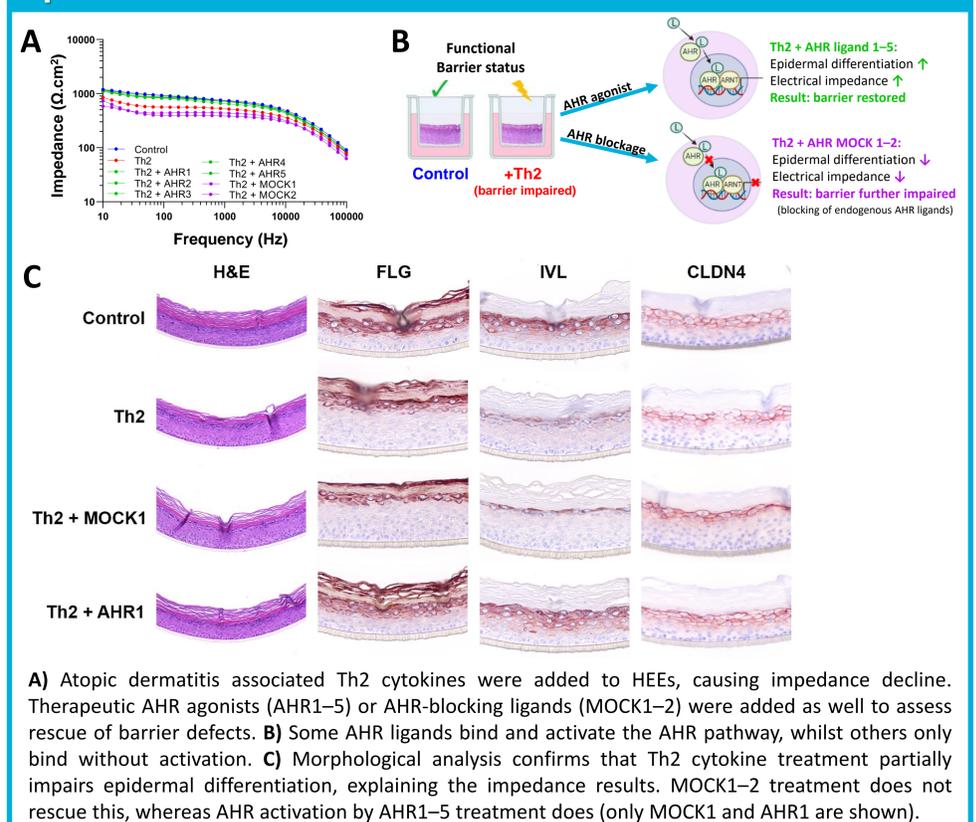
3 | EIS increases over time during HEE formation



4 | Barrier defects result in diminished EIS values



5 | Induction of differentiation restores EIS values



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.