Case study

Monitoring barrier integrity and quantification of CFTR function

Cells: **FRT with wild-type human CFTR**

Tools:
AtlaZ

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John Lueck, Associate Professor at University of Rochester Medical Center. His current research is focused on developing therapeutic engineered tRNAs for suppression of disease causing nonsense mutations, and one focus lies on evaluating CFTR mutant variants.



Kari Thrasher, PhD, Postdoctoral Fellow in John Lueck´s lab. Her current work focuses on designing and performing assays to evaluate CFTR mutant variants.

CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) and its primary role as an anion channel has been implicated in various cellular processes, including epithelial differentiation, proliferation, tight junction formation and cancer[1,2]. CFTR helps maintain the balance of ions and water across epithelial membranes and mutations in the CFTR gene result in cystic fibrosis[3]. TEER (Transepithelial Electrical Resistance) measurements are a highly effective technique for probing CFTR function in epithelial monolayers, because of the ability to resolve changes in transcellular (e.g. ion channels) and paracellular (e.g. tight junction permeability) ion conductance. Continuous, non-invasive recordings capture dynamic responses with high temporal resolution. This is crucial for evaluating how CFTR mutations, activators, or pharmacological correctors impact epithelial barrier function. Such investigations are of central importance for cystic fibrosis research and drug discovery.

ECIS (electrical cell-substrate impedance sensing) is a versatile technique allowing for TEER measurements and, in general, used to characterize the electrical properties of biological systems by measuring their impedance across a range of recording frequencies[4]. Among various cell-based assays, this non-invasive technique is particularly useful for studying cellular behaviors such as attachment, proliferation, and morphology changes.

Monitoring CFTR function

Real-time monitoring: Impedance-based TEER recordings serve as a label-free technique for quantifying barrier integrity and functional changes in epithelial or endothelial cell monolayers, making them especially relevant in studies of CFTR function and dysfunction. Importantly, this approach enables assessment of CFTR-mediated effects on barrier



Nanion's AtlaZ platform
AtlaZ enables label-free and real-time cellular research on barrier function, TEER and cell adhesion, cytotoxicity and morphology change. Up to six 96-well plates can be assessed simultaneously or independently.

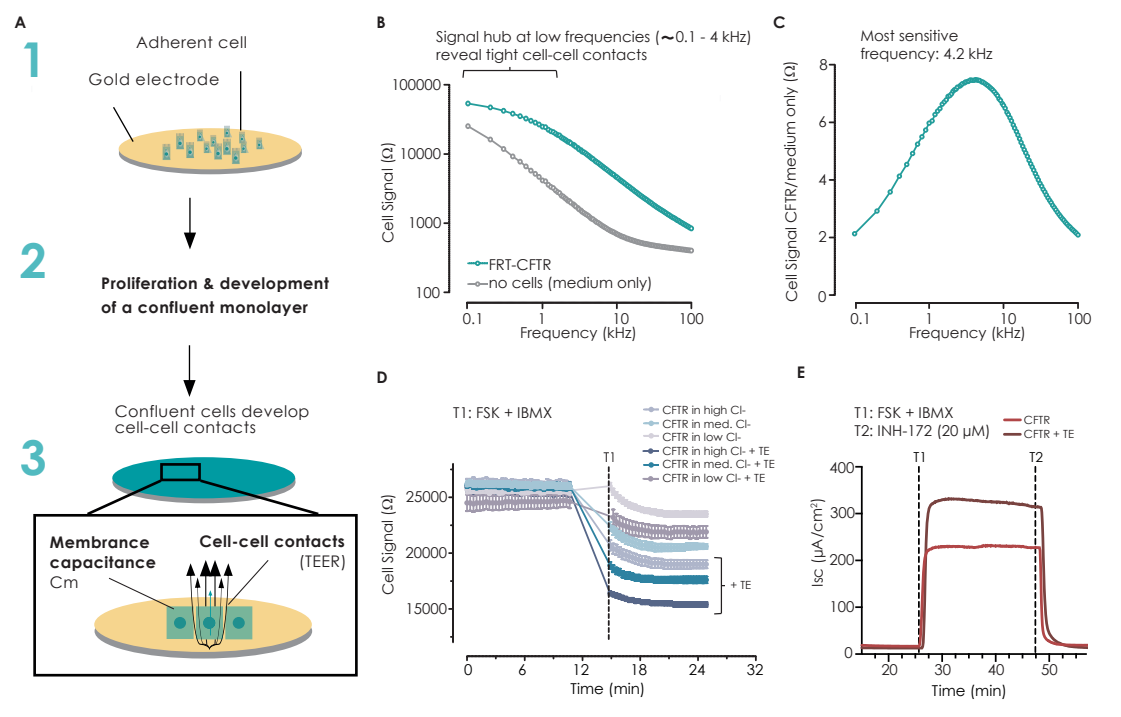
> **"CFTR-dependent ion transport changes epithelial transcellular resistance. AtlaZ captures these changes continuously, sensitively, and non-invasively, making it a powerful tool for studying CFTR biology and drug responses."**
>
> **John Lueck**, Associate Professor at University of Rochester Medical Center, USA

physiology, rather than solely on ion transport as measured in Ussing chamber assays.

CFTR function in disease-in-a-dish models: We used FRT cells which are an immortalized epithelial cell line derived from Fisher rat thyroid. They do not naturally express CFTR, thus they can be transfected with human CFTR to study its function[5]. Here, human CFTR wild-type was transfected into the cells. By quantifying the impedance or resistance (Cell Signal) of cells adhered on the substrate of AtlaZ recording plates, we could gain insights into the physiological and morphological changes occurring within the cells upon addition of activators, potentiators and inhibitors. Such an experimental outline is crucial for modeling diseases where CFTR mutations play a pivotal role.

In general, with AtlaZ, barrier function and cell junction integrity and transepithelial resistance can be studied, which are often compromised in disease states. The technology's sensitivity to detect changes at subnanometer levels[6] makes it an ideal tool for observing subtle cellular responses to treatments.

CFTR channel modulation: Forskolin (FSK) activates adenylyl cyclase, leading to an increase in intracellular cAMP levels. This rise in cAMP activates protein kinase A (PKA), which phosphorylates CFTR and promotes the opening of the channel. IBMX, a broad phosphodiesterase (PDE) inhibitor, prevents the breakdown of cAMP. When used in combination with FSK, it enhances the CFTR response. The CFTR inhibitor INH-172 binds to CFTR and blocks chloride conductance. Additionally, this selective inhibitor confirms that the observed current is mediated by CFTR channels.



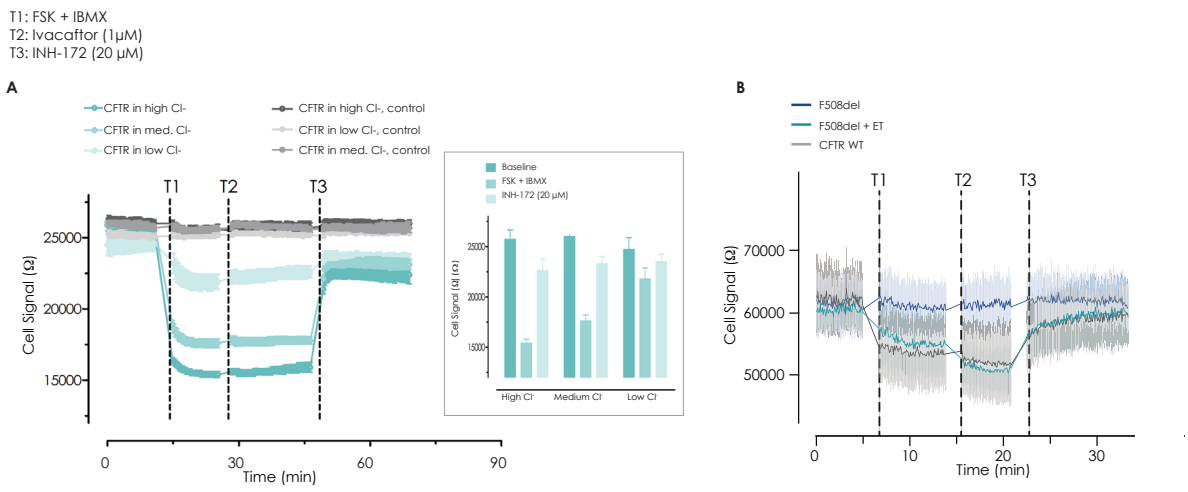
***AtlaZ recordings and CFTR-mediated conductance. A**, Once confluency of adherent cells is reached, cells form tight junctions sealing the paracellular gap. Cell-cell contact (TEER) and membrane capacitance (Cm) are measured in parallel to Impedance (Ω). **B**, Full Spectrum recording (0.1 - 100 kHz) reveals adherent cells and tight cell-cell contacts. The most sensitive frequency was 4.2 kHz (**C**). **D**, CFTR stimulation using 10 µM FSK + 110 µM IBMX at different Cl- concentration correlates with signal magnitude. Cell pretreatment (24 h before T1) with the drug combination TE (Tezacaftor + Elexacaftor) induced a potentiation of the FSK + IBMX effect. Vertical error bars represent SEM, n = 4 wells each. **E**, Exemplary Ussing Chamber measurements of the same cells showing stimulation via FSK + IBMX, a potentiation via TE and an inhibition using INH-172.*

CFTR potentiator Ivacaftor: Ivacaftor increases the CFTR open probability and is a drug approved for patients with specific, underlying genetic mutations. These are mutations where CFTR reaches the cell surface but doesn't open properly or has reduced flow. Tezacaftor is a CFTR corrector drug used to improve the function of the CFTR protein. It is often used in combination with other CFTR modulators like elexacaftor and ivacaftor in treatments designed to enhance the folding, trafficking, and channel activity of mutant CFTR proteins, thereby improving chloride ion transport in cystic fibrosis patients. This combination treatment helps restore CFTR function more effectively than single agents alone, contributing to better clinical outcomes in people with cystic fibrosis.

Aim and results of this study

In this study, the AtlaZ was used to monitor and quantify the resistive part of impedance values of FRT cells overexpressing wild-type CFTR. The cells were generated using a Flp-In system by the University of Alabama at Birmingham Cystic Fibrosis Foundation (CFF) Center, resulting in a single copy of CFTR integrated into the genome. The goal of this research was to determine the development of barrier properties and ion current effects in the presence of CFTR channel modulators.

- We show that changes in extracellular chloride concentrations correlate to recorded Cell Signal amplitudes. This proves that the signal is driven by Cl^- conductance.
- We could show here that tezacaftor in combination with elexacaftor (TE) is potentiating the IBMX and FSK effect. Comparable Ussing Chamber experiments are shown alongside the AtlaZ CFTR measurements to validate the functional equivalence of AtlaZ high-throughput platform with the gold-standard electrophysiology technique.
- Interestingly, after stimulation with IBMX and FSK, no further increase in signal was observed upon application of ivacaftor. This finding was consistent with parallel measurements performed in Ussing Chambers. The underlying reason for this lack of effect is not clear and



FSK + IBMX activation of wildtype CFTR after TE pre-treatment. *CFTR stimulation using 10 µM FSK + 110 µM IBMX in different external Cl^- concentrations correlates with impedance magnitude. Recorded traces (**A**) and bar graphs (inset) reflecting the signal amplitude upon application of FSK + IBMX, then ivacaftor, and then upon inhibitor application (INH-172, 20 µM). Cells have been pre-treated for 24 h with TE. Vertical error bars represent the SEM, n = 4 wells each. (**B**) Stimulation of FRT cells transiently transfected with F508del-CFTR or wild type CFTR, n = 3 +/- SD.*

"AtlaZ's high-throughput capability to track CFTR function allows us to screen even extensive sets of gene variants, making it an invaluable tool for advancing our research objectives."

John Lueck, Associate Professor at University of Rochester Medical Center, USA

warrants further investigation, however it may be due to the expression level of the CFTR channel. In general, ivacaftor acts by potentiating or rescuing the function of disease-causing CFTR mutants (e.g. G551D), but it does not enhance the activity of all variants.

- We also assayed CF-causing variants found in patients with CF, including the most common F508del-CFTR. Individuals harboring at least one copy of this allele are FDA approved for the ETI modulator therapies. As expected, when examined on the AtlaZ, F508del-CFTR without ET exhibited no FSK/IBMX dependent change in resistance. However, this variant was successfully modulated with ETI to be near WT-CFTR levels of activity. This indicates that using the AtlaZ, we can examine the function of CF-causing variants and their response to known CF therapeutics, as well as those under investigation.

Summary and conclusion

Measuring CFTR function with the AtlaZ platform offers major advantages when higher throughput, automation, continuous monitoring, and less invasive functional readouts are required, particularly in patient-derived airway models.

Ussing Chambers remain the gold standard for direct electrophysiological CFTR ion flux measurements, however AtlaZ expands what can be measured, especially barrier modulation, kinetics, and large-scale CFTR rescue profiling.

AtlaZ technology extends conventional TEER measurement setups by analyzing impedance across a range of frequencies, delivering richer insights into cell layer capacitance, resistance, and morphological changes. This technique can distinguish between transcellular and paracellular pathways, track real-time CFTR-dependent shifts in barrier properties, and monitor how pharmacological treatments or mutations impact epithelial function. The AtlaZ platform is a perfect choice if dynamic changes in epithelial barrier during CFTR activation should be tracked, for studying CFTR corrector/activator kinetics and performing drug screening or dose-response experiments.

In conclusion, this study shows that measurements derived from the AtlaZ system allow for a real-time quantification of CFTR function and response kinetics. Ussing Chamber side-by-side traces reveal nearly identical short-circuit current profiles, characterized by robust FSK/IBMX-induced activation peaks followed by sustained TE-mediated enhancement in patient-derived airway epithelia, demonstrating AtlaZ's ability to recapitulate clinically predictive CFTR rescue without compromising physiological fidelity. This correlation not only benchmarks AtlaZ's sensitivity to approved CFTR corrector-potentiator combinations but also instills confidence in its utility for rapid theratyping of novel modulators in cystic fibrosis research.

Outlook

In a next study, different CFTR variants based on diverse mutations will be transfected into the FRT cells, resulting in multiple cell types overexpressing each one CFTR variant. The high throughput of AtlaZ allows to identify which of the many gene variants are sensitive to the drugs, and importantly, AtlaZ enables HT-screening of novel compounds that could be used to treat patients bearing variant-CFTR genes that are not sensitive to current drug regiments.

References

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