Impedance spectroscopy; what and why?

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Impedance spectroscopy is a powerful analytical technique widely used in in vitro testing to evaluate the barrier function of cell cultures. One of the most critical applications of impedance spectroscopy is in assessing the integrity of epithelial and endothelial cell layers through measurements such as trans epithelial/transendothelial electrical resistance (TEER).

Fundamental concepts

To understand what impedance is, a couple of concepts need to be understood [1], [2]:

- Voltage (V): The electrical potential difference (signal) sent between electrodes with a specific amplitude.
- Amplitude: The peak magnitude of the voltage.
- Current (I): The flow of electrical charge in response to the applied voltage.
- Phase shift (θ): The delay between the applied voltage and the resulting current.
- Impedance (Z):
    - The ratio between the voltage over time to the resulting current over time.
    - The complex sum of the total resistance and total reactance
- Resistance (R): Resistance to the flow of electrical current. ; the amplitude reduces.
- Reactance (X): The storage and release of energy; the phase has changes.

To measure the impedance, a small amplitude AC voltage is applied across a frequency range, typically from 10 Hz to 100 kHz, and the resulting amplitude and phase response are measured. The change in amplitude and phase are dependent on, among others, the characteristics of the barrier function of the cell layer and properties of the culture medium [1].

Transepithelial electrical resistance (TEER) and barrier function

Transepithelial electrical resistance (TEER) is a purely resistive component of the total measured impedance in the cell layer, caused by the tight junctions between cells. In healthy barriers, the tight junctions inhibit the passage of the electrical signal, thereby resulting in a high TEER. On the other hand, compromised barriers have fewer malfunctioning tight junctions, which allow the signal to pass more easily and are therefore more permeable, leading to a lower TEER [3].

By performing impedance spectroscopy over a range of frequencies, researchers can distinguish between resistive and capacitive components of a barrier. High-frequency measurements primarily reflect cell membrane capacitance, while low-frequency data provide insights into the integrity of tight junctions [3].

Importance of impedance spectroscopy in in vitro testing

Impedance spectroscopy has a variety of applications in 2D cell culture as well as 3D cell culture. In 2D cell culture, its most most important use is for assessing the barrier function. This barrier function is important for applications such as disease modeling, drug efficacy, drug permeability etc. In 3D cell culture, impedance spectroscopy can also be used for several assays [4]. First, the barrier function of a fully grown 3D skin construct in a transwell can be measured [5]. Moreover, in cell suspensions (cells or spheroids in media or in Matrigel), impedance spectroscopy can be used for viability assays, which can also correlate with cancer therapy efficacy [4].

By integrating impedance spectroscopy into in vitro testing, researchers gain a non-invasive, real-time method to assess barrier function dynamically. This approach enhances the understanding of cell physiology and enables the development of advanced therapeutic interventions.

Conclusion

Impedance spectroscopy, with TEER as a key parameter, is a vital tool for analyzing the barrier function of in vitro cell cultures. By measuring voltage, current, impedance, and phase shift, researchers can assess the integrity of epithelial and endothelial layers, making this technique indispensable in biomedical research and pharmaceutical development. As advancements continue in 2D and 3D cultures, impedance-based methods will play an increasingly important role in improving the accuracy and efficiency of in vitro testing.

Join the conversation

Are you using impedance spectroscopy and/or TEER measurements in your research? Why or why not?

References

[1] B. Srinivasan, A. R. Kolli, M. B. Esch, H. E. Abaci, M. L. Shuler, and J. J. Hickman, "TEER measurement techniques for in vitro barrier model systems," *J Lab Autom*, vol. 20, no. 2, p. 107, Apr. 2015, doi: 10.1177/2211068214561025.

[2] "Resistance, Reactance and Impedance | Basic Alternating Current (AC) Theory | Textbook." Accessed: Feb. 11, 2025. [Online]. Available: https://control.com/textbook/ac-electricity/resistance-reactance-and-impedance/

[3] J. Schimetz *et al.*, "Automated measurement of transepithelial electrical resistance (TEER) in 96-well transwells using ECIS TEER96: Single and multiple time point assessments," *SLAS Technol*, vol. 29, no. 1, p. 100116, Feb. 2024, doi: 10.1016/J.SLAST.2023.10.008.

[4] T. Gerasimenko *et al.*, "Impedance Spectroscopy as a Tool for Monitoring Performance in 3D Models of Epithelial Tissues," *Front Bioeng Biotechnol*, vol. 7, p. 474, Jan. 2020, doi: 10.3389/FBIOE.2019.00474.

[5] N. J. M. van den Brink *et al.*, "Electrical Impedance Spectroscopy Quantifies Skin Barrier Function in Organotypic In Vitro Epidermis Models," *Journal of Investigative Dermatology*, Apr. 2024, doi: 10.1016/j.jid.2024.03.038.