Case study

Parametrizing phenotypes in 2D human cell models

Tools:
CardioExcyte 96, AtlaZ

Carlos Obejero-Paz,
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Carlos Obejero-Paz specializes in developing physiologically relevant approaches for preclinical drug screening. With over 20 years of experience at Charles River, US, he has also held roles in various academic laboratories, where he led research efforts in cardiac disease modeling and cancer drug discovery.

Imagine a technology that enables scientists to observe cellular events that remain undetectable by conventional methods - a system similar to a high-content reader, but capable of capturing even the most subtle changes in cellular behavior. Electric Cell-Substrate Impedance Sensing (ECIS) offers exactly this capability. Dr. Carlos Obejero-Paz has closely followed the technological evolution of ECIS over several decades and has applied it throughout his career at various institutions, including Charles River Laboratories and Stanford University.

ECIS is a versatile technique used to characterize the electrical properties of biological systems by measuring their impedance across a range of recording frequencies [1]. Among various cell-based assays, this non-invasive technique is particularly useful for studying cellular behaviors such as attachment, proliferation, and morphology changes. The technology allows for the real-time monitoring of electrical impedance alterations that correlate with cellular activities.

Parametrization of disease models in vitro

ECIS technology as it is implemented in the AtlaZ system offers significant potential for parametrizing cell-based models. By quantifying impedance, resistance, and capacitance of cells adhered on a substrate, researchers can gain insights into the physiological and morphological changes occurring within the cells. This capability is crucial for modeling diseases where cell behavior and interactions play a pivotal role. For instance, barrier function and cell junction integrity in e.g. epithelial cells can be studied, which are often compromised in disease states. The technology's sensitivity to detect changes at subnanometer levels [2] makes it an ideal tool for observing subtle cellular responses to disease conditions or treatments.

Investigation of cell phenotypes

When studying patient- or disease-specific cells, understanding how they interact with their environment

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

"The AtlaZ platform is a great tool to interrogate the properties and morphology of 2D cultures. We use the instrument to parametrize phenotypes of cell lines."

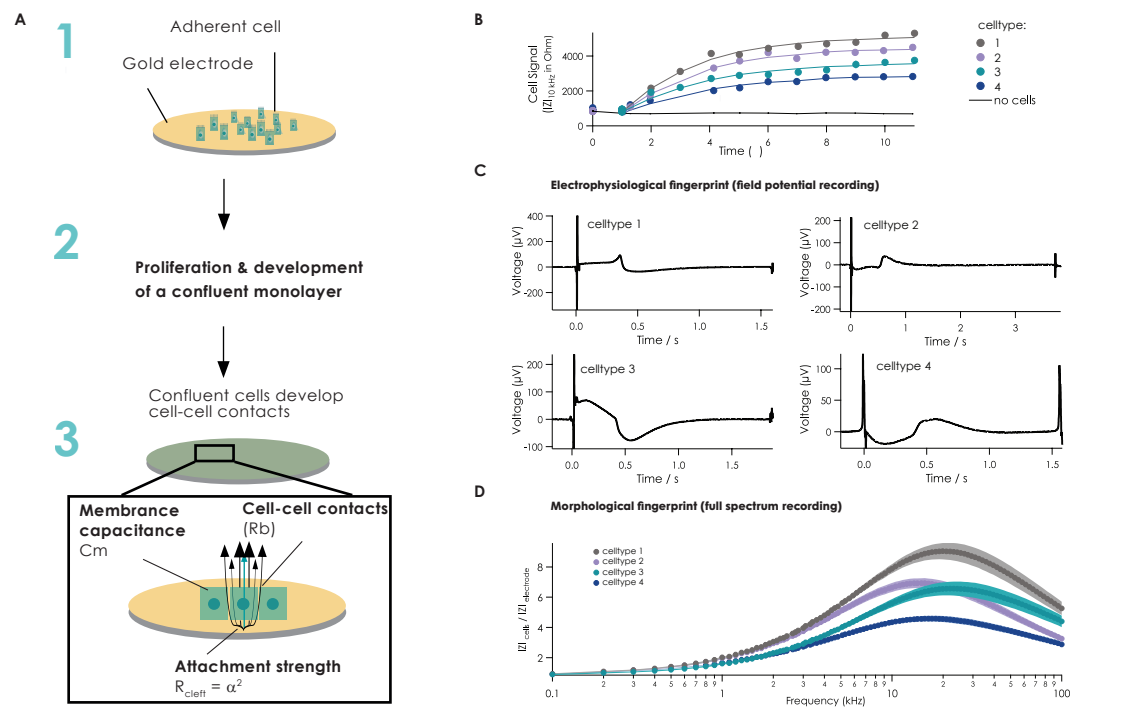
Carlos Obejero-Paz, Consulting Senior Scientist, CA, USA

and respond to various stimuli or drugs is critical. By processing acquired impedance data using the Giaever & Keese model [2], researchers can gain deeper insights into key aspects of cellular behavior and properties. For example, this modeling approach enables differentiation between cell-cell and cell-substrate interactions, which are essential for understanding cellular dynamics. In detail, ECIS impedance measurements can be dissected and translated into parameters that reflect cellular structure and behavior [3]. Recorded values reflect cell-cell junctions and barrier function (resistance), or cell-substrate interactions and amount of membrane (capacitance). To be able to gain such a wealth of information from just one measurement platform is an advantage compared to e.g. conventional endpoint assays. Further advantages of the technology are as follows:

Real-time monitoring: AtlaZ provides continuous data acquisition without disrupting the cell culture, allowing for dynamic observation of cellular processes over time.

Non-invasive measurements: The technique does not require any labels or dyes, preserving the natural state of the cells and avoiding potential artifacts introduced by staining procedures.

Quantitative analysis: By measuring electrical parameters such as impedance, resistance and capacitance, the system offers quantitative insights into cellular behavior that can be correlated with biological functions. The simultaneous measurement of the multiple cellular parameters provides a more comprehensive view of cellular responses compared to traditional single-readout assays.



***AtlaZ recordings and parametrization of phenotypes. A**, Once confluency of adherent cells is reached, cells start forming tight junctions which are sealing the paracellular gap. The cell-cell contact (Rb), membrane capacitance (Cm) and attachment strength (α) is measured in parallel to cell adherence & proliferation. **B**, Adherence and growth of 4 patient-derived iPSC-CM cell lines, each of them showing a specific EFP signal (**C**, recorded with CardioExcyte 96) or spectral characteristics reflecting the morphology (**D**) revealed by AtlaZ, 10 days after plating.*

Versatility: The method is applicable across various research areas for drug discovery and toxicity studies, as well as to understand cellular responses to environmental changes.

Aim and results of this study

In this study, in collaboration with Ana Kojic in Joseph Wu's Lab at the Stanford Cardiovascular Institute, we used induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from four healthy donors. The AtlaZ was used to monitor and quantify impedance values, while field potential recordings were performed using the CardioExcyte 96 system [4]. For analyzing and interpreting impedance data, the system independent Giaever & Keese model [2] was employed. This model assumes that confluent cells interact with each other and the substrate, creating two defined resistances: Rb, the resistance of the space between cells and α[2], the resistance of the space between the cells and the electrode where the cells are attached to. The model also includes the cell membrane capacitance (Cm).

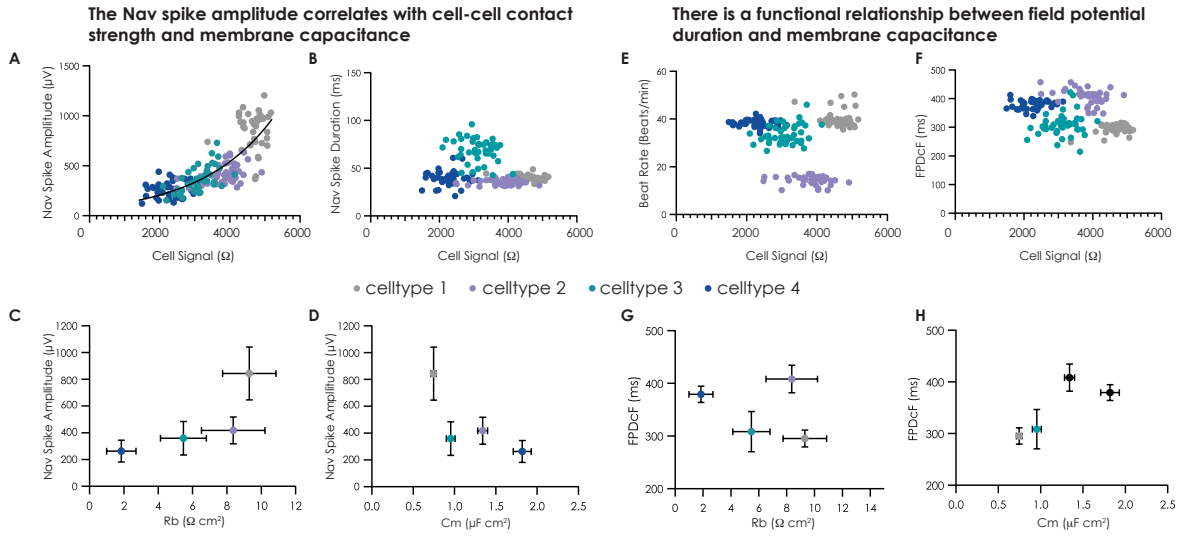
The goals of this research were to determine whether cardiomyocyte phenotypes can be defined by the strength of cell-to-cell interaction (Rb), the strength of interaction with the substrate (α), and the amount of cell membrane (Cm). Furthermore, we wanted to assess whether these parameters can be used to predict the electrophysiological properties of 2D-cultured iPSC-CMs.

• The data indicate that iPSC-CM cultures with stronger cell-to-cell contacts are associated with increased sodium channel activity.

• Additionally, larger capacitance values appear to correlate with longer field potential durations (FPDs).

Summary and conclusions

We found that the Giaever & Keese impedance model [2] for confluent cell cultures is a reasonable approximation to iPSC-CM cultures. The parameters α, Rb and Cm can be used to parametrize these cultures and serve as endpoints in drug discovery. Hence, the parameters correlate to cellular behavior and function. We conclude that a detailed



Correlation of parameters and functional relationships. *Na_v spike amplitude (**A**), but not duration (**B**), correlates with impedance magnitude of the different celltypes. Functional relationship between Na_v spike amplitude and fitted parameters Rb and Cm (**C** and **D**, respectively). Functional relationship between beat rate (**E**) and Fredericia-corrected field potential durations (FPDcF, **F**) with impedance magnitude. Relationship between FPDcF and the fitted parameters Rb (**G**) and Cm (**H**). Vertical error bars represent 95% CI. Horizontal error bars represent the SEM of fitted parameters.*

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Nerea Jimenez Tellez, Stanford University, CA, USA

profiling of cellular phenotypes using such an advanced approach provides a more comprehensive and predictive understanding of *in vivo* effects compared to traditional biochemical assays.

Looking ahead, the following considerations could be addressed in future studies: the Giaever & Keese model is based on specific assumptions [2], such as circular cell geometry and uniform cell layers, which may not fully capture the complexity of all biological systems. Hence, an independent confirmation of potential differences in cell shape, which could influence the parameter α [5], would be highly interesting. Furthermore, employing complementary methods could help validate these assumptions within the experimental setup or identify deviations that might impact result interpretation. Lastly, the limited number of cell lines used in this study restricts the generalizability of the observed functional relationships.

In conclusion, this study shows that measurements derived from the AtlaZ system and interpreted by the Giaever & Keese model [2] allow for a parametrization of 2D iPSC-CM cultures. The device platform stands out as a powerful tool for investigating adherent cells and parametrizing cell-based models. Its ability to provide real-time, quantitative data on cellular behaviors makes it indispensable in both basic research and applied biomedical sciences. By leveraging the detailed insights provided by these measurements, researchers can advance our understanding of complex cellular processes involved in cardiovascular diseases and cancer and develop more effective therapeutic strategies.

References

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