CASE STUDY

In Vitro Disease Modeling In Human Coronary Artery Endothelial Cells

CytoTronics

INTRODUCTION

Disease modeling in primary endothelial cells provides a powerful tool for understanding the intricacies of vascular pathophysiology. These cells, derived directly from donors, offer a physiologically relevant platform for studying various diseases, including cardiovascular disorders, inflammatory conditions, and cancer metastasis. However, the biological states of these cells can vary significantly based on donor characteristics, passage number, and the composition of the extracellular matrix proteins used for cell culture.[1] Such variations can profoundly influence cellular behavior, leading to distinct disease-relevant phenotypes. By carefully considering these factors, researchers can design assays to elucidate the underlying mechanisms of disease and develop targeted therapeutic strategies.

In this study, we leverage CytoTronics' Pixel technology[2] in order to explore the variability of human coronary artery endothelial cells (HCAECs) across donor background, cell passage, and ECM composition representative of potential assay-ready physiological states. The wealth of data collected using CytoTronics' longitudinal, multiparametric readouts highlight the inherent biological diversity of primary endothelial cells which can inform the development of more robust *in vitro* assays.

RESULTS

Effect of donor background on multiparametric readouts

Donor variability in *in vitro* assay design refers to the inherent differences in cellular responses observed among cells sourced from different donors. These variations can arise due to genetic backgrounds, age, health status, and environmental exposures of donors. Accounting for donor variability is crucial for obtaining robust and generalizable results, as it mirrors the heterogeneity present in human populations, thereby enhancing the translational relevance of *in vitro* findings to clinical settings. With CytoTronics' Pixel technology, multiple primary cell lines from different donors and/or lots can be efficiently assessed across a range of biological readouts within a single assay, ensuring robust evaluations of assay-specific requirements.

As shown in Figure 1, HCAEC cells sourced from two different

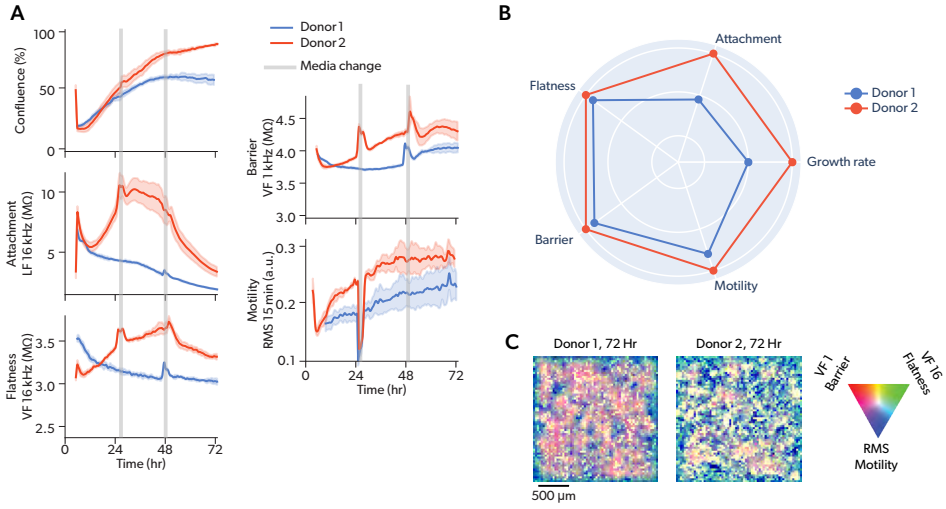

Figure 1. Longitudinal measurements of HCAEC cells from two donor backgrounds. (A) Confluence, attachment, flatness, barrier, and motility as measured over 72 hours from cell plating for donor 1 (blue) and donor 2 (red). Shaded regions indicate standard error of 3-6 technical replicates and grey bars indicate times of cell feeding (i.e. removal from incubator). (B) Radar plot displaying the relative donor fingerprint for the five measurements shown in panel A. (C) RGB images for both donors at 72 hours showing significant differences in barrier, flatness, and motility.

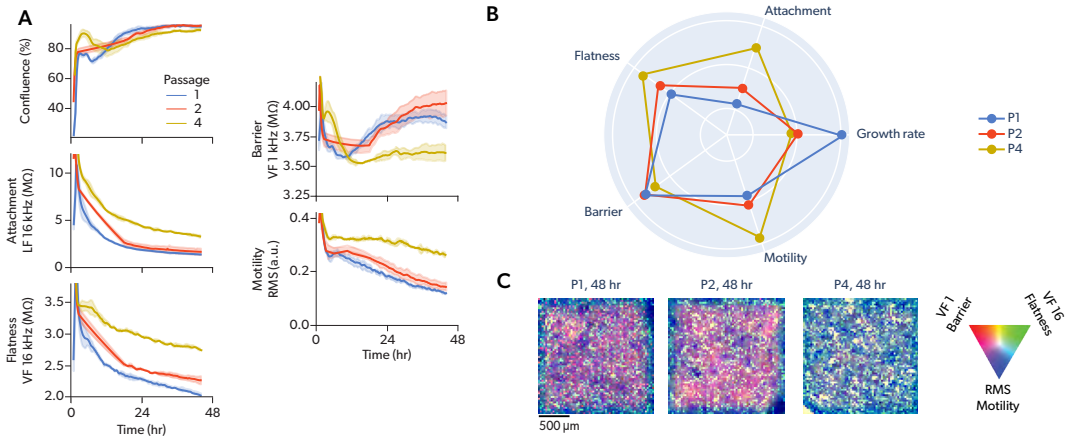

Figure 2. Longitudinal measurements of HCAEC cells from three passages of patient donor-1 patient backgrounds. (A) Confluence, attachment, flatness, barrier, and motility as measured over 48 hours from cell plating for passage 1 cells (blue), passage 2 cells (red), and passage 4 cells (yellow). Shaded regions indicate standard error of 3-6 technical replicates. (B) Radar plot displaying the relative donor fingerprint for the six measurements shown in panel A. (C) RGB images for all three passages at 48 hours showing significant difference in barrier, flatness, and motility.

donors (labeled as 1 and 2) were seeded at a density of 40,000 cells per well. Over a 72-hour period, the complete range of impedance measurements was taken at 15-minute intervals. Longitudinal measurements revealed donor-to-donor variability across all biological readouts including growth rate (derived from confluence), attachment, flatness, barrier function, and motility (Figure 1A). Notably, donor 1 exhibited higher impedance measurements for motility, barrier function, and flatness. Furthermore, longitudinal measurements highlighted the dynamic nature of attachment measurements for donor 1, characterized by a significant increase from 24 to 48 hours, followed by a notable decrease.

These distinctions between donors are further highlighted by additional visual representations shown in Figure 1B and 1C. In Figure 1B, the spokes of the radar plot illustrate the relative measurements for biological phenomena integrated over time for each donor, essentially generating a unique donor-specific fingerprint. In addition to temporal variation, the measurements can also monitor spatial variation or heterogeneity between donors. Figure 1C offers a spatial comparison using an electrical RGB image derived from three select measurements (in this case, barrier function, flatness, and motility), showcasing the impedance at the 72-hour mark for both donors. As previously mentioned, donor 2 has higher overall measurements as evidenced by the white coloring in the multiparametric image.

Effects of cell passage number on multiparametric readouts

Cell passage number is an additional factor that can modulate cell function and cause physiological variations, particularly in primary cells. Each time cells are passaged, they undergo cellular division, and with each division, there is a chance for genetic and phenotypic alterations to occur. As shown in Figure 2, primary HCAECs from donor 1 were evaluated over three different passages (P1, P2, and P4) with impedance measurements taken at 15-minute intervals. These longitudinal measurements reveal a passage-dependent change across biological parameters. In particular, attachment, flatness, and motility increase, while barrier decreases without significant differences in overall confluency. The ability to detect small changes in cell function and morphology speak to the sensitivity of electric imaging as a technique.

The radar plot shown in Figure 2B illustrates the passage-specific fingerprint, while the electrical images shown in Figure 2C show that cells from P1 to P4 show dynamic changes across the multiparametric readouts shown here.

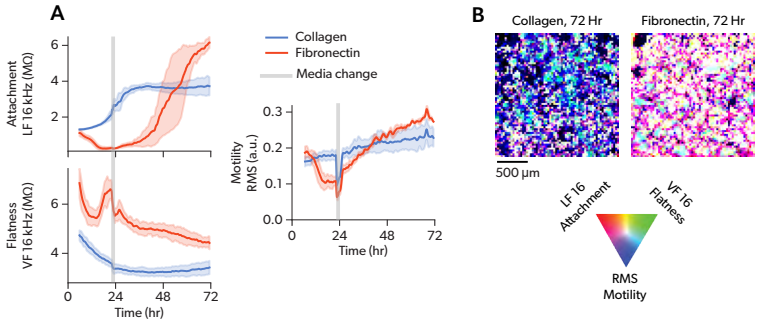

Figure 3. Longitudinal measurements of HCAEC cells from donor 1 plated on two ECM coatings: collagen, type I and fibronectin. (A) Attachment, flatness, and motility as measured over 72 hours from cell plating for cells plated on collagen (blue) and fibronectin (red). Shaded regions indicate standard error of 3-6 technical replicates and the grey bar indicates cell feeding (i.e. removal from incubator). (B) RGB images for both ECM coatings at 72 hours showing significant difference in attachment, flatness, and motility.

Effect of ECM coating protein on multiparametric readouts

Extracellular matrix (ECM) coating involves applying ECM proteins or components onto cell culture surfaces to replicate the natural microenvironment of cells *in vivo*. *In vivo*, ECM undergoes tight regulation and mirrors various functional states; for example, fibronectin secretion increases during repair processes, whereas collagen represents a steady state ECM condition.

In the experiments depicted in Figures 1 and 2, collagen type I served as the coating protein for the extracellular matrix (ECM). Figure 3 examines the longitudinal behavior of HCAEC cells on both collagen and fibronectin coatings, focusing on a subset of impedance measurements over a 72-hour culture period. As illustrated in Figure 3A, HCAEC cells from donor 1 exhibit notable longitudinal impedance differences, particularly in attachment, flatness, and motility when plated on different ECM coatings. These distinctions are further visualized in the electrical images presented in Figure 3B, where the white coloration indicates cells with higher impedance measurements across all three parameters shown here.

CONCLUSION

In this study, we employed CytoTronics' Pixel platform to monitor functional changes in primary endothelial cells based on donor background, cell passage, and ECM coating protein. The multiparametric readouts enabled rapid assessment of features critical in the design of *in vitro* cellular assays/models. By leveraging the real-time capabilities of the Pixel platform, we were able to comprehensively evaluate various cellular parameters, providing insights into the nuanced interplay between donor characteristics, cell passage number, and ECM coating composition. This comprehensive approach not only enhances our understanding of cellular behavior, but also offers valuable insights for optimizing experimental protocols and designing more robust *in vitro* models.

METHODS

Cell Lines

Primary HCAEC cells (CC-2585) were obtained from Lonza and maintained in a humidified incubator at 37°C and 5% CO_2. Cells were cultured in EGM™-2 media supplemented by the Endothelial Cell Growth Medium-2 Bullet Kit.

Measurement

Impedance measurements were taken at 0.25, 1, 4, and 16 kHz inside a humidified incubator at 37°C and 5% CO_2 every 15 minutes throughout the experiment. Cells were seeded at either 3,000 or 40,000 cells per well and allowed to grow for 72 hours. A half media change was performed at either 24 or 48 hours.

Data Analysis

The well median of each measurement (confluence, attachment, flatness, barrier and motility) was plotted over time, with the standard error calculated across 3 technical replicates. Radar plots reflect the steady-state levels of attachment, flatness, barrier and motility calculated in the last 24 hours of the experiment, and the growth rate calculated from the first 12 hours of the experiment.

REFERENCES

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2. Chitale, S. *et al.* A semiconductor 96-microplate platform for electrical-imaging based high-throughput phenotypic screening. *Nat Commun* **14**, 7576 (2023).

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