CytoTronics

APPLICATION NOTE

Spatial Resolution: Assessing Population Heterogeneity

Introduction

Biological systems are inherently heterogeneous. Tissues are composed of diverse cell types characterized by unique functions, morphology, and gene expression profiles. The recent move towards establishing *in vitro* models that better replicate physiological diversity is accompanied by the necessity to be able to monitor phenotypes in heterogenous cell populations. Even within homogeneous cell populations, variability in response to specific modulations is common, stemming from slight genetic variations, asynchronous cell division, or other contributing factors. Techniques such as single-cell sequencing have been leveraged to scrutinize responses at an individual cell level. However, few platforms exist that can observe real-time heterogeneity in larger populations of cells.

This study showcases our platform's capability to effectively observe and characterize heterogeneity within cell populations by harnessing spatial resolution in impedance readouts. The segregation of heterogenous populations is the first step in monitoring cell-specific responses.

Technology

CytoTronics' electronic microplate features a high-density electrode array per well, providing an exceptional spatial resolution. This feature allows for nearly single-cell spatial resolution across the entire spectrum of functional and morphological measurements.[1] Each pixel monitors the characteristics of closely-situated cells, while the platform's temporal resolution permits real-time tracking of changes within cell populations over time. The output values from each well can be obtained as a median value that represents the population average or as individual electrode values which depict the distribution of measurements within the population. This dual capability facilitates readouts of population averages and on an individual cell or group of cells basis.

Results

Impedance measurements can differentiate between cell populations

To study population heterogeneity in a biologically relevant context, we focused on epithelial to mesenchymal transition (EMT), a phenomenon with implications in cancer and fibrosis, making it a focal point of therapeutic interest. This process involves epithelial cells gradually losing their barrier-forming capabilities, undergoing morphological alterations, and acquiring heightened migratory and invasive properties. To simulate this cellular diversity within EMT, we examined two distinct cell lines: MCF7 cells, representing the luminal A subtype with epithelial-like characteristics[2] and a classic cobblestone morphology in a monolayer and MDA-MB-231 cells, originating from a triple-negative metastatic breast adenocarcinoma, which have undergone EMT[3] and display a spindle-like appearance with pronounced invasion and migration capabilities. In order to characterize and quantify the morphological differences, we seeded MCF7 and MDA-MB-231 cells separately at a density of 10,000 cells per well and observed their growth over a 72-hour period (Figure 1). These two cell types exhibited notable distinctions in various parameters, including growth rate, flatness, barrier formation, and attachment. Notably, MCF7 cells displayed higher levels of cell-cell adhesion, tissue barrier, and flatness, while MDA-MB-231 cells demonstrated increased growth rates and motility (RMS) (Figure 1A, B). This is further highlighted in the impedance images, which show a spatial comparison of motility in the two cell lines (Figure 1C).

Having established the unique signatures of each cell type in homogeneous cultures, we aimed to investigate our spatial resolution's capacity to differentiate between these distinct cell types within heterogeneous populations in the same well. As the two cell lines were robustly differentiated by their motility characteristics (i.e. RMS), we focused on that in our subsequent analyses of the spatial distribution of electrode (pixel) values in a single well at different time points. As illustrated in Figure 2, we observed the population distribution for both homogenous and heterogenous (1:1 co-culture) scenarios. Initially, the two homogenous cultures displayed overlapping distributions shortly after cell seeding, gradually diverging as cells adhered and proliferated. After 72 hours,

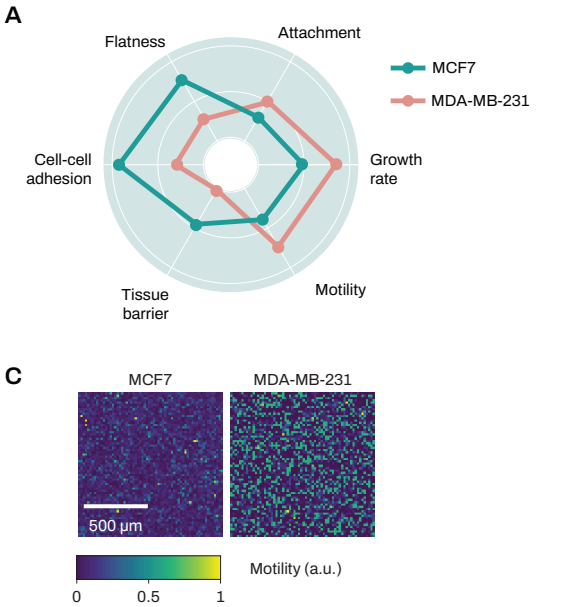

Figure 1. (A) Comparison of MCF7 and MDA-MB-231 cell lines across six different functional characteristics, based on steady state values calculated over 60–72 hours post seeding (grey shaded region in B). (B) Confluence, flatness, and motility of MDA-MB-231 and MCF7 cells measured from seeding to 72 hours. The shaded areas around the curves represent the standard error of well replicates. (C) Motility images of the two cell lines at 48 hours post seeding.

MCF7 cells exhibited low motility, while MDA-MB-231 cells displayed high motility. Notably, the heterogenous co-culture displayed an intermediate distribution during early growth, but ultimately converged with the distribution of MDA-MB-231 cells at the 72-hour mark. This shift can be attributed to MDA-MB-231 cells outpacing their counterparts in the co-culture due to their higher growth rate (Figure 1B).

To delve deeper, we aimed to explore whether population distributions could serve as predictive indicators for the contribution of each cell type. For this purpose, we seeded MCF7 and MDA-MB-231 cells at three different ratios: 1:3, 1:1, and 3:1, while maintaining a consistent total cell count of 10,000 cells per well. Each condition was seeded in a minimum of three wells. As depicted in Figure 3A, we monitored the population distribution over time for each co-culture ratio. To ascertain the proportion of each cell type within these mixed cultures, we compared the population distribution at various time points to the distributions of the individual parent cultures. The similarity between the distribution of the co-culture and each of the parent distributions was used to determine the number of cells from each type.

For instance, the 1:1 co-culture starts at a similarity score of approximately 0.5 for both cell lines and shifts toward an increasing MDA-MB-231 contribution (similarity score approaching 1) and a decreasing MCF7 contribution (similarity score approaching 0) over time. Co-cultures starting with fewer or more MDA-MB-231 cells displayed different initial contributions and extents of dominance by one cell type. The validity of this model was also confirmed through experiments with pure test cultures (Figure 3).

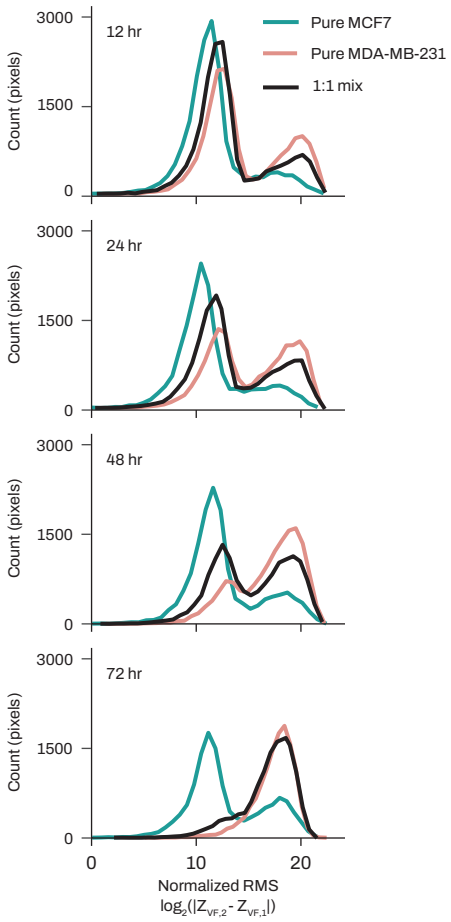

Figure 2. Population distributions of motility for the pure MCF7 (teal), pure MDA-MB-231 (coral), and heterogenous (1:1) co-culture (black) cells at the indicated time points post seeding.

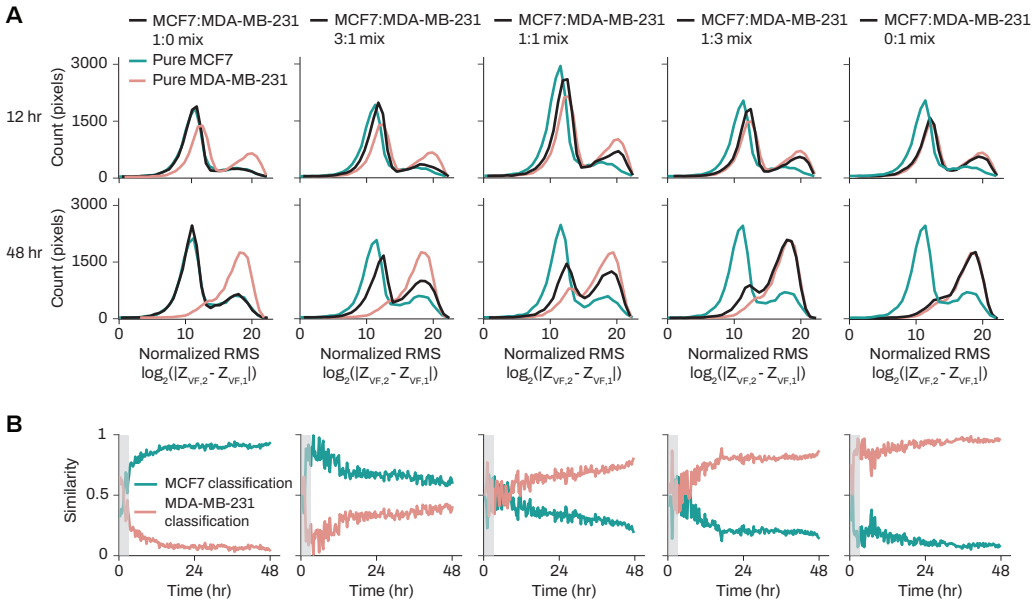

Figure 3. (A) Population distributions of motility for MCF7 (teal), MDA-MB-231 (coral) and MCF7/MDA-MB-231 co-cultures (black) at the varying ratios at 12 and 48 hours. (B) Similarity score of populations as compared to MCF7 (teal) and MDA-MB-231 (coral) cells over time for various co-culture ratios. The gray overlay indicates the model is not predictive until +5 hours after seeding (as determined by the pure population tests).

Conclusion

CytoTronics' platform provides a precise real-time assessment of a range of impedance parameters. The platform not only facilitates the analysis of populations and contributions of different cell types but also allows for the visualization of this heterogeneity through impedance images with high spatial resolution. This offers valuable insights into studying cells with distinct properties, making the platform an invaluable tool for cell analysis.

Methods

Cell lines and culture

Cell lines (MCF7, HTB-22 and MDA-MB-231, HTB-26) were obtained from ATCC, maintained in a humidified incubator at 37°C and 5% CO_2, and cultured in DMEM supplemented with 10% FBS and Pen/Strep.

Measurements

Impedance measurements were taken at 0.25, 1, 4, and 16 kHz inside a humidified incubator at 37°C and 5% CO_2. For the cell comparison experiment, MCF7 or MDA-MB-231 cells were seeded at 10k cells per well with impedance measurements taken every 15 minutes over 72 hours. For the 1:1 cell mixture experiment, MCF7 or MDA-231 cells were seeded at 10k cells per well along with a mixture of 5k cells per well of each type. Impedance measurements taken every 15 minutes over 72 hours. For the varying cell ratio experiment, the total number of cells seeded was kept constant at 10k cells per well. Conditions were plated as MCF7:MDA-MB-231 with ratios of 10k:0k, 7.5k:2.5k, 5k:5k, 2.5k:7.5k, and 0k:10k. Impedance measurements were taken every 15 minutes for 72 hours.

Data analysis

The well median of each measurement (confluence, flatness, and motility) was plotted over time, with the standard error calculated across 3 technical replicates. Population distributions were generated by binning motility values across all pixels in each well. The number of pixels in each bin (count) is used as the y axis. Similarity scores were determined by comparing population distributions using earth mover's distance derived from the Wasserstein metric.[4,5]

References

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