Utilizing In Vitro Bioelectronic Assays in Cancer Cell Characterization

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Introduction

Detection of metastatic breast cancer will not occur until after the cancer has spread to other parts of the body, resulting in delayed treatment and often, more advanced/detrimental cancer cases. Thus, it is critical to understand the fundamental mechanisms underlying the metastatic process and the complex interactions that occur between the tumor and the host during disease progression for detection of metastatic cells. Impedance-based cellular assays offer a sensitive, label-free, and non-destructive method to continuously monitor cancer cells in real time, allowing the assessment of cancer behaviors (Fig 1).

While the mechanosensing abilities of breast cancer cells are well known, the role that extracellular matrix (ECM) composition plays on breast cancer aggressiveness, separate from stiffness, is still a subject of study. In the present work, five breast cancer cell lines and two non-cancerous cell lines were cultured and evaluated using a bioelectronic impedance-based assay system to elucidate the relationship between ECM composition, cell phenotype, and 2D migration. Bioelectronic assays, like the one used in this work, offer a sensitive, label-free, and non-destructive method to continuously monitor cancer cells in real-time, allowing the assessment of multiple cancer behaviors simultaneously.

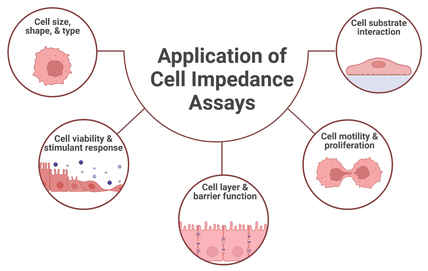

Figure 1. Application of cell impedance assays

Materials & Methods

- Cells were cultured using the Maestro-Z Impedance Assay System (Axion Biosystems)
- Cell proliferation was monitored via high frequency impedance
- Wells were coated with 20 µg/mL of ECM coatings (either collagen I, collagen IV, fibronectin, laminin, or Matrigel)
- At confluence, wells were scratched to create a wound to monitor the rate of wound closure (migration potential)

Table 1. Selected cell lines for analysis

Cell Line	Cell Type
Non-cancerous	
184B5	Normal mammary gland
MCF10A	Fibrocystic disease
Epithelial-like	
MCF7	Mammary gland adenocarcinoma(ER+, PR+, HER2-)
MDA-MB-453	Carcinoma (ER-, PR-, HER2-)
Basal-like	
HCC70	Primary invasive ductal carcinoma (ER-, PR-, HER2-)
HCC1806	Primary squamous cell carcinoma (ER-, PR-, HER2-)
Mesenchymal-like	
MDA-MB-231	Adenocarcinoma (ER-, PR-, HER2-)
BT-549	Papillary ductal carcinoma (ER-, PR-, HER2-)

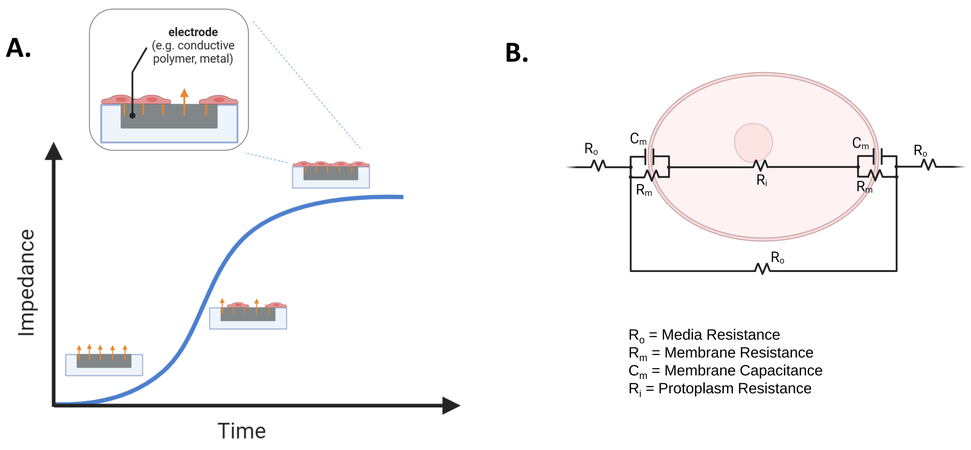

Figure 2. A. Schematic showing how cell surface coverage (i.e. proliferation, migration) can be measured by tracking changes in impedance. As cells cover electrodes embedded into the culture substrate, the flow of electrons is obstructed, leading to an impedance increase. **B.** Electrical circuit of an animal cell where R_o is resistance of the surrounding media, R_m is the resistance of the membrane, C_m is the capacitance is the cell membrane, and R_i is the resistance of the protoplasm.

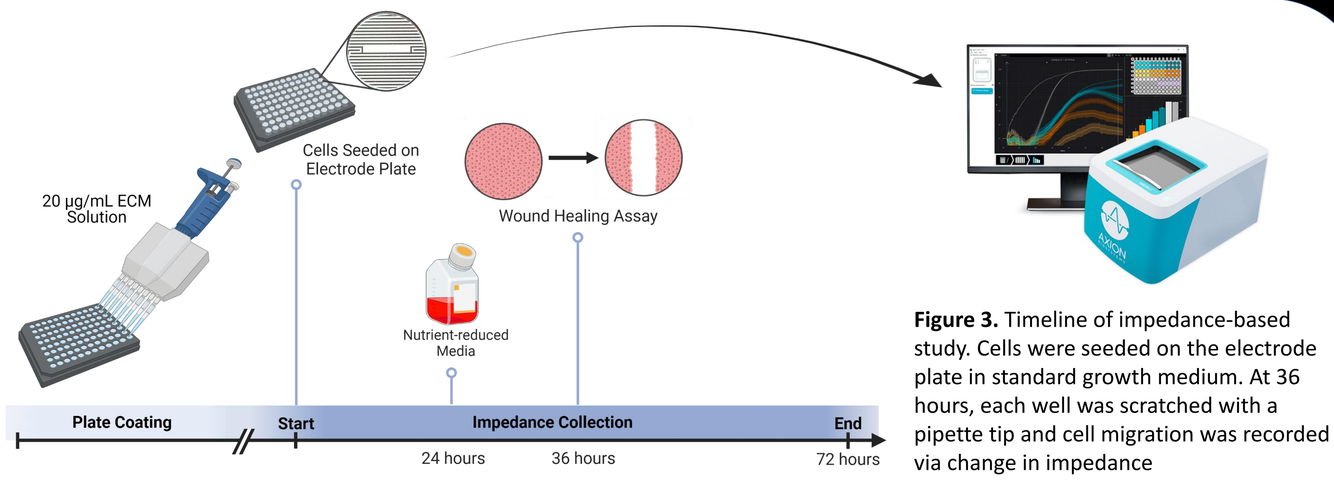

Figure 3. Timeline of impedance-based study. Cells were seeded on the electrode plate in standard growth medium. At 36 hours, each well was scratched with a pipette tip and cell migration was recorded via change in impedance

Results

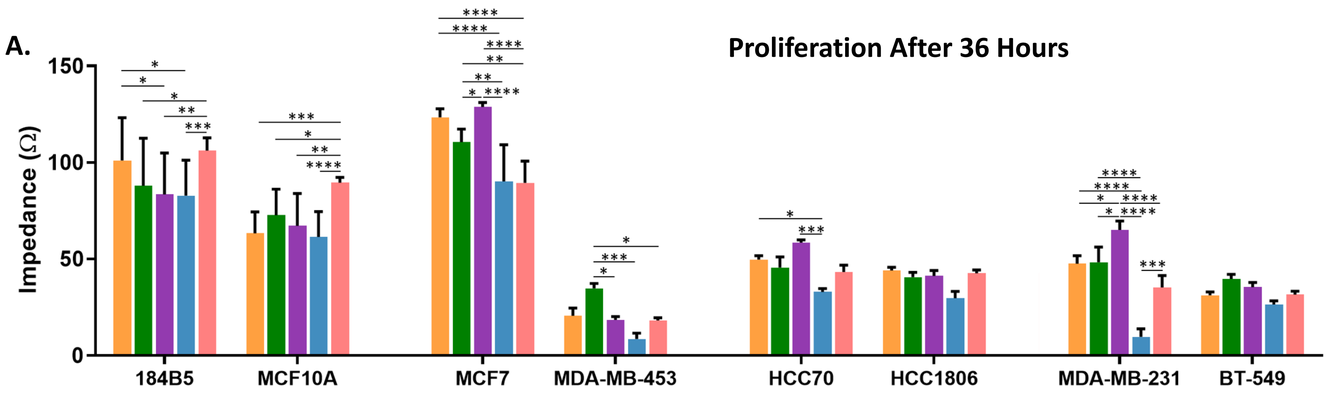

Figure 4. A. High frequency impedance after 36 hours of cell proliferation, representative of cell number and surface coverage. Cell proliferation was affected by ECM protein for most cell types, regardless of cell type/receptor status. **B.** Impedance, normalized to unscratched wells, for each cell type after 36 hours of migration. The dotted line indicates the corresponding normalized impedance of an unscratched well. While the triple-negative cell lines generally behave more aggressively to close the wound over 36 hours, there is little to no difference in wound closure with response to ECM protein expression across six of the cell lines. n=4-5. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, **** indicates $p<0.0001$, and # indicates significant wound closure over 36 hours.

Discussion & Conclusions

- ECM protein type did not meaningfully affect 2D migration however variations in proliferation response to ECM may be linked to phenotypic plasticity
- These findings demonstrate the feasibility of using an impedance-based assay for characterization of cancer cell metastatic potential using quantitative metrics
- Ongoing work includes performing additional studies to determine a correlation between cell response to ECM and morphological features
- This work creates a foundation for our long-term goal of improving current assessment methods to yield a more detailed and objective evaluation of cancer metastatic behavior

Acknowledgements

Many thanks to Axion Biosystems for allowing the Tissue RegenX Lab to use the Maestro TrayZ. Funding for this work provided by National Science Foundation CAREER Award #2145521. Figures 1-3 created with BioRender.com