Determining the Frequency that Leads to Optimal Detection of Cell Membrane Permeabilization

Dalia Fanik, Joseph Sherba, Jeffrey D. Zahn, David I. Shreiber
Department of Biomedical Engineering, Rutgers University, Piscataway, NJ

RUTGERS School of Engineering

Introduction

- In electroporation (EP), electric pulses are applied to cells to cause temporary cell membrane permeabilization, allowing delivery of molecules into cells such as DNA
- Cells must receive an appropriate pulse for successful, functional delivery
  - If the field is too weak or too short, the cell will not be permeabilized
  - If the field is too strong or too long, the cell will not recover and will die
  - The ”correct” pulse will vary between cell types and even from cell-to-cell
- Our lab is currently developing a continuous flow microfluidic system that detects when individual cells become permeabilized to avoid over exposure to the electric field. This is to avoid killing the cell.
  - Monitor changes in the electrical impedance of cells by measuring the current across the microfluidic channel
- The goal of this study is to identify the optimal frequency at which to monitor different cell types to detect permeabilization with the greatest sensitivity

Rationale

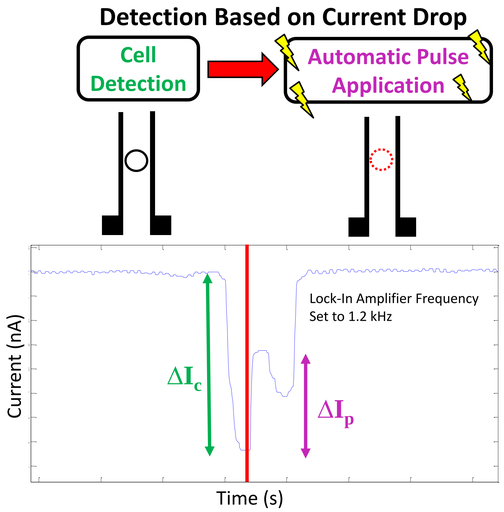
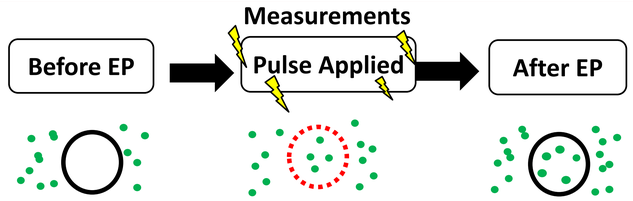
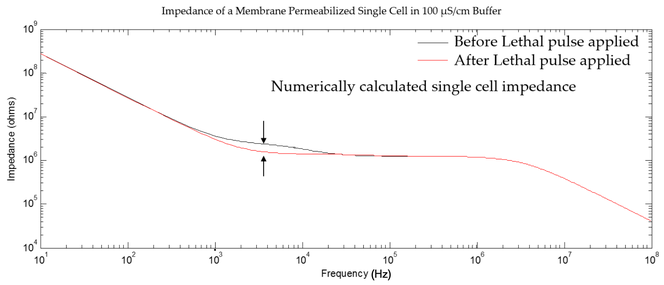


Figure 1: Current displacement due to cell entering region between electrodes and permeabilization

- Cells are suspended in a conductive buffer solution
- Electrodes measure the current across the microfluidic channel
- When a cell enters between the electrodes, the current drops (ΔI_c) because of the increase in impedance due to the presence of the cell
  - This drop triggers pulse application (red line)
- When the cell is permeabilized, the current increases (ΔI_p) because of the decrease in cell impedance
- We have modeled how this change in impedance depends on frequency and measure the change in NIH 3T3 fibroblast cells
  - There is an optimal frequency for detecting permeabilization

Research question: Does the optimal frequency to detect changes in impedance depend on the cell type?





Experimental Approach

Prior to each experiment, cells are suspended in a conductive buffer solution and placed into a syringe. This syringe is loaded into a syringe pump and connected to the microdevice through thin tubing. The microdevice is placed onto a microscope. The syringe pump is used to control the flow rate of the cells in the microdevice.

1) Before Electroporation:

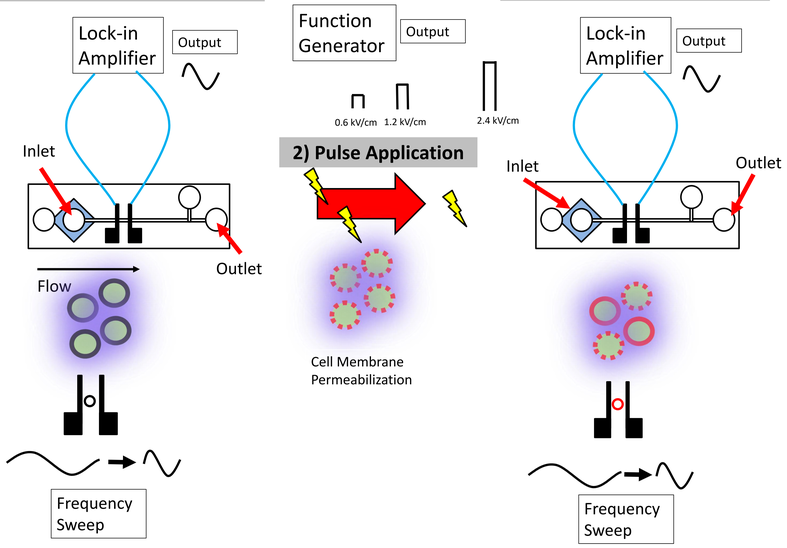
- Frequency sweep performed once a single cell is trapped between the electrodes
- Lock-in Amplifier measures the impedance at a set range of frequencies

2) Pulse Application:

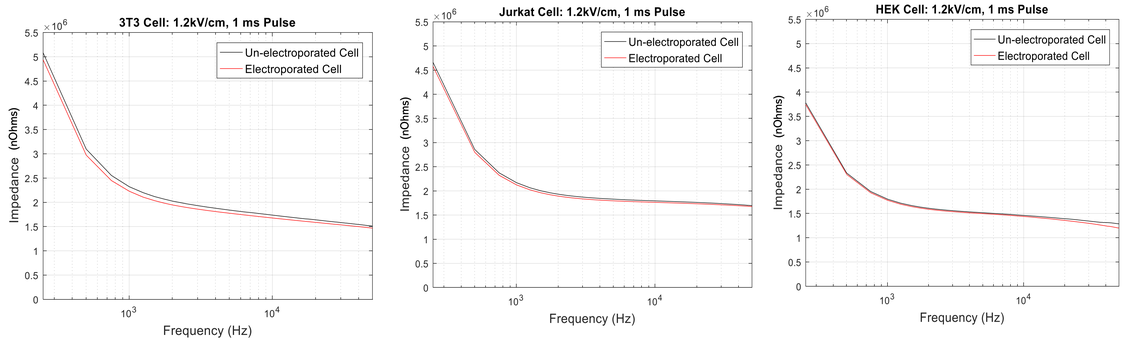
- Function generator outputs a square wave pulse
- Pulse strength used was 0.6 kV/cm, 1.2 kV/cm, and 2.4 kV/cm depending on the experiment
- Pulse duration is kept constant at 1 ms

3) After Electroporation:

- Lock-in Amplifier performs second frequency sweep



Results



3T3 cells:

- Maximum percent change within frequency range 1->10 kHz: **4.21%**
- Corresponding frequency: **1250 Hz**

Jurkat cells:

- Maximum percent change within frequency range 1->10 kHz: **2.10%**
- Corresponding frequency: **1000 Hz**

HEK293 cells:

- Maximum percent change within frequency range 1->10 kHz: **1.49%**
- Corresponding frequency: **1000 Hz**

Discussion

- Data on these three cell types suggests that optimal frequency for detection is around 1 kHz
- Difference in percent change between different cell types is possibly due to differences in cell size

Future Direction

- Increase the sample size on each cell type
- Collect impedance data on additional pulsing conditions
- Expand the variety of cell types

Acknowledgements

I would like to thank Dr. Shreiber for his guidance, Joseph for his mentorship, and the Electroporation Group for their support.

References

Zheng, M., Sherba, J. J., Shan, J. W., Lin, H., Shreiber, D. I., & Zahn, J. D. (2017). Continuous-flow, electrically-triggered, single cell-level electroporation.*Technology, 05*(01), 31-41. doi:10.1142/S2339547817500017

RUTGERS