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Electroporation Monitoring by Machine Learning and Single Cell Morphodynamic on Lab-on-Chip

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Summary:

Electroporation is a reliable, reproducible technique to induce biological cell membrane poration. Because of the biological and clinical interest in this technique, recently, many Lab-On-Chip platforms have been proposed to understand more about deep electroporation mechanisms. This led to the discovery of many electroporation side effects, such as cell contractivity and blebbing. In this work, we propose a new sensing system based on Lab-On-Chip and machine learning to correlate these side effects observed by brightfield time-lapse microscopy with electroporation efficiency.

Keywords: Electroporation, Lab-On-Chip, Machine Learning, Time-Lapse Microscopy,

Background, Motivation and Objective

Since its discovery in 1968, electroporation spread its applicability in biological and clinical research [1]. Delivering high electric fields on a biological tissue for short periods, usually nanoseconds or microseconds, permits the generations of nanopores on cells. Changing these parameters guarantees great flexibility of the technique in multiple applications, spanning electrochemotherapy, cell lysis, fusion, or bacteria inactivation.

Even though electroporation has been known for a long time, its inner mechanisms are still under study. For example, from the morphological point of view, it was discovered that cells react to the electric field application by contracting and blebbing [2].

These findings were significantly made possible by the slight transition in studying these phenomena from classical biological support (e.g., culture dishes) to Lab-On-Chip platforms, which allow the set-up of more complex experiments in a minimal environment.

Information extracted from these devices can sometimes be challenging to analyze without using more complex models, such as machine learning algorithms. Particularly in microscopy image analysis, these models permit extracting hidden features and intricate trends that are impossible to catch quantitatively by hand.

In this work, we present a sensing system based on a low-cost Lab-On-Chip platform able to correlate the electroporation efficiency with its side effects by applying a tailored machine learning algorithm on brightfield time-lapse microscopy images.

Method description

A Lab-On-Chip based on transferred Laser-Induced-Graphene (LIG) was designed and fabricated using a technique we recently proposed [3]. Briefly, LIG electrodes are first generated by laser scribing a polyimide sheet. Then, the printed geometries are transferred to a transparent, biocompatible PMMA substrate by surface solubilization.

The chip exhibited six pairs of interdigitated electrodes with different pitches, used to stimulate the U-87 glioblastoma cell line with several voltages and electric fields (Fig. 1). During the stimulation, fluorescence and brightfield time-lapse microscopy monitored calcium intake and cell contractility, respectively.

A custom machine-learning algorithm based on Particle Image Velocimetry (PIV) and cell segmentation computed single-cell contractivity and

internal calcium quantity tracking during the time-lapse [4].

Finally, the extrapolated peaks of cumulative velocity were thresholded and used to estimate the electroporation efficiency in the acquired frame. Similarly, we used the detected calcium intake spikes as the ground truth, calculating the real electroporation efficiency.

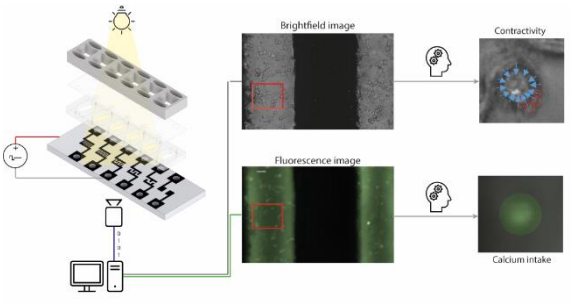

Fig.1. A Lab-On-Chip is designed to have six different interdigitated electrodes and separated chambers for cell culture. Each electrode pair is connected to a high-voltage pulse generator. Fluorescence and brightfield time-lapse microscopy are acquired at the same time. Each time-lapse frame is then analyzed using a tailored machine, computing cell contractivity and calcium intake.

Results

To better understand the behaviour of cell culture after electroporation, it was necessary to study cell movements and calcium presence before the stimulus. As expected, cells exhibited an independent movement, which was necessary to subtract from the one induced by the electric field application. Moreover, U-87 cells were demonstrated to contain a significant amount of calcium ions in their cytoplasm also before the poration stimulus.

Once the pulsed electric field was applied, cells started to contract and bleb, generating a peak in contraction detected by PIV. Calcium diffusion was observed to be almost instantaneous, while cell contractivity follows a second-scale dynamic. Moreover, as expected, the beginning of cell contractivity perfectly correlates with the fluorescence peak induced by calcium intake caused by cell poration (Fig. 2a).

Finally, velocity and fluorescence peaks from single cells were then separately thresholded and used to compute the electroporation efficiency of the experiment. As can be evinced from Fig. 2b, there is an almost complete correspondence between the efficiency computed by fluorescence and the one calculated from contraction ($R^2 = 0.931$ around the bisector). Moreover, these results were obtained by aggregating data from different electrodes and electric fields. This indicates that our sensing system can predict electroporation from brightfield time-lapse microscopy without additional fluorescent channels independent of the experimental conditions.

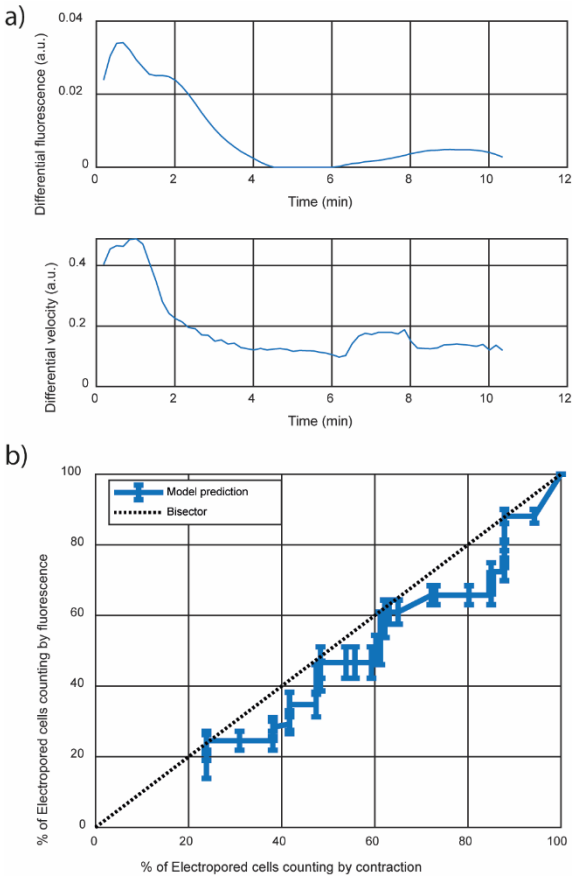

Fig 2. a) Fluorescence intensities from single cells during the time-lapse experiments are compared with the contractivity velocity calculated by PIV analysis. b) Average fluorescence intensity is correlated with the peak velocity. Cell population heterogeneity and different electrode geometries contribute to performance variability.

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