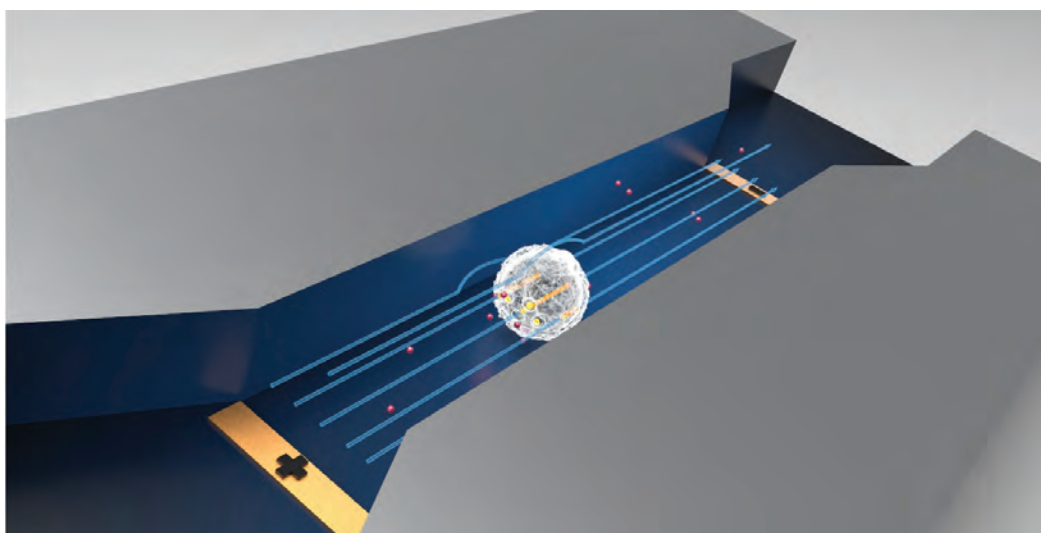


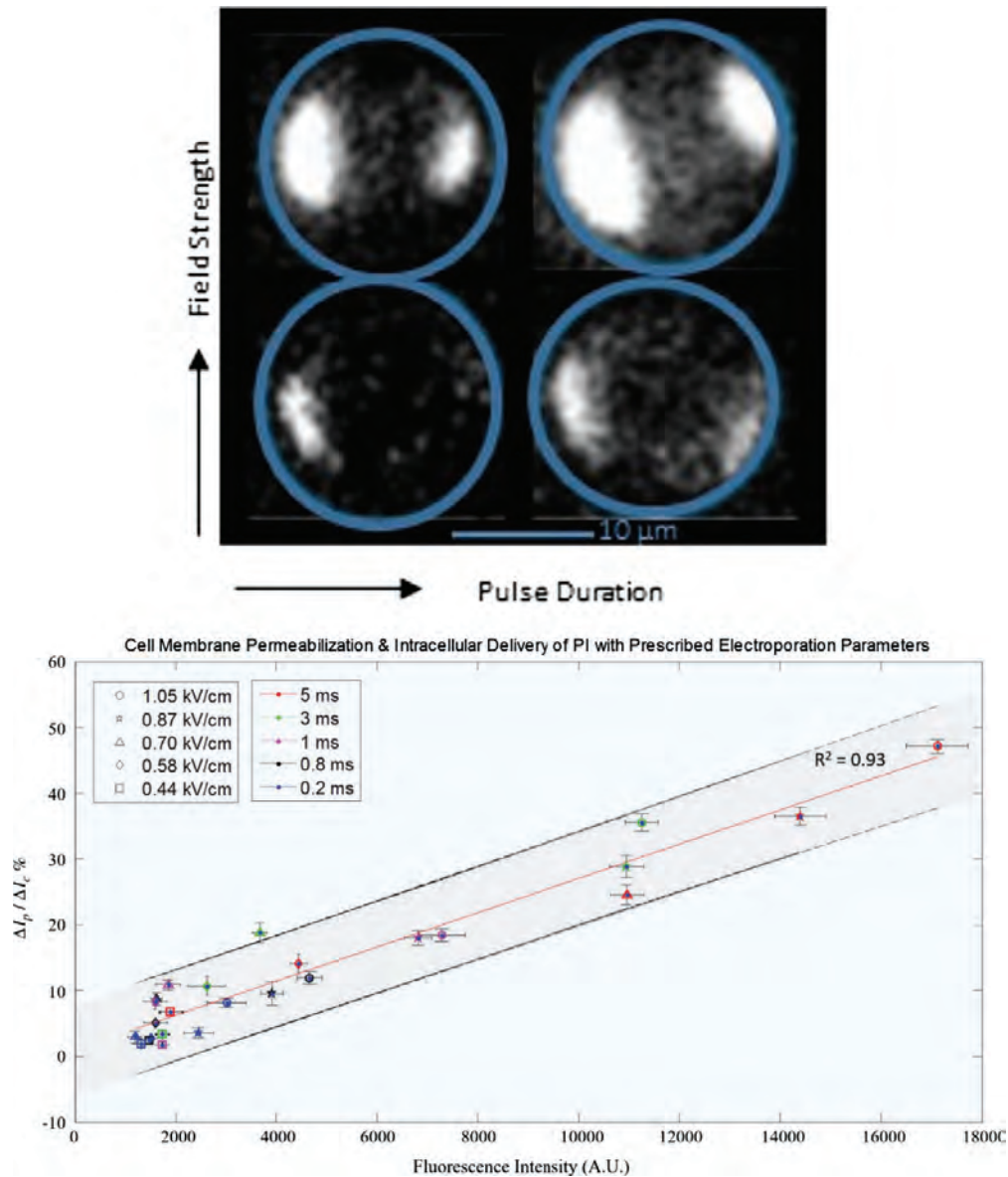
PRESS RELEASE

Continuous-flow, electrically-triggered, single cell-level electroporation

March 1, 2017 — A flow-based electroporation microdevice that automatically detects, electroporates, and monitors individual cells for changes in permeability and delivery enabling a high throughput, controlled electroporation platform.



(Top) 3-dimensional rendering of a cell passing through the electroporation zone while a pulse is being applied. The electric field lines which normally do not penetrate an insulating cell are able to penetrate the cell through the conductive pores. (Middle) Epifluorescence images of cells following delivery of a fluorescent dye, propidium iodide, following electroporation treatment at varying pulse strengths and duration. Propidium iodide is impermeant to the cell until the membrane is permeabilized via electroporation. The propidium iodide is then transported into the cell cytoplasm and fluoresces upon binding cytoplasmic nucleic acids. (Bottom) The degree of membrane permeabilization measured electrically via a change in cell membrane impedance is well-correlated to the fluorescence intensity of propidium iodide delivery, following electroporation treatment with varying pulse strengths and duration.



(Continued)

Graduate students Mingde ‘Jack’ Zheng and Joseph Sherba have developed a novel, microfluidic platform for monitoring electroporation and molecular delivery at the single cell-level as part of a collaborative research team led by Professors Jeffrey Zahn and David Shreiber in the Department of Biomedical Engineering and Professors Hao Lin and Jerry Shan in the Department of Mechanical and Aerospace Engineering at Rutgers, The State University of New Jersey, in Piscataway, NJ. Electroporation is a widely used, safe, non-viral approach to deliver foreign vectors into many different cell types. When a cell is exposed to an electric field of the appropriate strength, the membrane undergoes reversible electrical breakdown, where transient pores form in the membrane, which allows molecular transport into the cell. The controlled intracellular delivery of biomolecules and therapeutics enables the ability to study and engineer fundamental cellular processes and has therefore been a major focus in biomedical research and clinical medicine. According to BioMarket Trends, the electroporation market currently represents the second largest segment of the total, ~\$200 million transfection technology market in terms of revenues, behind lipid based technology¹. Consumers in the market include those in biomedical research in academic and industry labs and biotechnology and life science companies who aim to express

¹Witonsky, J. (2009) Transfection Market Clamors for Silver Bullet. BioMarket Trends Vol 29 No. 20.

specific molecules in a variety of cells. In addition, there is increased interest in using transfection technology clinically, especially with the advent of CRISPR technology for gene editing.

Successful cell transfection represents the rate-limiting step in numerous biomedical research and bioproduction workflows including: cell based therapies, RNA interference screening, and stem cell research. The challenges include variable and poor transformation efficiency, especially with hard-to-transfect cell lines such as primary cell lines and stem cell lines. One of the traditional bottlenecks to electroporation has been obtaining efficient delivery without compromising cell viability. Successful electroporation involves the optimization of a wide range of electric field and buffer parameters that are affected by the cell type and molecular payload to achieve an ideal balance of the efficiency of transfection (how much is delivered) with the damage produced (how many cells are damaged or die). Protocols are often identified through costly trial-and-error, and can vary significantly from lab-to-lab and application-to-application. The Rutgers interdisciplinary research and development team are focusing on translational research in developing next-generation electroporation technology for high-efficiency delivery into cells with superior viability.

The novelty of this report is the impedance detection of membrane permeabilization in a continuous flow environment at unprecedented sensitivity, an accomplishment not previously reported in literature. By monitoring changes in the electrical characteristics of individual cells when exposed to short, high-strength electric fields, the team was able to identify when a cell has become permeabilized and determine the conditions which led to molecular delivery while preserving cell viability. This technology will expedite the transfection process by eliminating trial-and-error electroporation protocol development in a safe and effective manner across cell types and applications.

The micro-electroporation platform was realized following extensive theoretical modeling. The team designed and fabricated a microfluidic device consisting of a converging microfluidic ‘electroporation zone’ and a set of electrodes capable of both pulsing the passing cell in transit and sensing the degree of cell membrane permeabilization. The electroporation microchip is integrated with a custom-built LabVIEW algorithm that continuously monitors the channel for the entrance of a cell into the electroporation zone. Upon detecting a cell, a prescribed electrical pulse is applied to the cell and the electrical signal is monitored for changes in membrane permeabilization, which ultimately determines the therapeutic payload potential.

A widespread parametric study was performed by altering both the electric field strength and pulse duration and electrically measuring the membrane impedance response immediately following pulse application. The degree of membrane permeabilization was dependent on the intensity of the pulse application, with a significant increase in permeabilization occurring at a pulse duration of 0.8 to 1 ms. This trend was also verified by optically monitoring the delivery of a fluorescent probe, propidium iodide, which is impermeant to cells with intact membranes but is transported into the cell upon compromising the membrane via electroporation. Cell viability trends were also shown to be dependent on the strength and duration of the pulse being applied.

Moving forward, the Rutgers team looks to continue the development of this technology into a “smart”, autonomous system that is capable of using these electrical signals to create a flow-through, feedback-controlled, single cell-level electroporation platform. Improvements in transfection efficiency will allow electroporation-based cellular transformation approaches to become more commonplace and supplant viral based approaches. They envision a product line comprised of a base equipment docking station and software that applies the electric pulses and monitors permeabilization in real time using disposable “chips” for cell handling and microfluidics. The end result will be a product line that is easy to use, reproducible, and robust, which will open up a wide range of applications by basic research laboratories, development and production laboratories in the biotechnology sector, and ultimately clinical entities interested in direct gene editing or transfection for transplantation and cellular therapies.

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