Identifying severity of electroporation through quantitative image analysis

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Electroporation is the formation of reversible hydrophilic pores in the cell membrane under electric fields. Severity of electroporation is challenging to measure and quantify. An image analysis method is developed, and the initial results with a fabricated microfluidic device are reported. The microfluidic device contains integrated microchannels and coplanar interdigitated electrodes allowing low-voltage operation and low-power consumption. Noninvasive human buccal cell samples were specifically stained, and electroporation was induced. Captured image sequences were analyzed for pixel color ranges to quantify the severity of electroporation. The method can detect even a minor occurrence of electroporation and can perform comparative studies. © *2011 American Institute of Physics*. [doi:10.1063/1.3575561]

When electric pulses of intensity in kilovolts-per-centimeter and of duration in microseconds to milliseconds are applied to a cell suspended in an ionic medium, numerous reversible hydrophilic pores are formed within the cell membrane.[1] This causes a temporary loss of the semipermeability of the cell membrane and is termed as electroporation. Electroporation can lead to ion leakage, escape of metabolites, and an increased uptake of drugs, molecular probes, and genetic materials. Applications of electroporation include controlled drug delivery, gene transfection, and vascular therapy.[2]

Severity of electroporation determines the amount of molecular uptake and the cell survival rates.[3] Electroporation is shown to be critically dependent on various parameters including the excitation durations, electric field magnitudes, orientations of the electric field, and buffer osmolarities.[4–7] However, a major challenge of studying electroporation is the lack of quantitative methods to measure and compare the severity of electroporation.[8] To quantify the severity of electroporation, we introduce and define the term "Flow Index of electroporation" based on an image analysis method. The corresponding initial results using experimental data from a fabricated microfluidic device are reported here.

Flow Index of electroporation is defined as a numeric indicator of the severity of electroporation and relates to the amount of fluidic exchange between an electroporated cell and its environment. A higher value of Flow Index of electroporation represents higher fluidic exchange. The minimum Flow Index value is 0 representing no occurrence of electroporation, and the maximum value is 1 representing complete exchange of fluids that usually occurs in the case of lysis.

A microfluidic device, as shown in Fig. 1, is designed using MEMSPRO tool from SoftMEMS Corp., CA, USA, and fabricated using SensoNit Fabrication Process from Micronit Inc., Netherlands. The fabricated device is 3 cm long, 1.5 cm wide, and 1.8 mm thick. The device contains integrated microchannels and three types of ports; fluidic ports, sample loading ports, and electrode ports. An electroporation chamber designed within the device is composed of five parallel microchannels of 400 μm wide, 12 mm long, and 40 μm deep each, and contains coplanar interdigitated electrodes at the bottom of each microchannel. The fingers of the interdigitated electrodes are 420 μm long and 10 μm wide, and have 10 μm gaps in between. Nanoport Assembly from Upchurch Scientific, WA, USA, are fixed on top of the fluidic ports to connect the microchannels with the microfluidic pumps using capillary tubes. The external electrodes are connected to the integrated electrodes through the electrode ports using silver conductive epoxy.

Prior to the initial use of the microfluidic device, the device wetting was performed with 10% detergent solution. A pulse generator was used as the excitation source and an oscilloscope was used to monitor and record the pulse waveform. Two microfluidic pumps were used to introduce buffer fluids in the electroporation chamber. Experiments were performed on the stage of an optical microscope connected with a charge-coupled device camera to capture the sequence of images at every 0.5 s interval in the bright field mode. The fixture for the experiments was custom made with a clean glass surface on which the device was secured with silicone to enable proper positioning on the microscope stage.

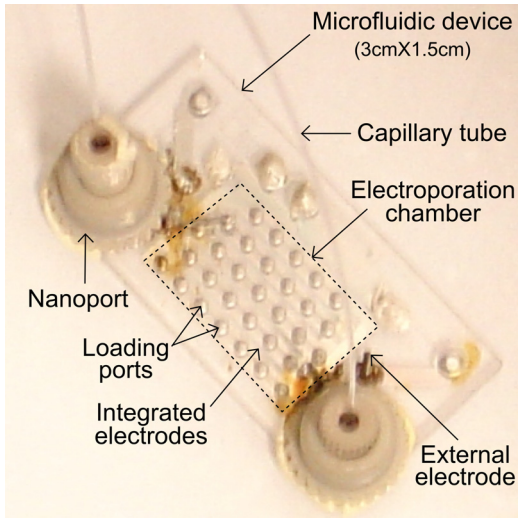

FIG. 1. (Color online) A photograph of the microfluidic device and test setup indicating the key components.

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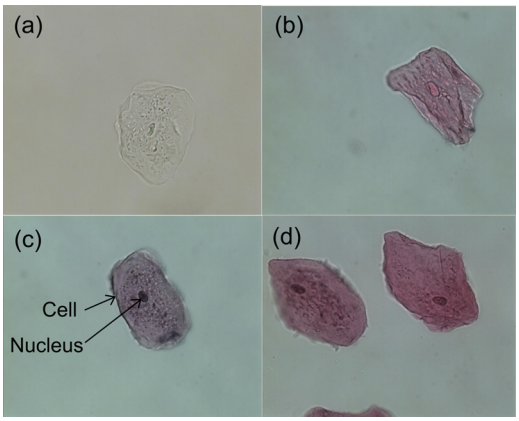

FIG. 2. (Color online) Optical microscope pictures in bright field mode with 40X objective lens depicting images of the sample buccal cells with various stains; (a) an unstained cell; (b) a cell stained with Eosin Y; (c) a cell stained with both Eosin Y and Haematoxylin; and (d) stained cells observed after a week of storage–showing that the stain does not degrade with time.

Buccal sample cells were collected with sterile swabs from volunteers. To detect electroporation, sample cells were stained with both Haematoxylin and Eosin Y as shown in Fig. 2. Haematoxylin stain colors basophilic structures, such as DNA, with a blue-purple hue. Eosin Y stain colors eosinophilic structures, like cytoplasm, in bright pink or magenta. These specific stains did not degrade after storage for a week.

The device was secured on the stage of a microscope using the fixture so that the electroporation chamber is in the vicinity of the focus area of the microscope. Buffer fluid in the amount of 10 μl was injected inside the electroporation chamber using the microfluidic pumps. Stained samples were then introduced to the chamber using the loading ports. Next, an excitation pulse was applied through the electrodes and simultaneously the image capturing sequence was initiated.

Figure 3 shows two sets of images for a control case and an electroporation case. As depicted in case 1 of Fig. 3, the stains of the control sample suspended in the buffer fluid remains unchanged. In contrast, as shown in case 2 of Fig. 3,

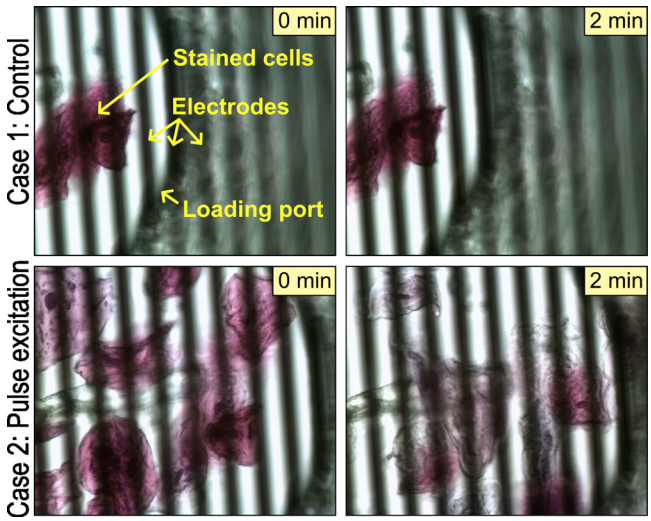

FIG. 3. (Color online) Two sets of pictures demonstrating the effect of an electroporation pulse. Case 1: cell stain remains unchanged after 2 min when no pulse is applied (control case). Case 2: a pulse of 20 V for 100 ms causes Eosin Y stain to reduce significantly due to electroporation.

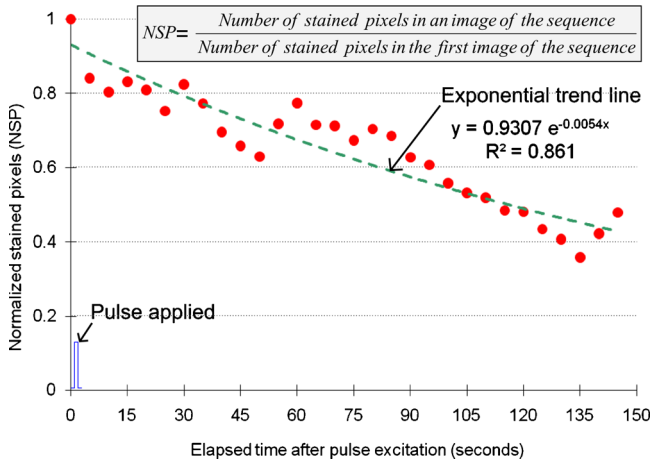

FIG. 4. (Color online) Normalized stain pixel plot shows an exponential reduction in stains with elapsed time. The experimental data is shown using dots and the trend line is indicated by the dashed line.

significant reduction in Eosin stain within 2 min occurred after a pulse of 20 V for 100 ms was applied. In fact, applying a pulse of a range of magnitudes and durations caused stains to decrease. Due to electroporation, stained fluids exit the cell into the buffer fluid causing dilution of stains.

To conclusively identify the occurrence of electroporation, the image sequence for each experiment was analyzed using a VISUAL BASIC script and a MATLAB code. The script compared each pixel of an image with a color range for Eosin Y stain to find the number of stained pixel. The normalized numbers of stained pixels were calculated by dividing the number of stained pixel in an image by the number of stained pixel in the first image of that sequence. The normalized stained pixel (NSP) from the image sequence corresponding to case 2 of Fig. 3 is plotted in Fig. 4. The plot shows an exponentially decreasing rate for the stained pixels in the image sequence closely matching the exponential trend line drawn using a curve fitting technique, as shown in the figure.

The final value of the NSP is proportional to the amount of fluidic exchange that relates to the defined flow index of electroporation. Hence, the Flow Index can be calculated by subtracting the final value of the NSP from 1. Mathematically,

$$Flow\ Index = 1 - final\ value\ of\ the\ NSP.$$

For the example, shown in Fig. 4, the Flow Index of electroporation is 0.57. This index allows a quantitative comparison between experimental electroporation results under different parameters and conditions.

Electroporation with microsecond duration pulses and small supply voltages were conclusively demonstrated and quantified with the fabricated microfluidic device and the developed method. The method along with the quantification term Flow Index of electroporation enables quantitative studies to relate various pulse parameters and environmental conditions to the severity of electroporation. This technique allows for the determination of optimal conditions for electroporation in various practical applications.

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