*Advanced Materials Research* *Online: 2009-01-20*
*ISSN: 1662-8985, Vols. 60-61, pp 44-48*
*doi:10.4028/www.scientific.net/AMR.60-61.44*


# A DC Voltage Driven Flow-Through Electroporation Microchip

Qiaole Zhao[1,a], Yu-Cheng Lin[2,b] and Qing-An Huang[1,c]

[1] Key Laboratory of MEMS of Ministry of Education, Southeast University, Nanjing 210096, China
[2] Department of Engineering Science, National Cheng Kung University, 701,Tainan, Taiwan
[a]zhaoqiaole@163.com, [b]yuclin01@ncku.edu.tw, [c] hqa@seu.edu.cn

**Keywords:** Electroporation, Electric field strength, DC voltage

**Abstract:** Differing with the traditional way to perform electroporation (EP) by using the DC electrical pulse, this paper proposes a new EP system by applying continuous DC voltages to generate proper EP electric field strengths utilizing the shape change of the channel. The fabrication of chip and set-up of system are clearly described and simulations also carried out utilizing CFD-ACE to study the electric field strength distribution and the time span when fluid passes through different electric field strengths. The fabrication of the proposed EP system is quite simple and low-cost.

## 1. Introduction and Principle

Electroporation is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA [1].

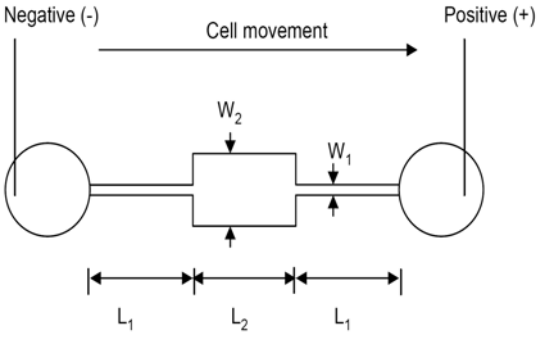
A one pulse microfluidic device fabricated on PDMS and determined the Chinese hamster ovary cells' electroporation threshold local field strength was reported [2]. Two electrical pulses induced by designed narrow sections were chosen to perform the cells EP, since more than two EP pulses in sequence would damage the cells [3]. Fluid in the channel can be treated as the resistances, the values of which depend on the resistivity, the length, and the cross section area of the channel. Thus, different resistances can be obtained by varying either the cross section area or the length of the channel. Based on Ohm's law, we are able to get different voltage drops on different sections of the channel due to the various resistances, further to get different electric field strengths in different sections along the microchannel. Thus the effective electric field strengths could be obtained by the designed shapes of the channel while the duration time for cells to stay in specific electric field strengths is controlled by the length of each section and the given velocity.

## 2. System Design

Fig. 1 shows the principle and the dimension of the chip with the dimension of: $W_1$ = 200 μm and $W_2$ = 1000 μm. The polymethylmethacrylate (PMMA) and Polydimethylsiloxane (PDMS) were utilized to fabricate the chip, as shown in Fig. 2. PMMA layers were the major structures to form the channel while PDMS helped to seal the channel so that the fluid would not leak. Thermal bonding at 120 °C for 90 minutes made sure that there was no leakage between two lower layers of PMMA. Screws were used to fix the upper layer of PMMA and PDMS with the lower PMMA layers. Samples were loaded to the inlet and flowed to the receiving reservoir through the Teflon pipe which connected with the output in a DC field. The positive electrode is set to be at the outlet due to the flow direction of negative charged DNA. The channel was fabricated utilizing a $CO_2$ Laser Micro-Machining System.

The schematic of the chip and the system setup are shown in Fig. 3 and Fig. 4. The samples were loaded into the channel with needles controlled by syringe pump at a fixed velocity. Utilizing needle inserted into the channel through PDMS instead of cutting a hole on PDMS for the sample was to make sure there was no leakage at the inlet, otherwise, it would be almost impossible to conduct the

sample into the microchannel due to the atmosphere presser. The outlet of channel was connected with a Teflon tube which led to the reservoir for the collection of the sample. Platinum (Pt) was chosen for the electrodes due to its low resist, to prevent the unwanted voltage consume at the electrodes. 550 V of voltage was applied in this system at the fluid velocity of 75 μl/s to meet the electroporation requirements: the electric field of 500 V/cm for electroporation.



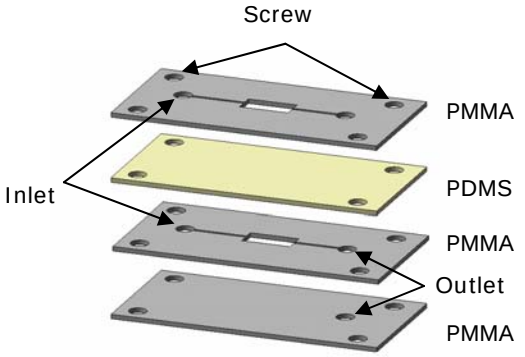




Fig. 1: Schematic of the electroporation channel. Fig.2: The expanded view of the designed chip.

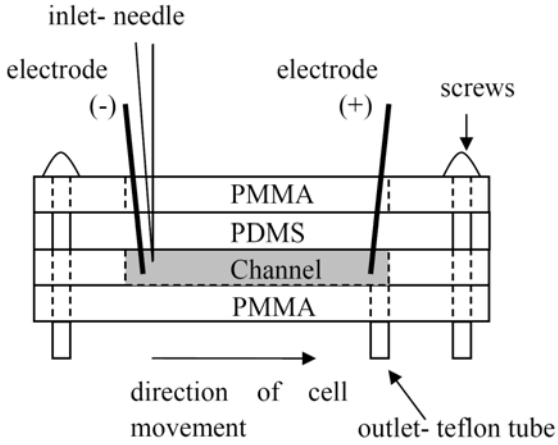



Fig. 3: The setup of the EP chip.

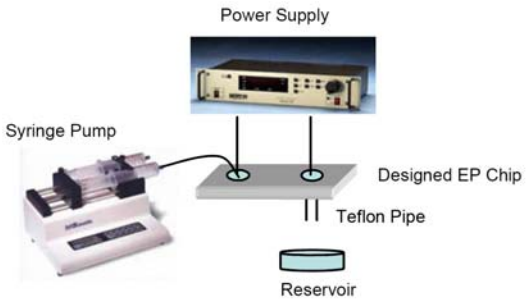



Fig. 4: The schematic of the setup of EP system.

## 3. Experiment

Jurkat cells were used in the experiment, which are an immortalized line of T lymphocyte cells. They are round cells growing singly or in clumps in suspension. The jurkat cells were cultured in RPMI containing 10% Fetal Bovine Serum (FBS), under the condition of 5% $CO_2$ at the temperature of 37 degrees. Plasmid DNA was extracted from bacterial cells by VIOGENE and stored at 4 degree. Before each experiment, the jurkat cells were pelleted by centrifuging for 5 minutes at the 1500rpm. Then we decanted the supernatant and removed all medium residues by pipet. Then cells then were washed by phosphate buffered saline (PBS) 3 times before put in RPMI without FBS. $1\times10^6$cells /ml and 40 μg/ml of plasmid were adopted in the experiment.

## 4. Simulation and Results

The Simulations were also carried out on the personal computer using the CFD-ACE to analyze different models. The electric field strength and the time span were studied in each model.

The electroporation microchannel model1 has the dimension of: $W_1$ = 200 μm, $W_2$ = 1000 μm, $L_1$ = $L_2$ = 5 mm, as shown in Fig. 5. Electrical field strengths were uniform in each region, and the intensity in the narrow region is 5 folds of that in the wide one and is proper to use in the experiment. The different colors also indicated the different electrical field strengths, the blue in the wide part showed the lower electrical field strength while the red color represented the higher electrical field strength. The comparison between the data and the model shape can be clearly seen in Fig. 6 (a). Since the electroporation is the effect of the high electric field strength on cell, the time

spans of the samples stay in different electric field strengths are therefore considered important in analyzing the electroporation effect, as shown in Fig. 6 (b). By controlling the time span in high electric field strength, we will be able to obtain a promising efficient EP without damaging the sample.

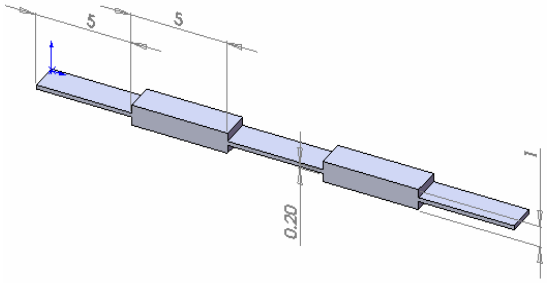
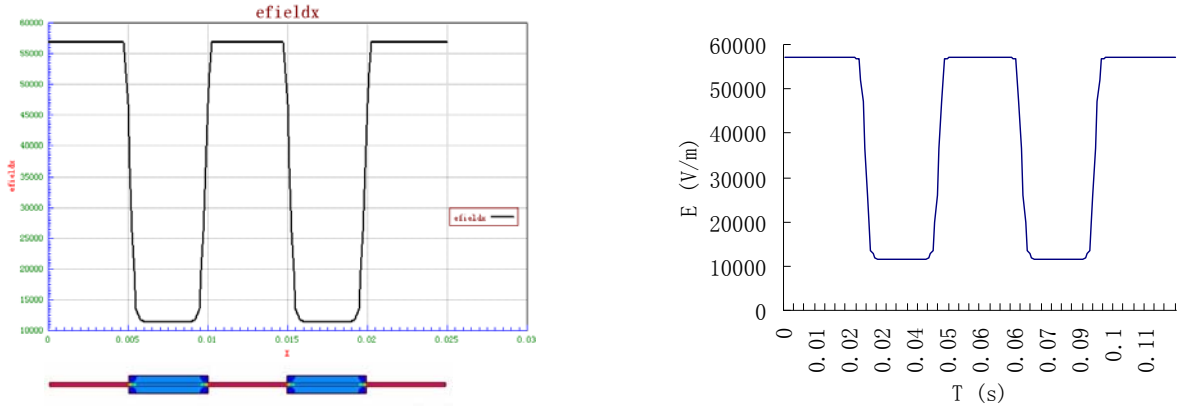



Fig. 5: Model1: the length ratio of narrow rectangle part to wide rectangle part is 1:1




(a) (b)

Fig. 6: Simulation results of model 1: (a) electrical field strength distribution, (b) the relationship of electric field and time span in model 1.

### 4.1 Length Adjustment

Different models were designed to observe the electroporation performances. Different length ratios of narrow part to wide part influence the time span when the samples are in the different electric fields, which will result in different electroporation effects. The electroporation microchannel model2 has the dimension of: $W_1$ = 200 μm, $W_2$ = 1000 μm, $L_1$ = 5 mm, and $L_2$ = 10 mm. Thus, the length ratio of narrow rectangle part to wide rectangle part is 1:2.

The electric field ratio between wide part and narrow part is decided by the section area ratio of those two parts. Therefore, when only changing the length of the wide part without modifying the width, the electric field ratio of the wide part to the narrow one remains the same, shown in Fig. 7 (a). However, the changed length of the wide part has a significant influence on the time span of the samples traveling through when the inlet velocity is fixed, as shown in Fig. 7 (b).

### 4.2 Shape Adjustment

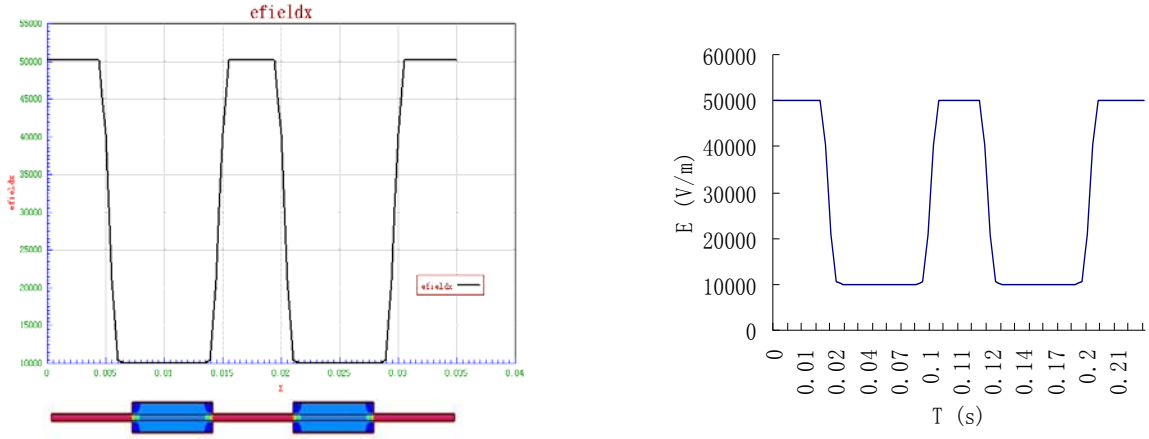
To study the influence of the channel shape on the electroporation, we change the wide rectangle part in model1 to triangle shape as shown in Fig. 8. And simulations were also carried out about this model. Both the electric field strength is not uniform in triangle part and the samples may go through a period of increasing electric field strength in that area, as shown in Fig. 9 (a) and (b), which will have an effect on electroporation.

## 5. Conclusion

In all, an EP system utilizing continuous DC voltages was proposed and simulated in the paper. The fabrication of chip and set-up of system were described and simulation of the proposed EP system was also shown in the paper. Simulations using CFD-ACE were also carried out on different models. The proposed EP system gains the advantages of low cost and easy fabrication and will provide potential application in the bio-MEMS fields.

**Acknowledgement**

This work was supported by a grant from National High-Technology Research Program (863) under contract No. 2006AA04Z302.




(a) (b)

Fig. 7: Simulation results of model 1: (a) electrical field strength distribution, (b) the relationship of electric field and time span.

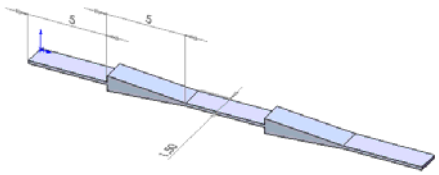
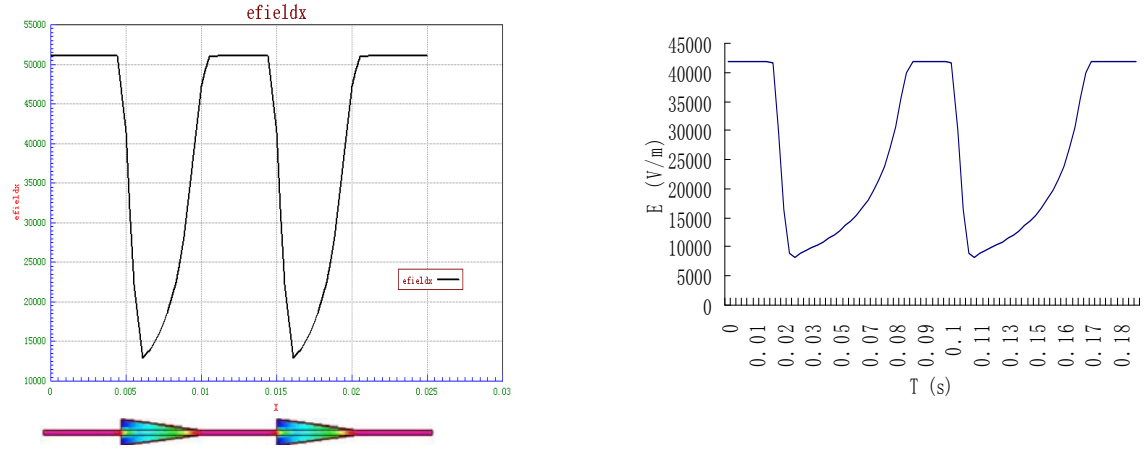


Fig. 8: Model3: the length ratio of narrow rectangle part to wide triangle part is 1:1.




(a) (b)

Fig. 9: Simulation results of model 3: (a) electrical field strength distribution, (b) the relationship of electric field and time span.

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