

AN OPTICAL-INDUCED PLATFORM FOR MULTIPLE GENES TRANSFECTION

Hsin-Tzu Kuo¹, You-Hsun Lee¹, Chih-Hung Wang¹, Chen-Min Chang² and Gwo-Bin Lee¹

¹*Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan*

²*Department of Engineering Science, National Cheng Kung University, Tainan, Taiwan*

ABSTRACT

Gene transfection is an important technology in biological applications. Electroporation is one of gene transfection methods that deliver extracellular genetic materials into cells by using a high electrical field to forming pores on cell surface. However, the high voltage provided by an electroporator may impair cells to survive and cause low efficiency of yield rate. In this study, a new platform was developed for gene transfection under a lower applied voltage by utilizing an optical induced non-uniform electric field. To obtain higher transfection efficiency, a multi-spot optical image was projected to the gene transfection chip, resulting in localized non-uniform electric fields generated from these optical patterns. The experimental results in this study showed that one or more plasmid carried fluorescence genes could be effectively transfected into mammalian cells and the cells successfully expressed foreign proteins by using the developed optical-induced gene transfection platform. Moreover, the transfection efficiency of optical-induced gene transfection platform was significantly higher than the traditional electroporation technology. This developed platform may provide a simpler and more efficient tool for gene transfection.

KEYWORDS

gene transfection, optical-induced dielectrophoresis (ODEP), microfluidics

INTRODUCTION

In recent years, several gene transfection methods, including physical (electroporation), chemical (calcium phosphate method) and biological (viral carrier) techniques, have been widely applied to change cellular properties by introducing foreign genetic materials into cells. The gene electroporation has widely applied in a variety of applications including gene therapy, DNA vaccine, RNAi and induced pluripotent stem (iPS) cell. Among them, electroporation has been commonly used in gene transfection which alters the permeability of cell membrane transiently for extracellular DNA entry. However, large-scale and expensive electroporation devices are used and more importantly, relatively high applied voltage (>190 volts) are require, which may limit the practical applications of this method. The high voltage may also cause cell damage and lead to extremely low transfection efficiency.

The iPS cell is a kind of cells which are generated from various somatic cells and can be reprogrammed to embryonic stem (ES) cells. The iPS cell technology has been demonstrated and commonly used by viral transduction with defined factors carried vectors [1]. However, the low yield of the iPS cells and virus-caused damage for therapeutic applications still remain to be an issue. To tackle these problems, effective and virus-free gene transfection platform is therefore of great need. In previous study, the optical-induced dielectrophoresis (ODEP) was used to manipulate and lyse cells in a non-uniform electric field by projecting optical images on a photoconductive material [2]. In this study, a new optical-induced gene transfection platform was developed to deliver extracellular DNA into mammalian cells, which may have great potential for iPS study. The new optical-induced platform could effectively and simply transfect one or more plasmids into cells. The ODEP-based platform may be demonstrated as a more effective and safe tool in iPS cell reprogramming process.

EXPERIMENT

The optical-induced gene transfection platform was comprised of a function generator, an ODEP chip, a fluorescent microscopy, a commercial digital projector and an image acquisition system, as schematically shown in Fig 1(A). Figures 1(B) and 1(C) show an exploded view and a photograph of the ODEP chip. This chip was consisted of a thin layer of amorphous silicon coated on indium tin oxide (ITO) glass as a bottom layer, 50- μm double-side tape as a spacer layer and another ITO glass with PDMS inlets/outlets as a top layer. The amorphous silicon is a photoconductive material whose resistivity may be changed significantly when illuminated by optical images [2]. The 50- μm tape formed a sealed gap between two ITO glass plates such that a cell chamber can be formed in the ODEP chip. The top ITO glass plate with two via holes constructed with PDMS was utilized for cells inlet and outlet. When 20- μl cells and 10 $\mu\text{g/ml}$ plasmid DNA were co-injected into the chip, a non-uniform electric field can be locally induced when illuminating optical images while an alternating current (AC) voltage was applied between two ITO glass plates.

In this study, in order to obtain higher transfection efficiency, a circular pattern composed of multiple optical spots was projected onto the chip, as shown in Fig. 2(A). The operating process for gene transfection performed on the chip was schematically shown in Fig. 2(B). The cells and plasmid DNA were first loaded into the chip. The cells were then locally electroporated by the induced non-uniform electric field and the fluorescence expressed plasmids entered into cells. Then, the optically-induced cells were replaced by sucrose solution and collected from the cell chamber. Finally, the cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) and high glucose medium and fluorescent protein expression were observed in treated cells after 60 hr.

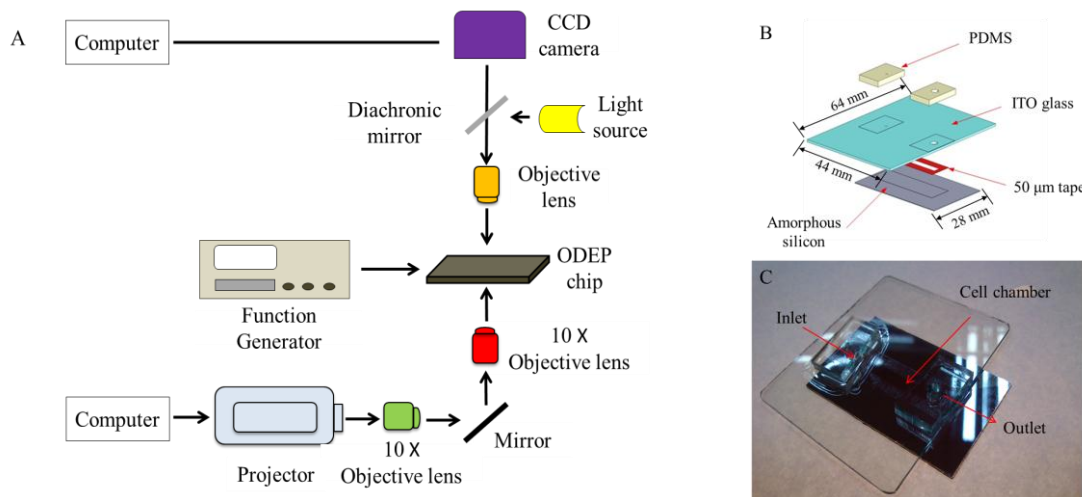


Figure 1. (A) Experimental setup of the optical-induced gene transfection platform, (B) exploded view and (C) a photograph of the chip.

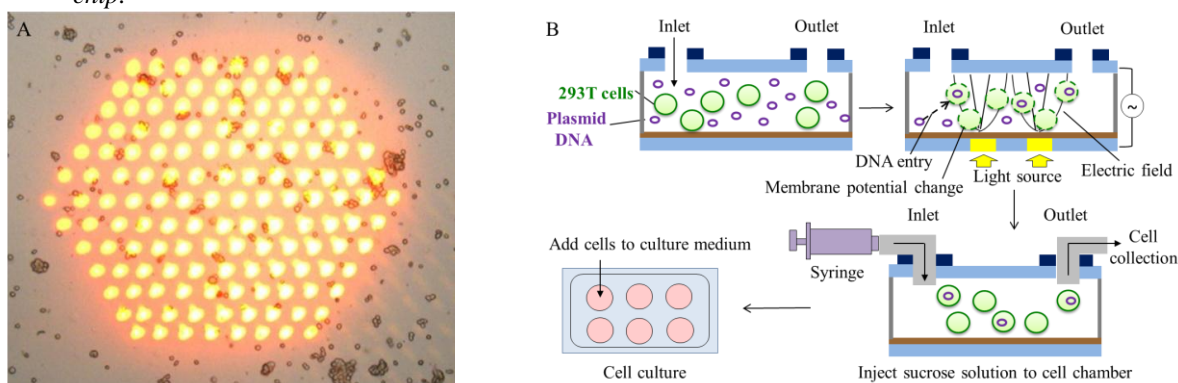


Figure 2. (A) The optical images observed under a microscope and (B) the operating processes of optical-induced gene transfection platform.

RESULTS AND DISCUSSION

The optimal operating conditions of gene transfection into embryonic kidney epithelial 293T cells were first explored. Different frequencies and voltages were first tested to induce ODEP forces. The results of ODEP response were listed in Table 1. The 293T cell line was found to exhibit a positive ODEP force under 100 kHz at 10 Vpp. However, negative ODEP response was observed under 15 Vpp at the same frequency. Furthermore, the enlarged cells were observed experimentally under these conditions, indicating that the permeability of cell membrane was altered. Furthermore, it was reported that the cells experienced negative ODEP force when they were damaged or dead [cite a paper], therefore the operating voltages between 10 to 15 Vpp were further tested to determine the optimal conditions for the gene transfection performed on the ODEP-based platform. The optimal conditions of electroporation were finally determined to be 13 Vpp under 100 kHz.

Table 1: ODEP responses of 293T cells at different operating conditions

Frequency (kHz)	Voltage (Vpp)	ODEP response
100	10	Positive
100	15	Negative
100	20	Negative

In order to further investigate the optimal illumination time of every multi-spot illumination, single pEGFPC vector which expressed green fluorescent protein was transfected into the 293T cells by using the optical-induced gene transfection platform. Illumination time ranging from 5, 10, 15, 20 sec was tested. Note that the intensity of the illumination was measured to be 0.29 mW. The transfection efficiency of the cells from the optical-induced platform was significantly higher than that obtained from a traditional electroporator (BTX ECM600, San Diego, CA, USA) as shown in Fig 3. The maximum efficiency of gene transfection could be as high as 6% when 15 sec illumination time was used when 13 Vpp and 100 kHz were applied. Note that the efficiency of gene transfection from the electroporator was only 0.05%.

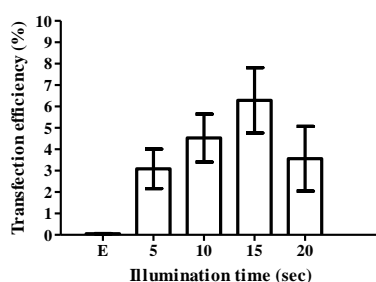


Figure 3. The transfection efficiency at different illumination time when 13 Vpp and 100 kHz were applied. Note that the case "E" is the result obtained from a traditional electroporator.

The capability of the developed platform for multiple plasmid transfection was also explored. Figure 4 shows the transfected 293T cells under fluorescent microscopy observation. The pEGFP-C1, pDsRed-express-1 and pECFP-H2B plasmids, which carried green, red and blue fluorescent genes, respectively, were transfected and used as markers to monitor the process of gene transfection. The single and triple plasmids co-transfection were successfully achieved by observing green, red and blue fluorescence in one single cell, as shown in Fig. 4. Note that the entire process only took 2 min. It is the first time that three plasmids can be transfected by using this optically-induced platform.

The developed system can be further used for single-cell gene transfection. If the light is illuminated on a single cell, then the neighboring cells are not electroporated. Since the light can be precisely illuminated on a selected cell, it also opens up a possibility to selectively lyse a single cell. Furthermore, continuous gene transfection may be feasible if a microfluidic device is integrated with this platform.

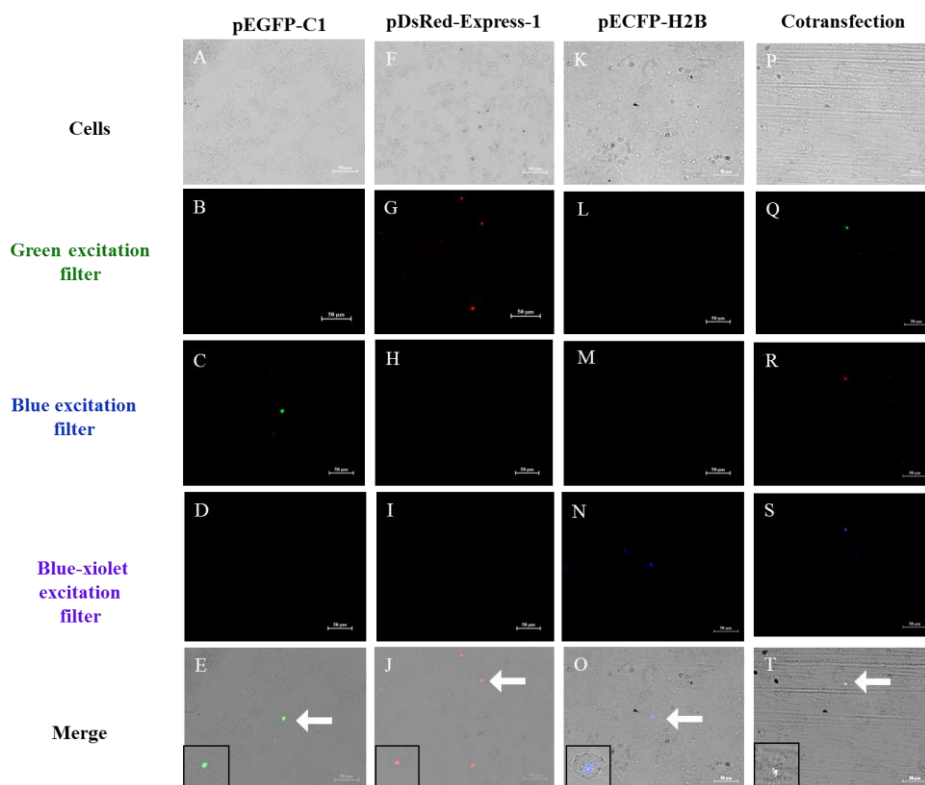


Figure 4. The 293T cells were transfected with plasmids by using the optical-induced gene transfection platform and visualized under fluorescent microscopy. Cells were transfected with pEGFP-C1 (A-E), pDsRed-Express-1 (F-J), pECFP-H2B (K-O) or triple (P-T) and visualized with green (B,G,L,Q), blue (C,H,M,R) or blue-violet (D,I,N,S) excitation filters. Transfected cells (white arrows in E, J, O, T) were distinguished in merged figures.

CONCLUSION

Gene transfection is a widely utilized tool and plays an important role in gene regulation research. The new optical-induced platform only requires 20- μ l of cell sample and a low voltage for gene transfection. Besides, one or more plasmids can be delivered into cells at same time by using this platform. The results suggested that the developed optical-induced platform is a more effective and simpler tool for gene transfection. The developed ODEP-based platform may be applied for iPS study.

ACKNOWLEDGEMENTS

The authors would like to thank the National Science Council in Taiwan for financial support and also appreciate Dr. J. J. Wu at Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University for providing access to electroporation device

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CONTACT INFORMATION

*Dr. Gwo-Bin Lee, Tel: +886-3-5715131 Ext. 33765; gwobin@pme.nthu.edu.tw