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A study on transfection into the cells of optimal condition using Laser Induced Emergent Stress Wave

レーザ誘起応力波による遺伝子導入の最適条件

Mieko Kogi† , Koji Aizawa , Syun Nishimura , Terue Takeuchi , Motoaki Nishiwaki , Yoshiaki Tokunaga (Kanazawa Institute of Technology)
小木美恵子† ， 會澤康治 ， 西村駿 ， 竹内光恵 , 西脇基晃 ， 得永嘉昭（金沢工業大学）

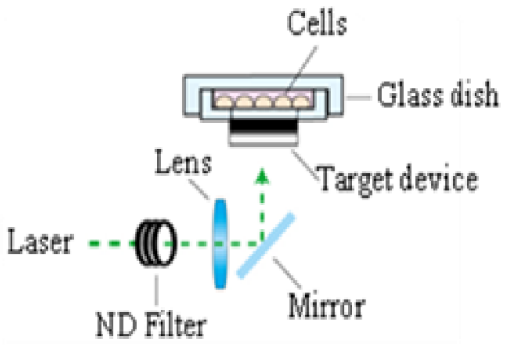
1. Introduction

Several studies have reported biological, chemical and physical techniques for the gene transfection [1-4]. However, it is necessary for the gene transfection to establish a way with much higher transfection efficiency and with higher safety in the transfection. We have developed a new method that has used the laser induced emergent stress wave (LIESW) generating by the Q switch Nd: YAG pulse laser irradiation to natural rubber. Tokunaga et al. have studied on the mechanism and principle of LIESW [5,6]. We reported an optimal condition of the concentration of plasmid DNA [7]. In this study, we found an optimal condition of the gene transfection efficiency by the number of cells and by LIESW.

2. Laser Induced Emergent Stress Wave (LIESW)

Figure 1 shows our apparatus of the transfection. Light source of laser was Q-switch YAG Laser (Spectra Physics, LAB-130). Laser fluence was adjusted 1.7J/cm^2 by ND filter. Target device was composed natural rubber (NR) and polyethlene terephthalate (PET). This device was adhered under glass base dish which immobilized HeLa cells.

Fig. 1 Transfection apparatus.



e-mail address: kogi@his.kanazawa-it.ac.jp

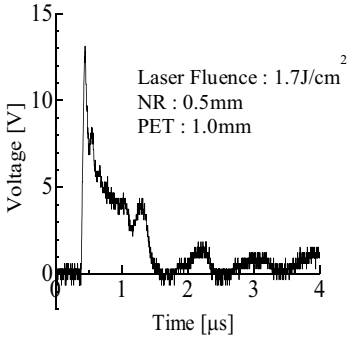


Fig.2 Temporal profile of LIESW.

In our experiment, the intensity of LIESW is observed as that of voltage signal by using a poly(vinylidene difluoride) (PVDF) transducer. Figure 2 shows a typical temporal profile of the transducer signal when the PVDF transducer is placed on the back of target device with the NR thickness of 0.5 mm.

3. Cells and DNA

HeLa cells plasmid DNA were used in our experiment. This plasmid was coded Enhenced Green Fluorescent Protein(EGFP). We cultured HeLa cells at the number of cells of 0.5×10^4, 1.0×10^4, 2.0×10^4 and 3.0×10^4 in E-MEM medium supplemented with 10% heat-inactived fetal bovine serum. HeLa cells were cultured in E-MEM, and 24h later, we changed for opti-MEM with 0.1μg/μl-pEGFP DNA(final concentration)[7]. After applied LIESW, we changed for E-MEM again and cultured for 24h. Table 1 shows the number of samples.

Table. 1 The number of sample.

10^4 (cells/200μl)	LIESW (-)	LIESW (+)
0.5	3	6
1.0	3	5
2.0	3	6
3.0	3	6

LIESW (-) : without LIESW
LIESW (+) : with LIESW

We observed the treated HeLa cells with a fluorescence microscope (Nicon,ECLIPSE 80i). Figure 3 shows expressed EGFP HeLa cells. We calculated transfection efficiency as a ratio of the number of GFP positive cells to all cells in a photograph (×100 folds-magnified).

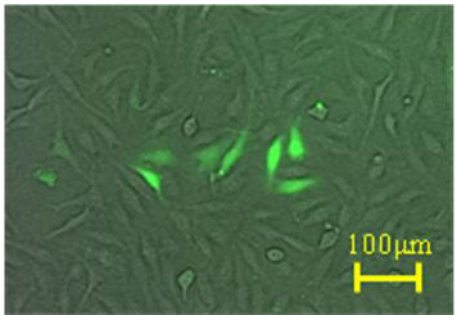


Fig. 3 Expressed EGFP cells.

4. Result

Figure 4 shows gene transfection efficiency at the number of cells.

1)The cells applied LIESW were clearly higher efficient of the gene transfection than the cells without LIESW.

2)The number of cells, 0.5×10^4 and 1.0×10^4 were higher efficiency than 2.0×10^4 and 3.0×10^4.

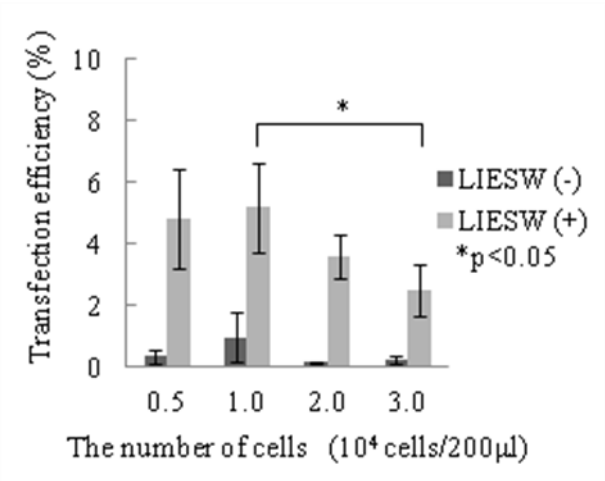


Fig. 4 Gene transfection efficiency by LIESW Values are expressed as means ± standard error(*p<0.05).

5. Discussion

According the study of Tokunaga et al., when LIESWs propagate through glass plate into the cells, the pressure on cells was calculated at approximately 20MPa.

1)We suggested that the cell membrane is temporality unstabilized with LIESW, therefore gene transfection efficiency increases.

2)The number of cells, 2.0×10^4 and 3.0×10^4 didn't have sufficiently large space to be cell devision. Therefore, the plasmid vector can not enter into nucleus

6. Conclusion

We found the optimal condition of DNA concentration for the gene transfection was 0.1 μg/μl at 1.0×10^4 cells.

7. Acknowledgement

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8. References

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