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Effect of Laser Induced Stress Wave on Mammalian Cells
レーザ誘起応力波が細胞に与える影響

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1. Introduction

Currently, a retro virus, virus vector adenoviral are known as a vector for gene therapy. And, the nonvirus vectors such as the liposome, polymer, and electropration are known as a nonviral vector. There are reports that retrovirus vector made to be T-cell acute lymphoblastic leukemia [1] and that the adenovirus vector killed the patients of gene therapy [2]. Although advantages of the nonvirus vector are low toxicity and antigenicity, and the nonvirus vector is expected its utility, the gene transfer efficiency is low, and safety to the cell has not been established. We have developed a new method that has used the laser induced stress wave (LISW) generating by the pulse laser irradiation to the solid material as gene delivery system up to now. Q-switch Nd:YAG pulse laser drove the LISW to human cultured cells. And, plasmid DNA transferred to mammalian cells [3]. But, we've not got study on plasmid DNA transfer mechanism, principle and the effect of LISW on mammalian cells besides.

Therefore, to evaluate the safety based on the intensity of LISW to human cultured cells and viability of those cells, we carried out an experiment of LISW injection into human cultured cells.

2. Laser induced stress wave (LISW)

Figure 1 shows our experimental system. We used Q-switch Nd:YAG Laser (Spectra Physics, LAB-130) as light source. This laser can emit second hermonics at wevelength of 532 nm and has 0.2 J/cm^2 as laser energy. A single pulse with 10-ns-duration passed through ND filter and lens. A foucused beam was reflected at the mirror in order to change optical path into upward direction. After that, a laser beam was irradiated on the target's surface.

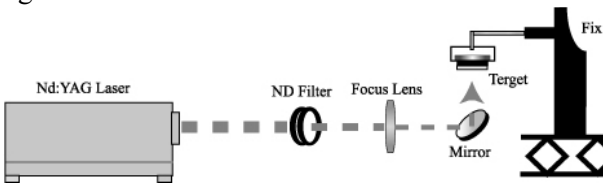

Fig. 1 Illustration of an experimental system.

Figure 2 illustrates an expansion of transfection part of our system. Target is constracted by natural rubber (NR) and polyethylene terephthalate (PET). We used glass-base dish in order to imprison the mammalian cells. LISWs propagate into the mammalian cells through glass plate from NR sheet.

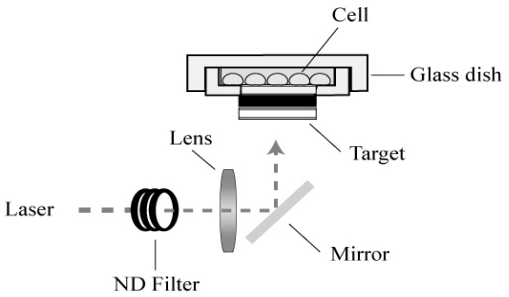

Fig. 2 Expanded illustration of transfection part.

3. Target cells

Two types of target cells were used: (a) HeLa cells, human womb cervix cancer cell. (b) HL60RG cells, which show a rapid growth phenotype, have been established from HL60 cells, human promyelocytic leukemia cell. Figure 3 shows HeLa cells and HL60RG cells.

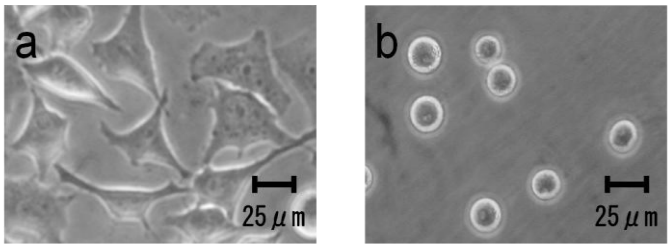

Fig. 3 Two types of target cells. a:HeLa, b:HL60RG

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HeLa cells were cultured in E-MEM medium supplemented with 10% heat-inactivated fetal bovine serum, HL60RG cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum. HeLa cells and HL60RG cells were plated cells onto dishes and 24h later, the medium were changed to opti-MEM. HeLa and HL60RG cells were cultured

to E-MEM or RPMI1640 for 24 hours. Figure 4 shows the typical time course in this experiment. After treatment of LISW, we calculated cell viability of HL60RG and HeLa cells peeled from culture dish by trypan blue exclusion.

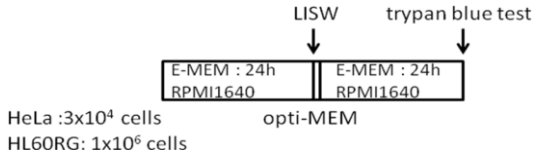

Fig. 4 Time course in this experiment.

4. Results

Figure 5 shows cell viability after LISW irradiation. Both cells viability of Hela and HL60RG cells were depended on laser fluence. Hela cells viability were 90%, 80% and 70% at 1.44J/cm^2, 2.40J/cm^2, 4.80J/cm^2 of laser fluence, respectively. HL60RG cell viability were 90%, 90% and 80% at 1.44J/cm^2, 2.40J/cm^2 and 4.80J/cm^2 of laser fluence. The results indicate that cell viabilities are dependent on laser fluence. We have done the gene transfer to HL60 by having used the electroporation so far, HL60 cells viability was about 15%.（Poring Pulse; Voltage 175V, Pulse width 7.5ms, Pulse interval 50ms, Driving Pulse; Voltage 20V, Pulse width 50ms, 10 repeats of driving pulse (Kogi unpublished, 2010). Despite irradiation to HeLa and 60RG cells, the cell viability of them was more than 80%. This suggests less physical damage in the cells. The LISW is compared with the electroporation, even the stress wave (laser fluence 2.40J/cm^2) shows the cell viability of 90% or more in HL60RG. Therefore, it is understood that physical damage to the cell is very few.

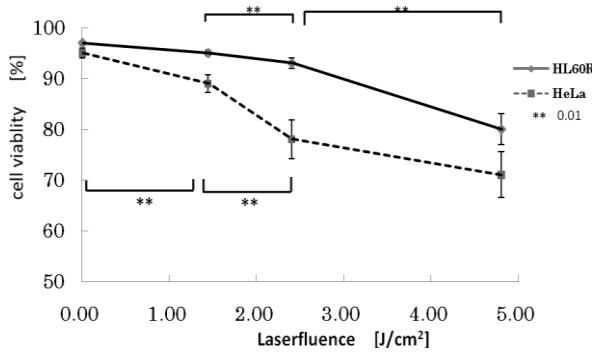

Fig. 5 Cell viability by laser fluence (1.44J/cm^2, 2.40J/cm^2, 4.80J/cm^2)

5. Discussion

HeLa and HL60RG cell viability were different with laser fluence (2.40J/cm^2, 4.80J/cm^2), HL60RG cell viability was higher than that of HeLa cell. This might be due to a type of cells. HeLa cell, epithelial cell, is originated human womb cervix cancer and a type of adherent cell. The cells express adherent genes. Because of infinite cell proliferation, many studies have employed this cell to investigate human cancer [4]. HL60RG cells are originated human promyelocytic leukemia cell, a type of blood cell and a suspended cell. This is an undifferentiated cell.

Currently, it is well-investigated that a process that hematopoietic stem cell differentiate to each blood cell, therefore many studies in biochemistry have applied the HL60RG cell to proliferation and differentiation [5,6]. Our results suggest that different properties between those cells, robustness of cell membrane, are attributed to LISW. We will investigate relationship between LISW and basal membrane, cell membrane.

6. Conclusion

We found that LISW was suitable to novel gene transfection to human cells.

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