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Effect of Laser-induced Emergent Stress Wave on cell adhesion
レーザ誘起創発的応力波が細胞接着に及ぼす影響

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1. Introduction

1.1 Background and motivation

We have developed a new method of gene transfection that has used laser induced emergent stress wave (LIESW). The gene transfer efficiency into the HeLa cells was about 6% which was not practical level for the clinical purpose [1, 2]. We have used LIESW to transfer Fluorescein isothiocyanate (FITC)-dextran into HeLa cells. The center of a culture dish was higher transfer efficiency, and those cells were removed [3]. That is to say, pressure distribution on the dish was not uniform. In this study, we investigated pressure distribution on the dish, and disclosed the relationship between that pressure distribution and cell adhesion.

1.2 Apparatus LIESW generation

Figure 1 shows our apparatus. Source of laser was Q-switch Nd-YAG Laser (Spectra Physics, LAB-130). Laser fluence was adjusted 0.5 J/cm^2 by ND filters. Target device was composed of Ethylene Propylene Diene Modification (EPDM、thickness 0.07 mm) and Polyethylene terephthalate (PET、thickness 1.0 mm). This device was adhered under a glass base dish.

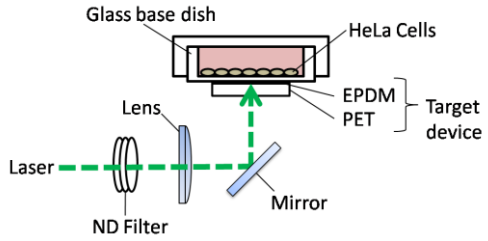

Fig. 1 Transfection apparatus.

2. Experimental procedure

2.1 Cells and transfection materials

HeLa cells were cultured at the number of 1.0×10^4 for 24 hours on glass based dish (IWAKI). Transfection material was used 4kDa FITC-dextran. Medium condition of this experiment was same as the condition of USE2012 (M.Kogi et al) [1].

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2.2 Measurement methods of pressure value

Pressure added to HeLa cells on the dish was measured by using PRESCALE (Fujifilm, measuring range: 10~50 MPa). PRESCALE is possible to quantitatively appreciate by color optical density. Pressure analysis of PRESCALE was conducted using FPD100S (FUJIFILM). Figure 2 shows pressure distribution measured with PRESCALE within a glass base dish. Pressure was lowered a around in the glass base dish [4].

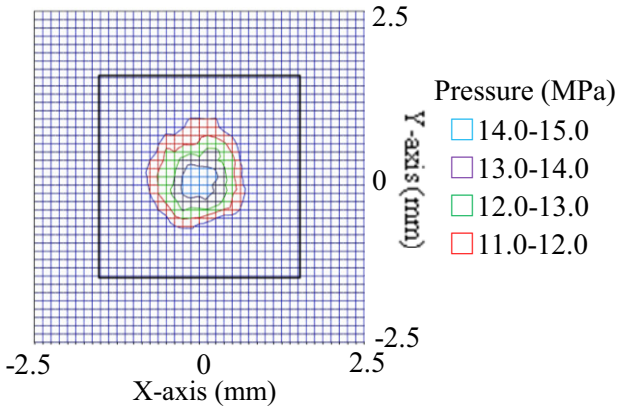

Fig. 2 Pressure distribution by PLESCALE

Pressure in water was measured by using a hydrophone sensor (Muller-Platte Needle Probe, tip diameter: 0.5 mm or less). Figure 3 shows pressure level that we measured with a hydrophone sensor. The biggest peak pressure was 15 MPa.

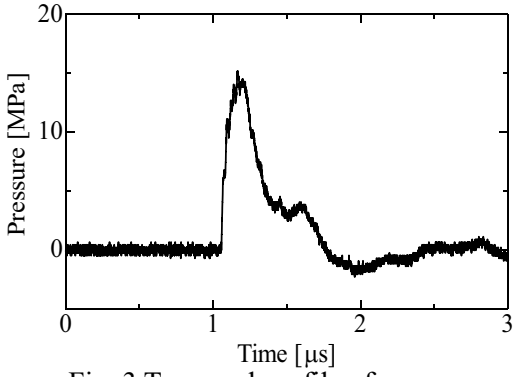

Fig. 3 Temporal profile of pressure

2.3 Measurement of adhesion and transfection

We observed under a fluorescence microscope (Nicon, ECLIPSE 80i) and took the photographed. Photography range was inside of the square of Fig. 2. The division was divided and took by 24 for 200 magnifications. We counted the number of the cells in photography and calculated HeLa cells adhesion rate.

3. Results and discussion

Figure 4 shows photographs of the HeLa cells before and after LIESW. The arrow heads show the cells that were removed LIESW. HeLa cells were deformed by LIESW.

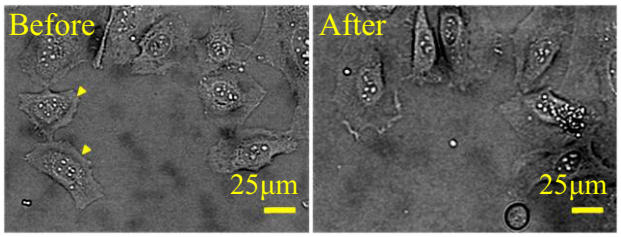

Fig. 4 The photograph before and after LIESW observed in one division

Figure 5 shows adhesion rate and transfection rate of each the square. Gray squares show adhesion rate 20% or less and transfection rate 80% or more. On the whole, gray squares were scattered.

Adhesion rate (%) Transfection rate (%)

31	24	0	20	93	87	0	100
20	44	22	13	75	77	80	71
19	35	18	20	80	85	67	100
43	25	29	25	90	79	71	77
26	15	20	8	91	86	100	100
51	17	4	24	79	100	50	73

54	42	32	4	58	53	76	100
40	27	36	12	62	80	35	63
36	59	13	4	85	69	67	100
19	21	10	4	100	100	0	100
33	27	9	7	75	100	86	100
31	18	5	10	80	70	100	71

32	47	11	10	100	95	80	100
18	16	22	43	63	100	75	94
23	17	14	24	90	88	83	88
21	16	5	21	100	86	0	100
26	32	46	36	71	83	100	100
27	35	16	13	100	100	86	67

Fig. 5 Adhesion rate and transfection rate at each square (%)

In summary, the pressure distribution, adhesion rates, and transfection rates of each division did not necessarily correspond. Relationship was not seen between adhesion rate and transfection rate.

Adhesion and transfection rates did not correspond with the pressure distribution by LIESW, the displays that there is not simple linear relationship between our result and the effect of LIESW and EPDM expansion and the secondly effect of the LIESW. The limit of control of circumstance for the cell, and no identical phase of the cell cycle, might cause different shapes and internal conditions the cells.

4. Conclusion

We found that there was no relationship between Adhesion rate of the HeLa cells and the pressure distribution that we measured through PRESCALE.

Acknowledgements

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