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Role of adhesive cell proteins in the gene transfer using LIESW

レーザ誘起創発的応力波を用いた遺伝子導入時の接着タンパク質の役割

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1. Introduction

1.1 Background and motivation

We have developed a new method for gene transfection, the method has used the laser induced emergent stress wave (LIESW) generating by the pulse laser irradiation to the solid material [1]. We have thought that this gene transfection was useful for many kinds of the cell. We have shown that this method was effective for HeLa cells (adherent cell derived from uterine cervix cancer) and not for HL60 cells (floating cell derived from myeloid leukemia) [2.3]. The efficiency of gene transfection for HeLa cells was depending on areas in the dish. It appears that HeLa cells are affected by the complex physical factor of LIESW [4]. We therefore examined the influence to LIESW on the adhesive protein of HeLa cells. Here we discussed the role of the adhesive cells protein of gene transfection in the present study.

2. Material and Method

2-1. Apparatus LIESW generation

Figure 1 shows our apparatus. We generated the LIESW by irradiating a target device of the second harmonic of the 532nm Q-switch Nd-YAG Laser. Pressure of LIESW in water was measured by using a hydrophone sensor (Muller-Platte Needle Probe, tip diameter : 0.5mm or less), and was 30MPa. Target device was composed of Ethylene Propylene Diene Modification (EPDM, thickness 0.07mm) and Polyethylene terephthalate (PET, thickness 1.0mm). This device was adhered under the dish [5].

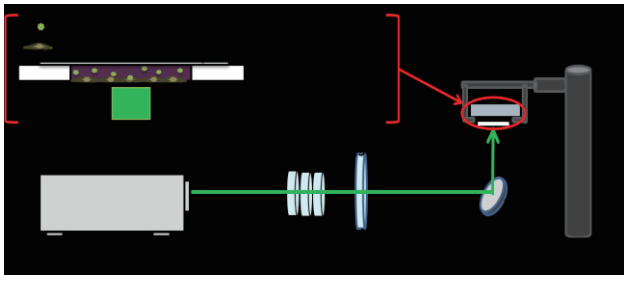


Fig.1 experimental apparatus

2-2. Condition of the experiment

HeLa cells were cultured at the number of 3.0×10^4 and, then they have incubated for 72 hours on cover glass. HeLa cells were maintained in E-MEM medium containing 10% heat-inactivated FBS and P&S (penicillin and streptomycin) at 37℃ under moisturized air containing 5% CO_2.

2-3. immunostaining

We analyzed immunostaining collagen type Ⅳ which was the largest amount of expression of the adhesion proteins of the HeLa cells. The HeLa cells were immunostained by a primary antibody (Anti-Collagen IV antibody: Rb pAb to Collagen Ⅳ, abcam ; ab21295) and a secondary antibody (Alexa Fluor 594 goat anti rabbit IgG [H+L]: life technologeis ; A-11012). HeLa cells were fixed in 70% paraformaldehyde. The nuclei of the HeLa cells were stained with DAPI (Hoechist33342).

2-4. Measurement of adhesive proteins

We observed and photographed the collagen type Ⅳ by a fluorescence microscope (Nicon, ECLIPSE 80i). Figure 2 shows a specification of three areas (A,B,C). The original microscopic image was modified as their binary images. We calculated a size of collagen type Ⅳ of the HeLa cells from the binary images by image J.

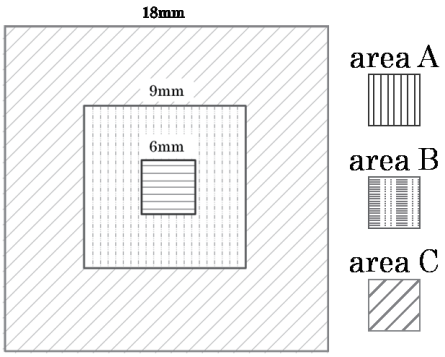
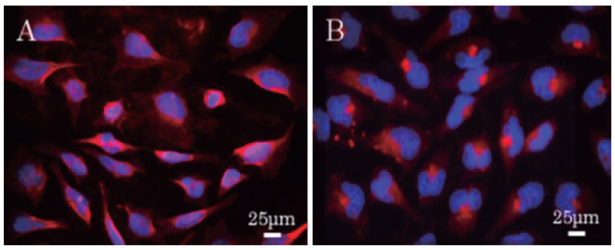


Fig2. A specification of three areas (A,B,C)

3. Result

Figure 3 shows Photographs of immunostaining collagen type Ⅳ of HeLa cells before and after LIESW processing.



A: before LIESW B: after LIESW

Fig.3 Examples of collagen type Ⅳ images (Red;collagen type Ⅳ, Bule; DAPI nucleus)

The HeLa cells decreased in area A after the LIESW. Figure 4 shows a size of the collagen type Ⅳ. Average size after LIESW decreased in the area A. There is no significant difference between before and after LIESW in the area B and C. LIESW was the most effective to the HeLa cells in area A.

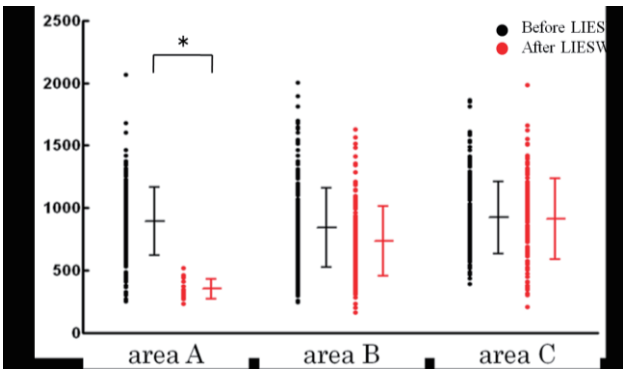


Fig4. Average size of the cells

4. Discussion and Conclusion

Figure 4 shows pressure distribution measured with PRESCALE (Fujifilm, measuring range: 10~50 MPa) in the glass base dish [5].

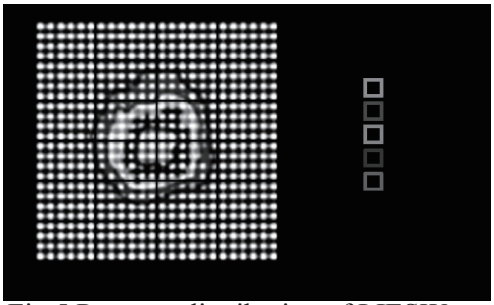


Fig.5 Pressure distribution of LIESW

Pressure was the highest in the center of the dish, and gradually lowered toward to the surround of the dish. Results from Fig.4 and 5 indicate that higher pressure causes the effect of LIESW on the HeLa cells. To explain these results, we propose a hypothesis of collagen type Ⅳ transformed in figure 6.

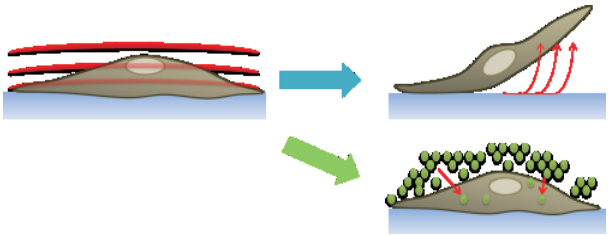


Fig.6 The partial detachment of the HeLa cells [6]

We conclude that the gene transfection into the HeLa cells progress by the affecting of on the cell membrane mediated by high pressure .

Anknowledgements

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Reference

1. M. Kogi, K. Aizawa, S. Nishimura, T. Takeuchi, M. Nishiwaki, Y. Tokunaga, Processing of Symposium on Ultrasonic Electronics, Vol.33 (2012) 101-102

2. M. Kogi, N. Sugiyama, T. Yanagisawa, S. Nishimura, K. Aizawa, Y. Tokunaga, IEICE Technical Report, US2013-55 (2013) 11-14

3. M. Kogi, T. Takeuchi, K. Aizawa, Y. Tokunaga, Abstract book of the 12th Congress of The Japanese Society for Regenerative Medicine, Vol. 12 Suppl. 2013, M-5-3 (2013) 303 [in Japanese].

4. S. Nishimura, M. Kogi, T. Yanagisawa, S. Orisaka, K. aizawa, Y. Tokunaga, Proceedings of Symposium on Ultrasonic Electronics, Vol.34 (2013) 345-346

5. S. Orisaka, Y. Tokunaga, K. Aizawa, Meeting of Acoustical Society of Japan (2013)

6. M. Kogi, T. Yanagisawa, S, Nishimura, N. Sugiyama, T. Sugimoto, K. Aizawa, Y. Tokunaga, Abstract book of the 14th Congress of The Japanese Society for Regenerative Medicine Vol. 13 Suppl (2014) 358 [in japanese]