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Challenging discussion for effective transfection of plasmid DNA into HeLa cell using laser-induced emergence stress wave

レーザ誘起創発的応力波を使う HeLa 細胞へのプラスミド DNA の高効率的導入のための検討

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1. Introduction

Barrier problem of 3% to transfect plasmid DNAs into human cervical cancer cells (HeLa cells) by laser–induced emergent stress wave (LIESW) is shown in our experiment. [1]

Up to now, we have studied reasonable value on peak, rise-time formed an acoustic signature of LIESW and could get them. Then, we begin to sum up deeper knowledge on human normal cell (cell) and the HeLa cell. In this paper we describe difference with cell and HeLa cell and then discussion on transfection process by LIESW.

2. Normal cell and HeLa cells

A normal cell may be about 20 μm on size and it is composed of many organelles (elements) such as the nucleus, cytoplasm, cell membrane. [2]

Furthermore, a domain of cytoplasm is made up of many smaller elements which are mitochondria, endoplasmic reticulum, Golgi body and so on. [3]

The individual elements play an important role as "a basic unit of life" due to macroscopic behavior determined by the interaction with each other. For ultrasonic researchers, understanding the detailed roles (microscopic roles) of the individual elements is very difficult since the elements interact with each other such as a matrix form. For better understanding a cell, we try to perform paradigm shift from concept of bio-chemical field to ultrasonic field usage. We treat a cell as "an open system" in a concept of complex system. We assume that the cell may be composed of cell membrane as input gate, mitochondria as energy supplier and nucleus as a transfection center as shown in **Fig.1**.

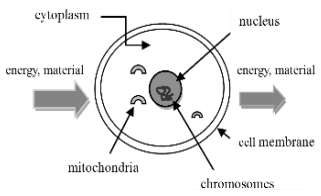

Fig. 1 A cell as a complex system.

It is said that remarkable characters of cancer cells are redundancy and diversity unlike normal cells. [4] **Figure 2** shows a photograph of HeLa cells distributed on surface of glass-based dish at 25 ℃.

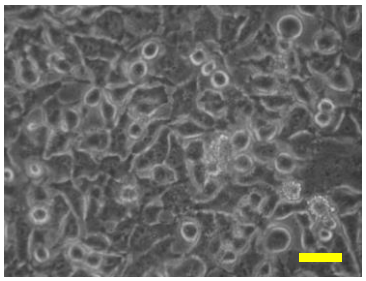

Fig. 2 Photograph of HeLa cells. (bar: 25 μm).

It was found that cells having various shapes and sizes were abundantly present. We consider that these variations depend on growth rate of individual cells. As interesting fact on HeLa cells, apoptosis (natural death) does not occur, as long as the nutriments are supplied, to continue to proliferate indefinitely. Individual HeLa cells are so difference on speed of the growth processes such as cell cycle (G1-S-G2-M) which are logarithmic growth phase, stationary phase, mitotic phase.

3. Sample and injection method

A number of HeLa cells seeded on a glass-based dish was 1 x 104 cells / 200 μl. HeLa cells had about 30 – 100 μm on size. Sample preparations were in similar with that in ref. [5].

Figure 3 shows a scheme of LIESW injection to HeLa cells. HeLa cells have strong adhesion on glass-based dish with 12 mm diameter and 100 μm thickness. Surface area of membrane without adhesion was surrounded by plasmid DNA suspension. Size of a plasmid DNA may be several ten [nm]. This size is so smaller than a size of HeLa cell. Usually, a plasmid DNA cannot pass through the cell membrane.

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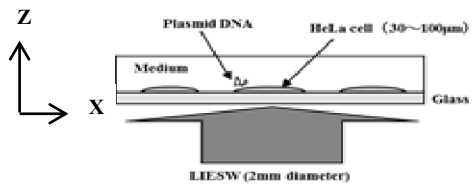

Fig. 3 A principle of LIESW injection method to glass-based dish. Cells are contacted closely with surface of glass-based dish.

4. Transfection mechanism of plasmid DNA

Up to now, no mechanism of gene permeation through the plasma membrane by LIESW has been clear. In order to overcome limit of 3 % transfection rate, we should study a mechanism which plasmid DNA can pass through plasma membrane. We must show here our proposed model on mechanism transferred plasmid DNA into HeLa cells.

Energy and momentum transfer from LIESW to HeLa cells were carried out by the method as shown in Fig.3. In this case, instantaneous movement (microsecond order) in the z-direction of the central region (about 2 mm diameter) of glass -based dish is given by LIESW' shock momentum and HeLa cells that adhered to the glass-based dish are forced by same movement. By this effect, a route for passing through a cell membrane of plasmid DNA is established. Therefore, separation between the lipid bilayers and neighbor lipid bilayers in the x direction is increased instantaneously. In this case, if HeLa cell is in cytokinesis, plasmid DNA entered in cytoplasm can be easily passed through nuclear membrane.

Figure 4 shows a relation of LIESW and HeLa cell in the mitotic phase.

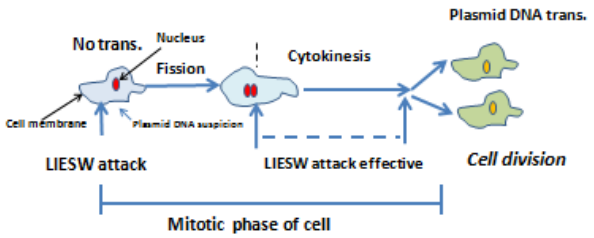

Fig. 4 A process until two cells from a cell by cell division in mitotic phase.

In a mitotic phase, processes until cytokinesis are as following; condensation of chromosomes, disappearance of nuclear membrane, alignment of chromosomes, and chromosomes segregation.

We consider that transfection of plasmid DNA into HeLa cells by LIESW is so effective by using mitotic phase of HeLa cells and LIESW attack to HeLa cells during cytokinesis seems to be the best choice. Because nuclear membrane will be disappearing temporarily and Chromosomes become defenseless. In addition, the size of cell membrane of the original cell is inflated in order to perform cytokinesis and then cell division begins. In this state, momentum and energy of LIESW play important and effective roles on transfection of plasmid DNA into a nucleus. This is our consideration in the present stage.

Unfortunately, we cannot propose specific and good experimental answers on it in present stage.

5. Conclusion

In this paper, we discussed barrier problem to high transfection rate (higher than 3% barrier) from viewpoint of HeLa cells. First, a normal cell was discussed from complex system approach. Next, Character of HeLa cell was explained. Finally, transfection mechanism of plasmid DNA into HeLa cells by LIESW was discussed. We consider that transfection of plasmid DNA into HeLa cells by LIESW is so effective by using mitotic phase of HeLa cells. In particular, action of LIESW to HeLa cells during cytokinesis may be the best choice.

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References

1. M. Nishiwaki, M. Kogi, K. Aizawa, and Y. Tokunaga, IEICE Tech. Rep. US2012-6 (2012-4) 29-32 [in Japanese].
2. J. Sakamoto, "Biology, for physical sciences and engineering" SHOKABO, (2011) 16 [in Japanese].
3. "Life science", ed. Tokyo Univ. Bio-chem. kyokasyo hensyu iinnkai (2012) [in Japanese].
4. H. Kitano, K. Takeuchi, "Introduction to bio-logical robustness" Chap.3, Diamond Co. Ltd. (2007) [in Japanese].
5. T. Takeuchi, M. Kogi, K. Aizawa, S. Nishimura, M.Kimura, M. Nishiwaki, and Y. Tokunaga IEICE Tech. Rep. US 2012-64 (2012-10) 5-8 [in Japanese].