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Spontaneous and Genome-Integration Free External Molecular/Gene Introduction to Cells Triggered by Complex Stimulus Generated by Plasma

Masafumi Jinno[1], Yoshihisa Ikeda[1], Hideki Motomura[1], Yugo Kido[1,2], Susumu Satoh[1,3]
[1]Department of Electrical and Electronic Engineering, Ehime University, Ehime, Japan.
[2]Pearl Kogyo Co. Ltd., Osaka, Japan.
[3]Y's Corp., Tokyo, Japan.
E-mail: mjin@mayu.ee.ehime-u.ac.jp

The authors have been working on exploring the mechanism of the introduction of molecules into cells by plasma irradiation and on developing this technique since the discovery of this phenomenon in 2002. In our plasma gene/molecule introduction method cultured cells on a well in which large molecules such as plasmids DNA or dextran were dropped were sandwiched between a high volt applied fine microelectrode of several tens of μm and a grounded flat copper electrode. The extracellular molecules are transferred into the cells simply by applying the discharge plasma generated by the sinusoidal high voltage of several tens of kHz for a short time of several ms. The transfer of molecules into cells by the plasma is mainly performed by endocytosis, one of the cell-specific functions taking extracellular molecules inside [1]. This spontaneous uptake of target cell is caused by the complex stimulus of plasma [2]. The plasma generate the electrical stimulus and the chemical stimulus due to radicals such as ROS and RONS. If one of the electrical and chemical factors is missing, no uptake occurs [1,2].

Since the plasma is quite small and irradiated for a short time of ms order and since this method only triggers the spontaneous uptake function of cells, the damage to cells is very low. Though genes are randomly integrated with extremely high probability into chromosomes in cells (genome integration) when they are introduced into cells by electroporation and lipofection method, genome integration rarely occurs with the plasma method. Therefore, when passage is repeated, the introduced molecules and genes by plasma will eventually dissociated by autophagy. This means plasma method is safe and suitable for medical application, breeding in agriculture and fishery, etc. As shown in Figs.1 and 2 by using discharge plasma we have already succeeded in molecular introduction to plant cells and fish eggs and adult fish. We expect that our plasma method is the introduction method suitable for genome editing because of the safety described above.

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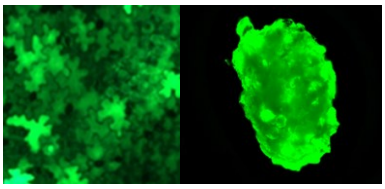

Fig.1 Fluorescent images of FITC-dextran introduced tabacco cells (Left: Leaf, Right: Callus)

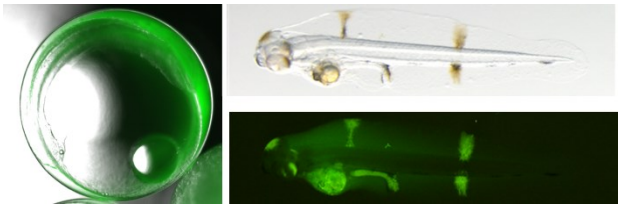

Fig. 2 Fluorescent images and bright image of FITC-Dextran introduced Suma-fish egg(left) and hatched fry(right)

References

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[2] M Jinno *et al.*, *Plasma Sources Science and Technology*, **26** (6), 065016 (2017).