Study on Control of Macromolecular Drug Transfer to Epithelial Cells Using Non-Invasive Microplasma

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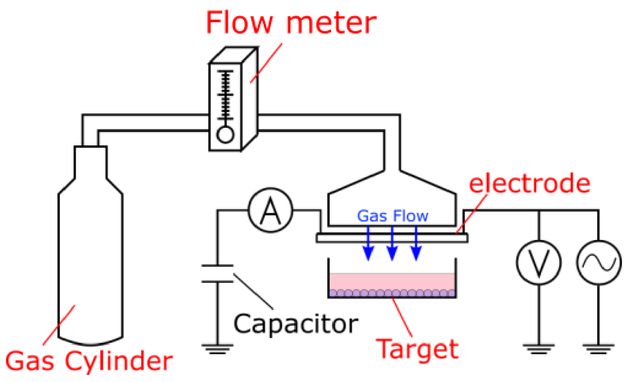
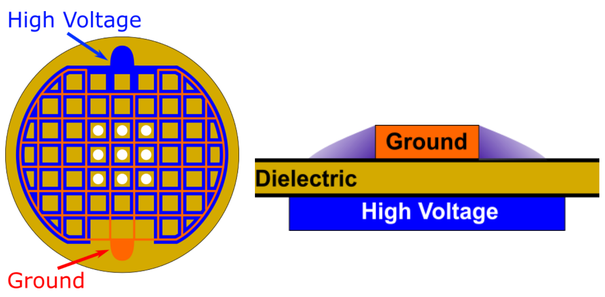
In recent years, drug introduction using Atmospheric pressure and low-temperature plasma has been studied as a method for introducing polymer drugs into epithelial cells.[1][2] In this study, with the miniaturization of the device in mind, we investigated using a film-type electrode that can irradiate a wide area using air at atmospheric pressure, unlike the conventional plasma jet. In this presentation, as a preliminary step to examine the introduction of drugs into normal cells, we report on the effects of plasma irradiation on normal cells in the upper intestine using fluorescent dye.

Experimental method



Experimental circuit configuration

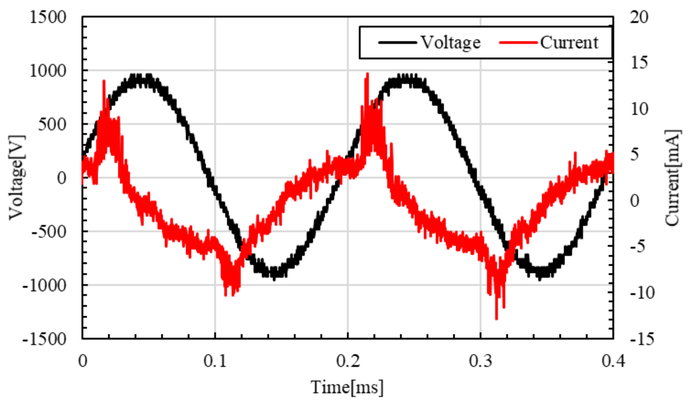


(a) Surface (b) Cross section

Electrodes used in the experiment

Experimental conditions

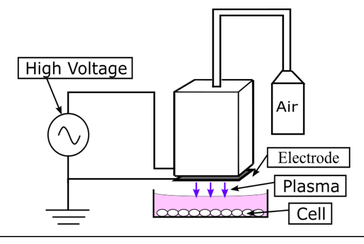
Cell name	RCB0993 : IEC6
Cell characteristics	Rat small intestinal epithelial cell line
Culture form	Adherent cells
Culture medium	DMEM (low glucose) + 5% FBS + 4μg/ml Insulin +Penicillin-Streptomycin Solution 100mg/ml
Fluorescent dye	5μM YOYO-1, 5μM FD-4, 0.1 mM FD-150
Medium volume	3ml
Carrier gas	Dry Air
Air Flow	2 L/min
Treatment time	0 - 120 s
Frequency	10 kHz
Voltage	940 $V_{0\text{-}p}$
Distance	5 mm



Applied voltage and current

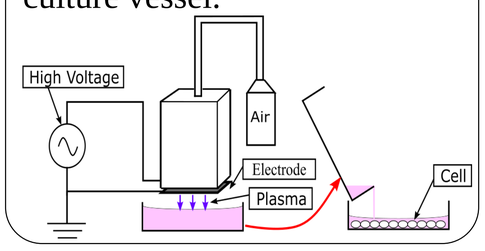
Direct plasma irradiation(DPI)

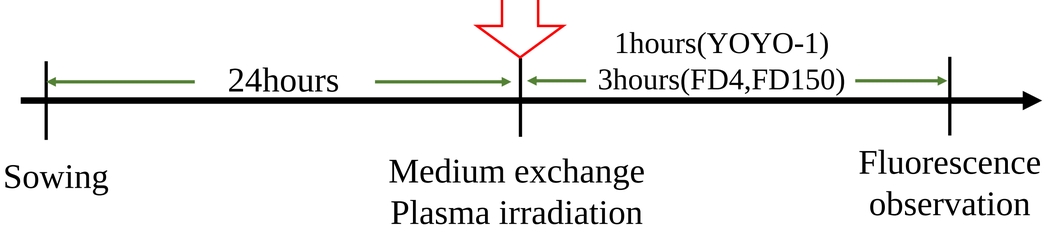
A method of irradiating the culture solution with the cells adhered to the culture container.



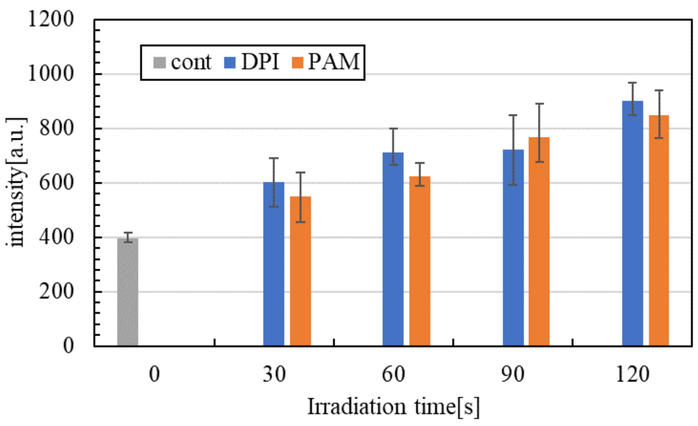
Plasma-activated medium(PAM)

A method of dropping a culture solution that has been irradiated with plasma in advance into a culture vessel.

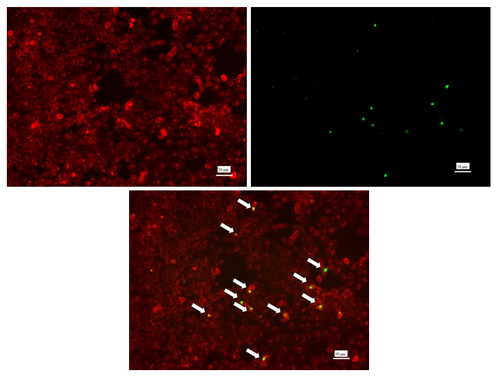




Results



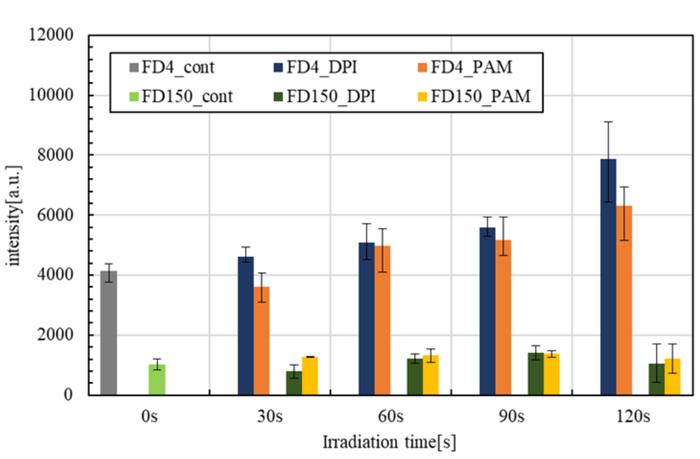
Fluorescence intensity of YOYO-1



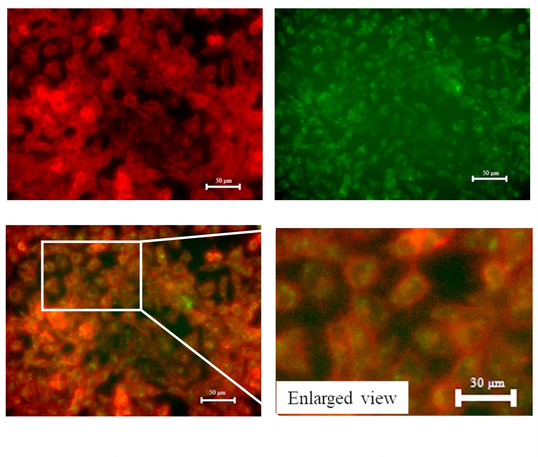
Fluorescent image of YOYO-1

(Scale bar is 50 μm)

In the case of YOYO-1, there is no difference in fluorescence intensity between direct irradiation(DPI) and Plasma-activated medium(PAM). When the plasma was irradiated for 120 seconds, the fluorescence intensity was about twice that of Cont.



Fluorescence intensity of FD4 and FD150



Fluorescent image of FD4

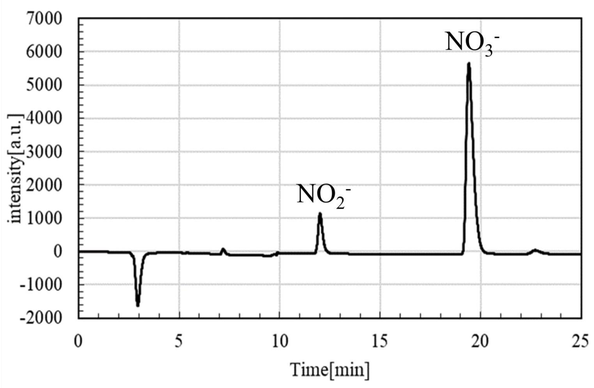
(Scale bars in a,b,c are 50 μm, and in d is 30 μm)

In FD4, the fluorescence intensity increases with the irradiation time, and direct irradiation tends to be higher than indirect irradiation.
It is considered that FD150 was not introduced into the cells because the fluorescence intensity did not change with the irradiation time.

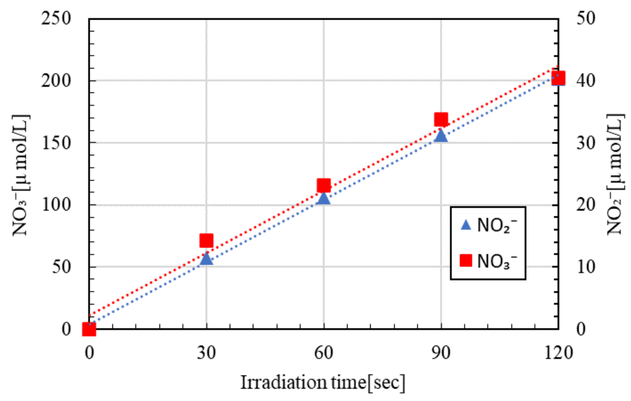
Nitrogen active species produced in the liquid

NO radical is highly reactive and forms NO_2^- ,NO_3^- in water. After that, it reacts with H_2O_2 to produce peroxynitrite ($ONOO^-$), which has high oxidizing power. [3]

Nitrogen active species produced in the liquid were quantitatively evaluated using an ion chromatograph. An increase in the concentration of NO_2^- and NO_3^- in the liquid was observed according to the plasma irradiation time.



Ion chromatograph measurement results

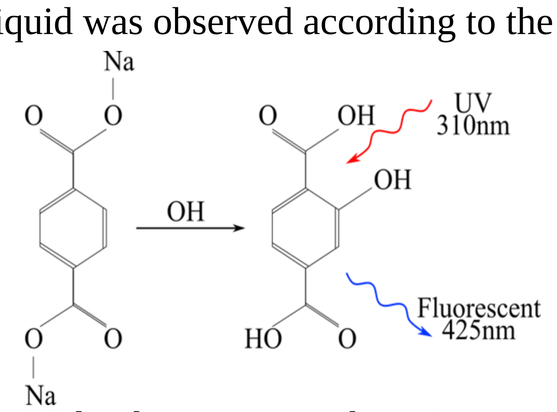


Amount of NO_2^- and NO_3^- generated

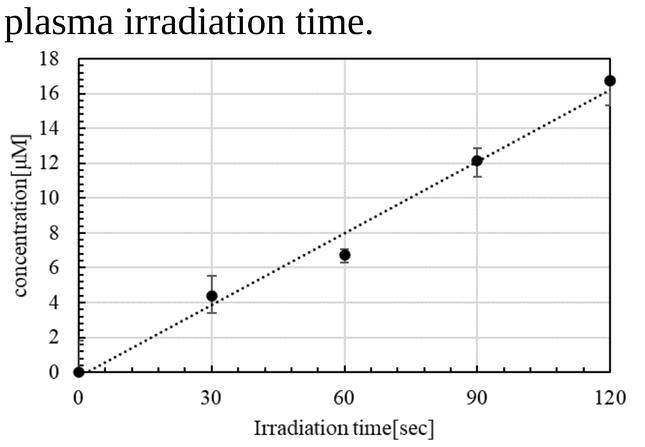
Oxygen active species produced in the liquid

OH radical improves cell membrane permeability and forms cell membrane pores by oxidizing membrane lipids.[4]

OH radical produced in the liquid was quantitatively evaluated using sodium terephthalate (NaTA). An increase in the concentration of OH radicals in the liquid was observed according to the plasma irradiation time.



Molecular structure and reaction mechanism of NaTA and HTA



Amount of OH radicals generated

Conclusions

- Up to 4 kDa drug uptake was observed in the cells by plasma irradiation. On the other hand, no uptake of FD150 was observed.
- Since the fluorescence intensity increases with the irradiation time, it is considered that the uptake of the drug is promoted by plasma irradiation.
- An increase in the amount of NO_2^-, NO_3^- and OH radicals produced were observed depending on the irradiation time.

[1] S. Sasaki, M. Kanzaki , T. Kaneko, “Highly efficient and minimally invasive transfection using time-controlled irradiation of atomospheric-pressure plasma,” Applied Physics Express 7, 026202, 2014.

[2] T. Kaneko, S. Sasaki, Y. Hokari, S. Horiuchi, R. Honda , K. Makoto, “Improvement of cell membrane permeability using a cell-solution electrode for generating atmospheric-pressure plasma,” Science & Technology of Materials, Interfaces, and Processing, 2015.

[3]W. H. Koppenol, “THE CHEMISTRY OF PEROXYNITRITE, A BIOLOGICAL TOXIN,” Química Nova. 21 (3): 326–331, 1998.

[4]A. Zerrouki, M. Yousfi, A. Rhallabi, H. Motomura , M. Jinno, “Monte Carlo Poration Model of Cell Membranes for Application to Plasma Gene Transfection,” Plasma Process. Polym. 2016, 13, 633–648, 2016