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大気圧低温プラズマによるPC12細胞へのCa^{2+}流入促進効果の評価
Evaluation of Ca^{2+} influx promotion on PC12 cells using nonthermal atmospheric pressure plasma

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1. Introduction

A novel nerve cell culture system is developed to enhance an axonal-extension and promote regeneration by using non-thermal atmospheric-pressure plasma (NTAPP). Until now, many regeneration methods to treat the injured central nervous system (CNS) have been proposed and examined, however the effective treatment has not been established. We focused on the plasma medicine for cell therapy. Sasaki et al. [1] reported that reactive species generated by plasma irradiation induces calcium ion influx via the TRP channel. In addition, Shibasaki et al. [2] reported that axonal extension is enhanced by promoting calcium ion influx. Therefore, we investigate the effect of axonal extension enhancement induced by Ca^{2+} influx into the rat pheochromocytoma cell (PC12) by NTAPP.

2. Methods

For stimulation of PC12 cells, a plasma activated medium (PAM) is used. We adopted a bubbling method to generate PAM. We used a damage-free multi-gas plasma jet apparatus (PCT-DFJM-01, Plasma Concept Tokyo Co., Ltd.). FIG. 1 shows the fabrication process of PAM in the container using the plasma bubbling system.

PC12 cells were seeded in a 35mm dish with at a cell density of 3.0×10^3 cells/cm^2. DMEM medium supplemented with serum and antibiotic was used as the culture medium. PC12 cells were cultured for 24 h in the culture medium and maintained in an incubator. After incubation, the culture medium was removed and 2.0 ml of PAM and 50 ng/ml NGF were added to it. PC12 cells were stimulated in the PAM containing NGF in incubators. After stimulation, the PAM was removed, and 2.0 ml of culture medium and NGF was added. The incubation time was 72 h from the start of PAM stimulation. PAM production conditions and stimulation conditions were: gas type: air, flow rate: 3.0 L/min, solvent: 20 ml DMEM containing only antibiotic, bubbling time: 15, 30, 45, and 60 s, stimulation time: 1.0, 2.0, 3.0, 4.0 and 5.0 h.

In addition, we verified whether the influx of Ca^{2+} into PC12 cells by PAM was promoted using Fluo-4.

3. Results and discussion

We measured axonal extension of PC12 cells at 72 h from the start of PAM stimulation. We search an optimum condition using response surface method to maximize the axonal extension length under two design variables, such as the bubbling time and the stimulation time (total 21 conditions). Fig. 2 shows the response surface and the optimum point, where the bubbling time is 18 s and the stimulation time 3.9 h, and a significant axonal extension compared to the case of control was obtained.

Fig. 3 shows the time histories of the luminance ratio (F_t / F_0) with the standard error in cases of the Control and the PAM stimulation. The luminance ratio was obtained by dividing the luminance value F_t at the observation time t s with the luminance value F_0 at the start of observation. As shown in FIG. 3, at the period of 60 s, the increase ratio in case of control was about 1.2, and 1.6 the PAM. In addition, a significant difference was confirmed in the period after 60 s.

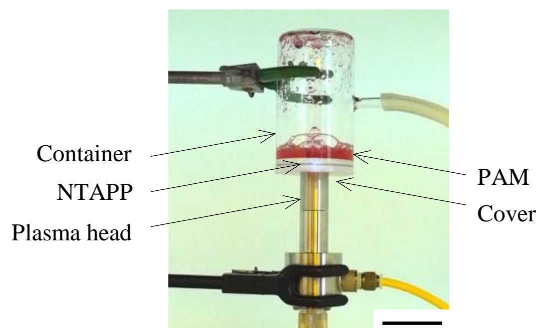

Fig.1 Plasma bubbling system to generate PAM (Bar 50 mm)

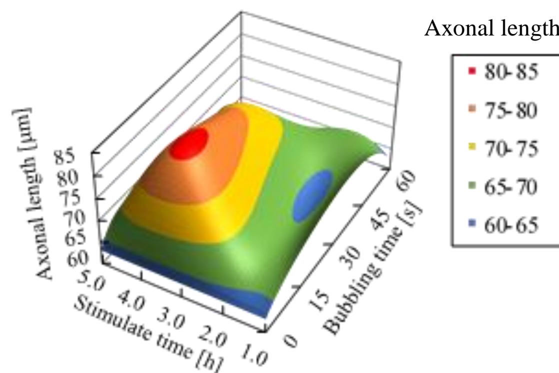

Fig.2 Optimum condition search using the response surface method

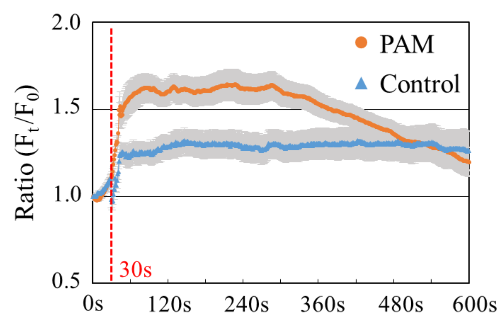

Fig. 3 Time history of luminance ratio, which evaluates the change of intracellular Ca^{2+} concentration compared with the initial value.

References

[1] S. Sasaki et al., Sci. Rep. **6**, 25728 (2016).
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