Plasma-generator-supplied Nanosecond Pulsed Current Effects on Fibrosarcoma Cells

Chia-Hsing CHANG[1], Ken-ichi YANO[2], Takehiko SATO[3]

[1] *Department of Mechanical System Engineering, Tohoku University, Japan*
[2] *Institute of Pulsed Power Science, Kumamoto University, Japan*
[3] *Institute of Fluid Science, Tohoku University, Japan*

Abstract: Plasma medicine is a novel promising medical field that utilizes atmospheric pressure plasma. Although most previous studies have been focused on biological effects of plasma-derived chemical species, the influence of electrical transmission processes on cellular activities remains to be fully investigated. In this study, we speculate that electrical factors (field, charge, current, etc.) of nanosecond pulsed current supplied by a plasma generator may be critical determinants of the HT-1080 cellular responses.

Keywords: Plasma Medicine, Electrical Factor, Nanosecond Pulsed Current, Cell Response

1. Introduction

Recently, plasma medicine, using atmospheric pressure plasma (APP) to medical research and health care field, has been known as highly potential to become the powerful device in wound healing, sterilize bacteria, inactivate cancer cell, cell proliferation, to name a few [1, 2]. By controlling types of APP, we can apply for different medical treatments. To date, plasma and liquid interactions have been mainly reported, also treatment of targets usually covered with liquid. Hence, to investigate the phenomenon of plasma in liquid is necessary [3]. APP effectively generates factors such as ions, electrons, reactive species, electric field, etc. However, most experiments focus on reactive species (RONS) in aqueous phase produced by APP, electrical factors transmission processes are not developed completely [4, 5]. Furthermore, tumor invasion is required for metastasis, yet the molecular mechanisms are still poorly understood.

To investigate the biological effect of the current injection and accumulated charges from the plasma, it is essential to exclude other chemical species besides cells. Thus, we constructed the stimulation system which could not only physically isolate cells from pH variations and reactive faradaic products of electrolysis, but also simulate same current supplied by a plasma generator [6]. This configuration was in order to elucidate electrical factors to induce cellular responses. In this study, we used a power source without plasma generation to model only the effect of a nanosecond pulsed current supplied by a plasma generator on cell activities.

2. Methods

In our experiment, the stimulation system had two parts, the cell culture chamber and the salt bridge setup, shown in fig. 1. First, the cell culture chamber was composed by cover glass slide, 10 × 20 × 10 mm (W × L × H) filled with 1.2 ml of the culture medium. Each chamber was cultured for one test, where one chamber was selected for the stimulated group and another for the control group. All experiments were done in the incubator at 5 % CO_2 and 37 °C. The cell used was HT-1080 (JCRB, human sarcoma cell line). Then, the salt bridge setup was for the current transfers via the Ag/AgCl chloride electrodes and agarose bridges; internal agarose bridges were constructed by filling 8 mm glass tubing filled with 2 % agarose to connect between the chamber and the reservoirs filled with phosphate buffered saline (PBS). On other hand, the power supply was designed by our lab, switched on from function generator, setting burst mode at a frequency of 100 Hz, 33.333% duty. For the maximum voltage was 900-1000 V, and maximum current was 200 mA which was less than 10 ns for the rising time. During the treatment, pH value and temperature were constant based for all conditions including the control sample without stimulation. Cells were injected into the chamber at 100000 cells ml^{-1}, allowed to adhere for 4 hours, then starting for treatment. Cells seeded into chambers receiving no electric stimulation served as controls. All of the experiments were repeated three times.

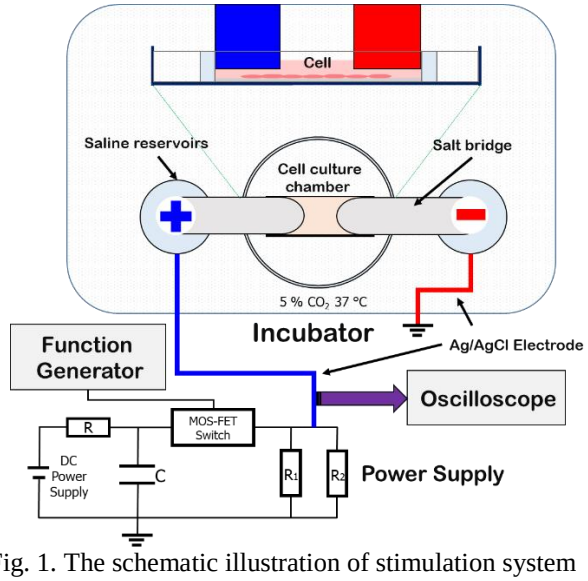

Fig. 1. The schematic illustration of stimulation system

The immunofluorescence protocol has several steps, including fixation, permeabilization, blocking, and immunostaining. Paraformaldehyde phosphate buffer solution, triton X-100, and bovine serum albumin were used for fixation, permeabilization, and blocking, respectively. 4′,6-diamidino-2-pheny-lindole (DAPI) is for DNA stain with blue-fluorescent; α-tubulin monoclonal antibody is for the intracellular distribution of tubulin with green-fluorescent; Alexa Fluor 488 dye is for secondary antibodies with green-fluorescent; Rhodamine Phalloidin is for F-actin probe with red-orange-fluorescent. Besides, to calculate mitochondria, we applied MitoRed dye which in living cells is stained in red.

3. Results & Discussions

By immunofluorescence images, we could observe details inside the cells. The control group after 24 hours was showed in fig. 2. Comparing with the stimulated group treated with nanosecond pulsed current in fig. 3., we discovered actin stress filaments in treated cells were remodeling during the time increased; simultaneously, cell shape performed flat and became larger overall size, and this indicated a change in cytoskeletons. However, there were no difference in tubulin (which is not showed in this paper). Moreover, MitoRed fluorescent, which is represented the amount of mitochondria, was gradually increased in the stimulated group as showed in table 1, and the highest difference was 1.8 time after 24 hours. We noticed that the increasing number of mitochondria should be related to influence on actin filaments, which has been reported to be involved in the cytoskeletal remodeling [7]. Besides, actin filament growth is connected with adenosine triphosphate (ATP) that also would be produced from mitochondria. By serval reasons, this demonstrated the relationship between mitochondria and actin filaments should be the mechanistic effects on cells.

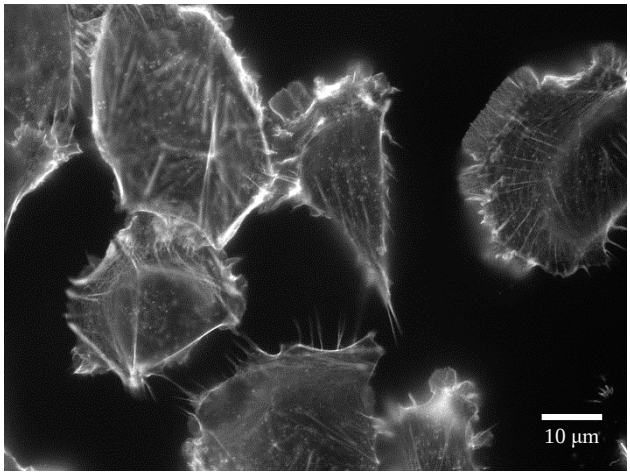

Fig. 2. The immunofluorescent image of the control group after 24 hours in actin filament stained by Rhodamine Phalloidin

In addition, calcium flux had been mentioned in serval reports, so the calcium ion concentration could be another mechanism to cause those cell responses in our results. In the cell, Ca^{2+} is present in organelles, mainly in the endoplasmic reticulum or mitochondria, also involved in cytoskeletal reorganization. Moreover, mitochondria could accumulate Ca^{2+} to accelerate ATP production and finally occur polymerization of actin filaments [8, 9].

On the other hand, mitochondrial respiratory chain generated superoxide anion ($O_2^{\bullet -}$) which is one of ROS. ROS play important roles in modulation of signaling pathways in tumor invasion and migration, as regulate the signal pathways responsible for lamellipodia formation, actin cytoskeleton remodeling, and so on. In fig. 6, the stimulated cells increased amount of lamellipodia formation and promoted protrusion branching, compared with the control cells showed in fig. 5. According to the paper, $O_2^{\bullet -}$ also could be a key element of fibrosarcoma cells through the activation of extracellular signal-regulated kinases (ERKs) and reorganization of the cytoskeleton [10].

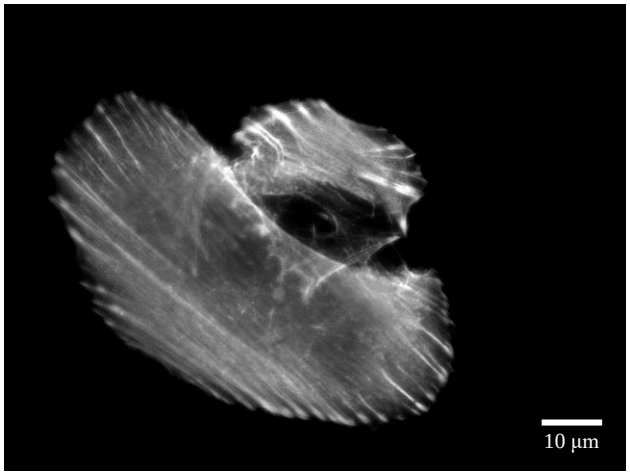

Fig. 3. The immunofluorescent image of the stimulated group after 24 hours in actin filament stained by Rhodamine Phalloidin.

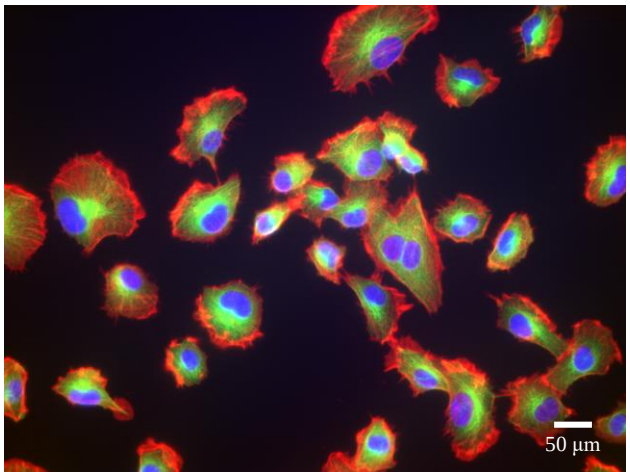

Fig. 5. The immunostaining image (merged) of the control group after 24 hours in cell nuclear stained by DAPI, tubulin stained by α-tubulin monoclonal antibody and actin filament stained by Rhodamine Phalloidin

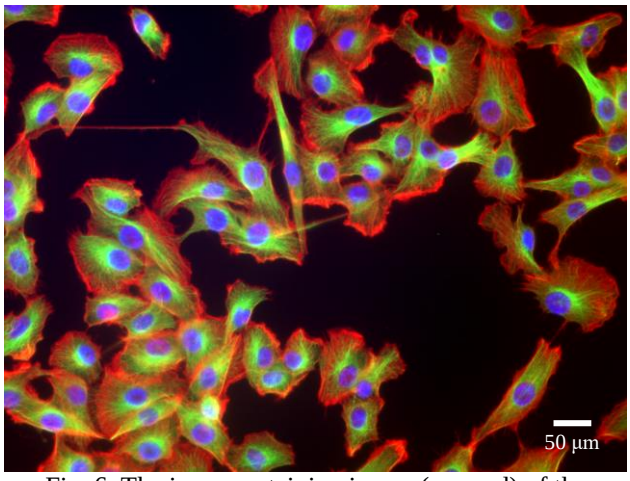

Fig. 6. The immunostaining image (merged) of the stimulated group after 24 hours in cell nuclear stained by DAPI, tubulin stained by α-tubulin monoclonal antibody and actin filament stained by Rhodamine phalloidin

Hence, we propose that the nanosecond pulsed current induced depolarization on the membrane and reorganized cytoskeleton.

Table 1. MitoRed fluorescent area increase ratio for 6, 12 and 24 h treatment

MitoRed Area Ratio	6 h	12 h	24 h
$\frac{\textit{Stimulated Group}}{\textit{Control Group}}$	1.042 ± 0.019	1.189 ± 0.027	1.846 ± 0.25

4. Summary

In this study, we used this plasma generator, providing the specific parameter to explore fibrosarcoma cell responses. We built up the stimulation system for the purpose of preventing non-reversible cytotoxic reactions near to cells for treating the nanosecond pulsed current. We successfully demonstrated a novel phenomenon, namely increased fibrosarcoma cells size caused by pulsed electric current under plasma-generating setting. Yet no inactivation or electroporation was discovered in stimulation treatment, due to its low energy consumption by ultra-short duration time of the current, such that certain mechanisms would not be applicable. Our results suggest that electrons accumulate on cell surfaces over time, the charge of the membrane components become crucial factors. After 24 h, cell size was gradually enhanced by stimulation. Cells appeared to be of an overall average size with increased lamellipodia formation and promoted protrusion branching.

To conclude, our experiment focused on impact from plasma-generator-supplied nanosecond pulsed current, and provided ionic mechanisms to accelerate cell attachment and expansion, also reorganized cytoskeletons from actin filaments. Last but not least, our final goal is to compare the results obtained in this study with those obtained by plasma treatment, investigating the mechanisms from not only reactive species but also electrical factors.

5. References

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6. Acknowledgements

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