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Effect of atmospheric-pressure radical irradiation on cell growth of mouse NIH3T3 fibroblast

マウスNIH3T3繊維芽細胞の増殖能に対する大気圧ラジカル照射の効果

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In this study, we investigated the irradiation effects of neutral oxygen radicals on the cell growth of NIH3T3 mouse fibroblasts using an atmospheric-pressure radical source. As a result, we have found the cell growth of 15% in the suspension irradiated by only neutral oxygen radicals with low dose, whereas the cells were inactivated in the high-dose region.

1. Introduction

Recently, nonequilibrium atmospheric-pressure plasmas have been widely applied in the medical fields such as sterilization, blood coagulation, apoptosis of malignant cells and so on. However, the each effect of species in plasmas to biological tissue has not been clarified yet because various factors such as ions, electrons, UV, and neutral radicals exist in the plasmas.

By measuring the oxygen radical densities such as ground-state atomic oxygen [$O(^3P_j)$] and singlet oxygen molecule [$O_2(^1\Delta_g)$], and ozone in the plasma, it was clarified that O (^3P_j) is closely related to the inactivation. [1] Accordingly, we have focused on the effects of O (^3P_j) on the growth of budding yeast cells quantitatively. As a result, we observed the activation in the growth of budding yeast cells under low-dose condition. The most optimal dose of O (^3P_j) for the activation was around 1.0×10^{17} cm^{-3}. [2]

In this study, we have studied the effects of the neutral oxygen radicals on the proliferation ability of NIH3T3 cells using atmospheric-pressure radical source, which can supply only neutral radicals.

2. Experimental procedure

NIH3T3 were maintained in high glucose Dulbecco's Modified Eagle's Medium (DMEM) with 13% fetal bovine serum.

We employed an atmospheric-pressure radical source (Tough Plasma :FUJI MACHINE MFG Co., Ltd.) to treat suspensions including NIH3T3 cells. As a unique point of the source, it can irradiate only neutral species extracted from the various species generated in the plasma.

In this study, the total flow rate and the O_2/ (Ar + O_2) flow rate ratio were maintained at 5 slm and 0.6% respectively. The distance from the radical exhaust slit to the surface of the suspension was fixed at 10 mm. In order to prevent the radicals from reacting with the ambient air, the treatment region was surrounded by a plastic cover and purged with Ar.

Figure 1 shows the experimental procedure. As for plasma irradiation, cells cultured in 10-cm dish were washed with phosphate buffered saline (PBS), and detached with 0.1% trypsin. After trypsinization, the cells were suspended by 3-ml PBS and seeded into 3.5cm dish. The dish was placed on the movable stage scanned at 4 mm/s, and the cells in the dish were irradiated by oxygen radicals immediately and recovered by medium. After centrifugation, the medium was replaced with fresh medium. After that, the cells were incubated on 96 well plates at 37℃ and 5% CO_2. After incubation, living cells were evaluated by MTS assay.

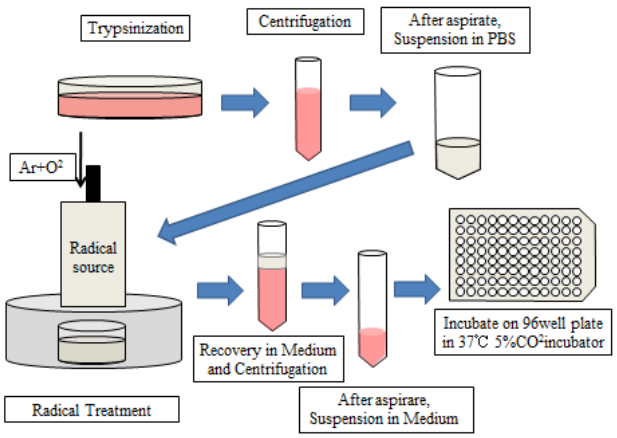

Fig.1 Experimental procedure.

Moreover, for investigating the effects under high-dose condition, the amount of PBS and the treatment distance were reduced to be 1.2 ml and 7 mm, respectively.

3. Results and Discussion

Figure 2(a) shows cell growth after radical irradiation. Cells were irradiated for 60 s and slightly inactivated at 0 hours after the radical irradiation. Proliferation in cell number was not observed at 36 hours after the radical irradiation. However, proliferative effect was slightly observed at 60 hours after the irradiation.

The growth rates of the treated cells to control cells at 60 hours after the irradiation are shown in Fig. 2(b). At the irradiation time of 20 and 30 s, we have found the proliferative effects of 15% compared to the control cells. The oxygen radical treatment for 20 s corresponds to the most effective growth at the dose of O (^3P_j) of 1.3×10^{17} cm^{-3}. On the other hand, in the study of budding yeast cells, the most effective dose was around 1.0×10^{17} cm^{-3}. [2] The dose of O (^3P_j) was calculated from the suspension volume and the irradiation flux.[1] Over the most effective dose, the growth effect was repressed as for budding yeast cells. Similarly, proliferative effect of NIH3T3 cells was also repressed in the irradiation time region longer than 45 s.

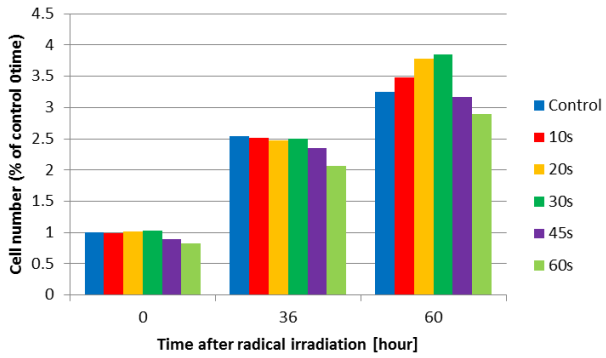

Fig. 2(a) Cell numbers to control at 0, 36, and 60 hours after radical irradiation.

Figure 3 shows cell viability under high-dose condition. In order to set up high dose condition, the treatment distance was set to be shorter to be 7 mm, and the PBS volume to suspend the cells was reduced to be 1.2 ml. As shown in Fig. 3, we have found that the number of cells after irradiation was reduced, and the growth promotion was not observed under high-dose condition. It seemed that most cells were inactivated at treatment time for 180s by excess doses.

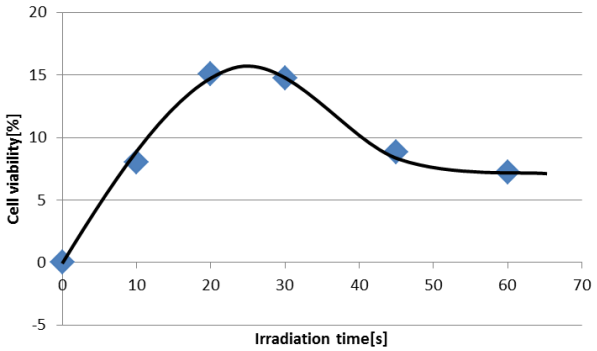

Fig.2(b) Cell viability as a function of treatment time at 60 hours after irradiation.

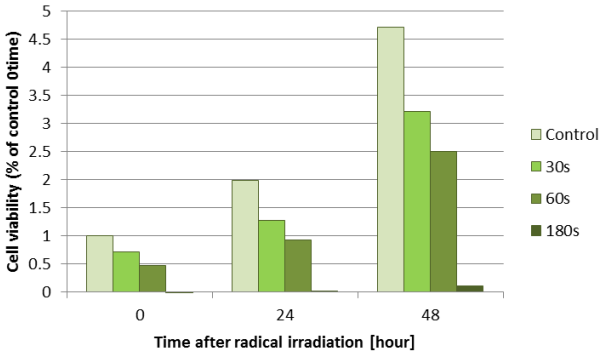

Fig. 3 Cell viability at 0, 24 and 48 hours after radical irradiation under high-dose condition.

4. Conclusion

We have studied the effects of oxygen radicals on cell growth of mouse NIH3T3 fibroblast. The cells were grown in low-dose region of oxygen radicals, which corresponded to the O (^3P_j) dose of ~ 1.25×10^{17} cm^{-3}. In contrast, the cells were inactivated under the high-dose condition.

Acknowledgment

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