Comparison of OH generation by radiation exposure and APPJ treatments in cells

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1. Introduction

Many studies utilize cold plasma for medical applications such as bacterial deactivation, wound healing, and cancer treatment by generating reactive oxygen species (ROS), especially OH radicals [1,2]. In these experiments, plasma dose was determined by setting operational parameters such as treatment time, applied voltage, discharge gas, and gas flow rate based on empirical data. Plasma mainly generates OH radical at the plasma plume and transfers the radical by diffusion from the medium surface. Considering that ROS produced in extracellular area are not threatening to the cells, it is noteworthy to measure the ROS produced at intracellular area to determine dose of plasma.

X-ray, which is low linear energy transfer (LET) radiation, induces cell death via water radiolysis by producing mainly OH radical [3]. Considering that X-ray produces ROS in both extracellular and intracellular area, X-ray dose could be used as reference dose in the point of ROS generation. OH production in extracellular medium and intracellular area were measured after each X-ray exposures or atmospheric pressure plasma jet (APPJ) treatment.

In this study, plasma treatment was quantitated in terms of equivalent radiation dose. Increasing applied voltage and treatment time induced increase in medium OH production linearly, and induced lower increases in intracellular OH production.

2. Methods and Results

2.1 X-ray exposure and APPJ treatment to cells

Cells in the culture medium were exposed to X-rays at 2–50 Gy by operating the X-ray tube (450-D08, YXLON, Hamburg, Germany) at 350 kVp and 10 mA. Dose rate was 3.68 Gy/min.

Plasma production was controlled by varying the voltage applied to the pin electrode with a function generator (33220A, Agilent, Santa Clara, CA, USA) at 20 kHz and a voltage amplifier (20/20C, Trek, New York, NY, USA) at 2000 times amplification The applied voltage was changed from 5 kVp to 7 kVp and to 9 kVp. The helium gas-flow rate was fixed at 2 LPM using a mass flow controller (TN2911V-4S, Celerity, Allen, Texas, USA).

2.2 Measurement of OH radical

The spin trap of 5,5-dymethyl-1-pyrroline-N-oxide (DMPO, D5766, Sigma Aldrich) was added into the medium for measuring OH radical. DMPO maintains a rather long half-life about 870s. After irradiation or plasma treatment, the samples were analyzed by ESR spectrometer (JES-TE200, JEOL) at NICEM in SNU. ESR measurement was taken 6 minute after X-ray exposure or plasma treatment.

Cells were incubated with medium containing 50 μM H2DCF-DA (ab113851, Abcam, Cambridge, UK) for measuring intracellular OH radical. The H2DCF-DA loaded cells were treated and then incubated for 30 min. Cells were analyzed using a flow cytometry system (FACS Aria, BD Biosciences) to measure the fluorescence.

2.4 Cell line cell culture methods

MECs (CRL-2161, ATCC, Manassas, VA, USA) were cultured in T-25 flask (Nunc, Roskilde, Denmark) containing Dulbecco's modified eagle medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco). All cells were incubated at 37℃ in a humidified incubator (MCO-230ALC, Panasonic, Gunma, Japan) with 10% CO2.

3. Results and Discussion

3.1 Extracellular OH production by X-ray and APPJ

Fig.1 presents the DMPO-OH signal intensities observed after X-ray exposures and APPJ treatments. The ESR signal intensity increases with increased DMPO-OH concentration. The DMPO-OH intensities increased linearly with increasing radiation dose (R_2=0.99).

From APPJ treatments, the DMPO-OH signal intensity from 2 min of APPJ treatment increased proportionally with the applied voltage at fixed helium flow (R_2=0.99). When the voltage and helium flow rate were fixed at 7 kVp and 2 LPM, respectively, the DMPO-OH signal intensity increased with APPJ treatment time (R_2=0.99).

3.2 Intracellular OH production by X-ray and APPJ

In MECs, DCF fluorescence intensity was observed to increase with increased X-ray dose in the range from

2 Gy to 8 Gy. However, after plasma treatment, the DCF fluorescence intensity in MECs limitedly changed by enhancement of the applied voltage to plasma source device and extension of plasma treatment duration. Two times of plasma exposure duration (condition 3 vs. condition 2) resulted in approximately two times of DCF fluorescence intensity. This result is in contrast with the change to more than thrice in DMPO-OH signal intensity.

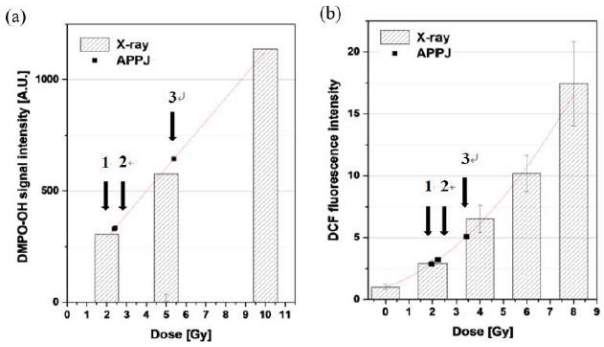

Fig. 1. (a) DMPO-OH signal intensities from X-ray exposures and APPJ treatments and (b) DCF fluorescence intensities from X-ray exposures and APPJ treatments.

Table I: DMPO-OH signal and DCF fluorescence intensities under three different parametric setups of APPJ device operation

	condition	X-ray dose for comparable DMPO-OH production	X-ray dose for comparable DCF production
1	(5 kVp, 2 LPM, 2 min)	2.37 Gy	1.96 Gy
2	(7 kVp, 2 LPM, 1 min)	2.43 Gy	2.22 Gy
3	(7 kVp, 2 LPM, 2 min)	5.39 Gy	3.42 Gy

3.3 Comparison of X-ray exposure and APPJ treatment in ROS production

Table I summarizes that the equivalent doses of conditions 1, 2, and 3 for the comparable DCF production (1.96, 2.32 and 3.42 Gy) are lower than for the comparable DMPO-OH production (2.34, 2.27 and 5.39 Gy), respectively. The low equivalent doses of plasma treatment for DCF production implies that plasma treatment is less efficient in intracellular ROS production as compared for OH production in the culture medium. OH radical generated from plasma recombined to form highly stable H_2O_2 and the H_2O_2 can diffuse into the cells and produce intracellular OH radical [4,5].

3. Conclusions

Both X-ray and cold plasma produce OH radical and transfer energy to the cells. Due to different OH production mechanism, plasma has less efficiency for producing intracellular ROS. By measuring intracellular ROS with equivalent radiation dose, quantitation of plasma dose would be possible for medical purpose.

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