Quantitation of OH Radical Production from X-ray and Plasma Treatments Seeking for Radiation-Equivalency of Plasma in Clonogenic Cell Killing Effect

Jin-Han Lee[a, †] Min-Ho Lee[a], Sung-Young Yoon[b], Gon-Ho Kim[b], Eun-Hee Kim[a, *]
[a]*Radiation Bioengineering Laboratory,* [b]*Plasma Application Laboratory*
Department of Nuclear Engineering, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, Korea
[†] *mystic2j@snu.ac.kr* [*]*Corresponding author: eunhee@snu.ac.kr*

1. Introduction

Application of plasma in medicine has been studied in various ways. Floating electrode dielectic barrier discharge (FE-DBE) plasma is used to sterilize human living tissues and to coagulate blood in wound treatment [1]. Non-thermal atmospheric pressure plasma is also used in dentistry for tooth bleaching [2].

Plasma production can be controlled by setting the operational parameters of plasma device. Medical use of plasma is made rather empirically in terms of plasma delivery to body parts under treatment. The following interactions of plasma with components of body parts result in the treatment effect. Production of hydroxyl radicals (·OH) is known to be a key process mediating the interactions between plasma and the body [3]. Hydroxyl radicals mediate the interactions between low-LET radiation and the body as well [4].

In this study, we estimated the OH radical production from plasma injection into distilled water and compared it with that from irradiation of distilled water with X-ray beam. We found out the operational set-up for plasma production that is comparable to radiation dose in clonogenic cell killing effect.

2. Methods and Results

2.1 Measurement of OH Radical Production

Hydroxyl radicals produced by irradiation of water have short lifetime (~200 μs) [5] and thus 5,5-dimethyl-1-pyrroline *N*-oxide, as known as DMPO, was utilized to hold the produced OH radicals. One DMPO molecule reacts with an OH radical and produces a DMPO-OH radical, which has rather longer half-life (870 s) [6]. DMPO-OH radical was measured by electron spin resonance (ESR) spectrometry (JEOL), in which the intensity is quantitated even though its unit is arbitrary.

We assessed the stability of DMPO-OH radicals after production in 2 ml of 100 mM DMPO solution via radiation exposure. Total 50 Gy of radiation dose was delivered at 0.5 Gy/min via operation of the YXLON 450-D08 X-ray beam tube at 450 kVp and 10 mA. In Fig. 1, the intensity of DMPO-OH peaks in ESR spectroscopy shows an insignificant change within 9 min after radiation exposure. Thus, our measurements of DMPO-OH production were completed at 6 min after subsequent exposure to either radiation or plasma.

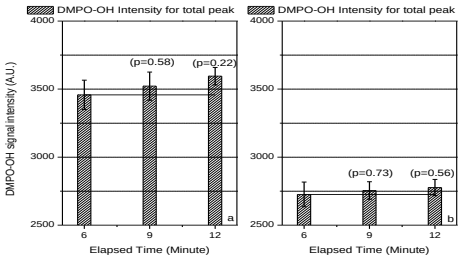

Fig. 1. DMPO-OH signal intensities from DMPO solution at a varying time lapse after X-ray exposure at 50 Gy.

2.2 Production of DMPO-OH by Radiation Treatment

The production of OH radicals possibly would increase with the radiation dose on the condition that the DMPO supply is sufficient. The relationship of OH radical production with radiation dose was assessed with 2 ml of 100 mM DMPO solution by delivering radiation at 2, 5, 10, 20, 35 and 50 Gy. In Fig. 2, the production of DMPO-OH radicals shows a strong linear correlation (r^2 = 0.98, for both the sum of 2 and 3 peaks and the sum of all four peaks).

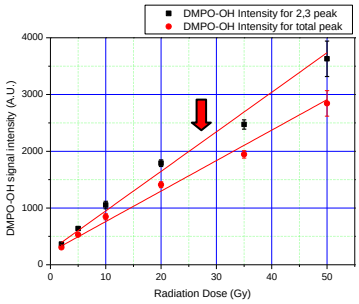

Fig. 2. DMPO-OH signal intensities varying with the radiation dose at 6 min after radiation exposure.

2.3 Production of DMPO-OH by Plasma Treatment

A device for plasma treatment was operated at rf-frequency of 15 kHz to produce atmospheric pressure plasma jet (APPJ). The voltage and helium gas flow rate were controllable within certain ranges. The production of OH radicals was considered to increase with the voltage and total gas flow. The test operational set up was 5.5 Vp and 1 L/min (LPM) of gas flow rate. The DMPO solution was prepared with 100 mM DMPO in 2 ml distilled water.

In Fig. 3, the duration of plasma treatment shows a strong linear correlation with the DMPO-OH production

(r^2 =0.97 for both intensity data sets). Regarding the gas flow rate, the DMPO-OH production at 1.5 LPM of helium gas flow rate was comparable to that at 1.0 LPM, but the production at 2 LPM was different ($p<0.05$) (see Fig. 4). To level the DMPO-OH production of plasma down to that of radiation, the voltage was lowered. A strong linear correlation (r^2 =0.98 for both intensity data sets) was observed between DMPO-OH production and the applied voltage in Fig. 5.

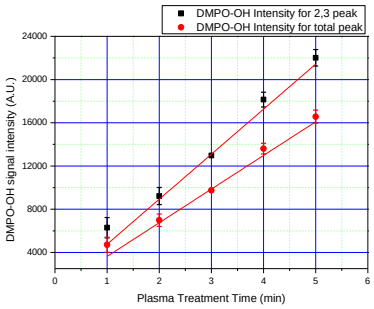

Fig. 3. DMPO-OH signal intensities varying with the duration of plasma treatment at 5.5 Vp and 1 LPM of helium gas flow rate.

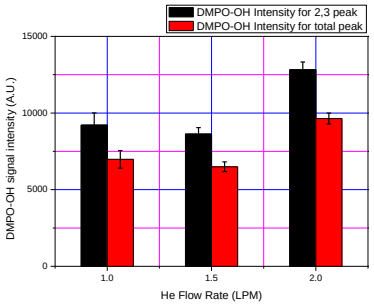

Fig. 4. DMPO-OH signal intensities after 2 min plasma treatment at 5.5 Vp with 1, 1.5 or 2 LPM of helium gas flow.

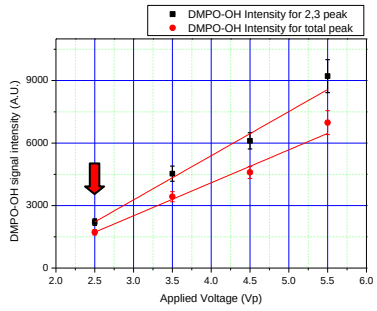

Fig. 5. DMPO-OH signal intensities varying with the applied voltage to the plasma device operating at 1 LPM of helium gas flow rate for 2 min.

2.4 Comparison of Clonogenic Cell Killing Effect

The cytotoxicity of plasma was investigated in terms of clonogenic death of mouse endothelial cells (MECs) (ATCC, CRL-2161), to be compared with radiation toxicity. The plasma device was operated at 2.5 Vp and 1 LPM of gas flow rate for 2 min plasma exposure (indicated by the arrow in Fig. 5), which was comparable to 27 Gy radiation treatment (see the arrow in Fig. 2) in DMPO-OH production.

It turned out that the cell killing effect by plasma treatment was significantly different from that by radiation treatment at 27 Gy. As marked in Fig. 6, the comparable cell killing was read at 7 Gy instead. On the presumption of the linear correlation between plasma treatment time and DMPO-OH production, additional data of clonogenic cell killing were collected by 1 and 3 min of plasma exposures. Data from 1, 2 and 3 min plasma exposures show a strong linear correlation (r^2 = 0.93) with data from 6, 7 and 8 Gy radiation treatments.

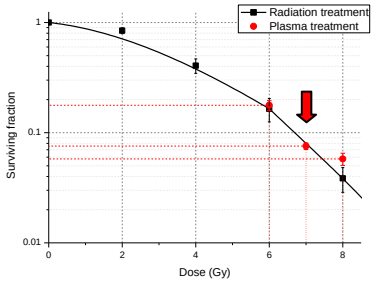

Fig. 6. Clonogenic surviving fractions of mouse endothelial cells treated with either X-ray beam or plasma. The solid line fits the experimental data (black squares) from radiation exposure. Data points of red dots were collected from 1, 2 and 3 min plasma exposures at 2.5 Vp and 1 LPM of helium gas flow rate.

3. Conclusions

The production of OH radicals can be used to infer in some degree the cytotoxic effect of plasma or radiation treatment. According to our observations, plasma and radiation treatments come along with different elements involved in their clonogenic cell killing effects in addition to the OH radical production.

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