Distribution and penetration of reactive oxygen and nitrogen species through a tissue phantom after Plasma Gun treatment

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Abstract: The transport and distribution phenomena of reactive oxygen and nitrogen species (RONS) into biological tissue following non-thermal plasma treatment have received much attention. The aim of this study is to test the efficacy of Plasma Gun (PG) on transporting the reactive species in an agar composed tissue phantom. RONS are generated on the agar gel by the plasma treatment and they continue to spread in the depth after plasma exposure. The amount of RONS after passing through the tissue phantom are strongly depending on discharge parameters such as distance to the target and capillary shape.

Keywords: Plasma Gun, RONS, tissue phantom, target gap, nozzle shape.

1. Introduction

Atmospheric pressure plasma jets have attracted a considerable attention for numerous applications in medicine and biology. The biomedical effects of plasma are strongly linked to the plasma generated RONS. An important issue in application of cold atmospheric plasma for tissue treatment concerned the penetration of RONS into biological tissue [1]. Because of the unique properties (soft and hydrated nature), RONS distribution and penetration can vary from tissue to tissue. While human skin is able to resist the penetration of many molecules, smaller molecules and species can pass through the corneal layer of the epidermis [2].

The tissue properties such as roughness and conductivity can influence the plasma treatment. Also, the gap distance (i.e., distance between the tip of the capillary and the target) may lead to variation of the plasma characteristics such as concentration of reactive species and gas temperature which can in turn change the effectiveness, distribution and penetration of the plasma on/in the tissue.

In the present work, we have focused on the RONS transportation and distribution in an agar tissue phantom after PG treatment. This agar model is very significantly different from real biological tissue but it can allow to get some precious information on transport of RONS in water containing media. Our results clearly and visually indicate that RONS are accumulated in the tissue phantom after the plasma treatment, and that continue to diffuse over and across the tissue. The influence of different nozzle shapes and gap distances on the distribution and penetration of RONS is studied.

2. Material and Method

Figure 1 shows a schematic of the experimental setup and two different nozzles. The plasma studied in this work is Plasma Gun (PG) composed of a coaxial dielectric barrier discharge reactor (Fig. 1) [3]. A 2 cm long powered electrode is set inside the 12 cm Pyrex capillary, 4 mm inner diameter and 6 mm outer diameter. In straight tube (Fig. 1 (a)), the inner diameter of the nozzle is 4 mm but in a tapered one (Fig. 1 (b)), the inner diameter at the end of the tube is 1.5 mm.

The helium flow rate through the reactor capillary is 0.5 slm. In this work, the PG device is driven by µs duration voltage pulses (positive polarity, repeated at 2 kHz). The applied voltage is measured using a high-voltage probe (Tektronix P6015A) and the discharge current on the agar target is measured using a 100 Ω shunt resistor. Also, discharge power is measured from V-Q Lissajous figures obtained using a 1.5 nF capacitor connected to the grounded electrode in series.

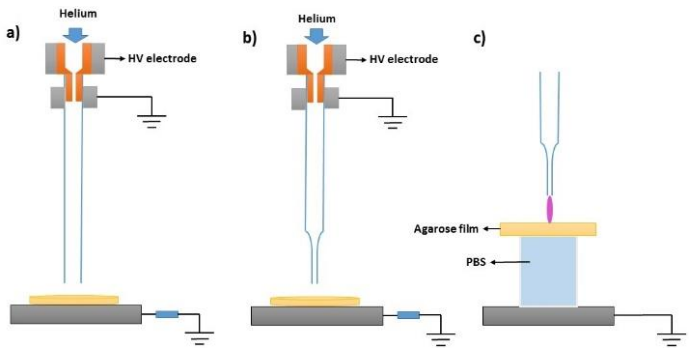

Fig. 1. Schematic diagram of the experimental set-up for a) straight nozzle and b) tapered nozzle c) RONS delivery through agar gel.

Figure 1 (c) shows the setup which can allow to visualize the penetration of RONS through the tissue phantom. A tissue phantom, made of a 2 mm thick agar gel, is placed on top of a 96-well plate; each well is filled with phosphate buffered saline (PBS, pH 7.4). The plasma plume produced by the cold atmospheric PG contacts the top of the agar surface. Then, the concentrations of RONS generated in

PBS are measured. The experiments are repeated at least three times for each condition.

Preparation of agar target

The agar target is prepared by dissolving 1.5% agar powder in physiological saline solution. The solution is heated on a conventional heating magnetic stirrer until all the powder is dissolved. Agar gels of 2 mm thickness are prepared by pouring 4,4 mL of solution into an 53 mm diameter polystyrene Petri dish. The agar is stored at 4ºC for 12 hours before use. Square sections of approximately 15 x 15 mm were cut using a scalpel and removed with a lab spatula. These agar squares, used for RONS penetration experiments, were laid on the wells of a 96 well-plate overfilled with the recipient medium.

In addition, the agar gel with KI-starch is used to evaluate the spatial distribution of oxidation reactions induced by PG. The agar target is prepared by dissolving 1.5% agar powder, 0.3% potassium iodide and 0.5% starch ($(C_6H_{10}O_5)_n$) in physiological saline solution. The KI-starch gel reagent can detect hydroxyl radicals (OH), atomic oxygen (O), O_3, H_2O_2, and HO_2 radicals.

H_2O_2 measurement in PBS solution

To measure the concentration of hydrogen peroxide passed through the gel into PBS after PG treatment, the Amplex™ Red assay kit was used. The Amplex red molecule is converted in the highly fluorescent resorufin in presence of H_2O_2 and peroxidase (HRP). For fluorescence intensity measurement, we used a microplate reader spectrofluorometer (Victor 3V, PerkinElmer), excitation wavelength at 531/25 nm and emission wavelength of 605/10 nm .

Not treated (NT) and helium treated gels were used as controls. Fifteen minutes after each treatment, the agar gel was removed and 50 μl of PBS containing diffused ROS were collected and transferred into individual wells of a 96-well plate. The working solution of 100-μM Amplex red reagent and 0.2-U/mL HRP were prepared, following the manufacturer's protocol. Then, 50 μL of the working solution was mixed with the same volume of the loaded sample. The 96-well plate was incubated in the dark at room temperature for 30 min and then read with the microplate reader.

RONS measurement in PBS solution:

The RONS concentrations passing from the agar gel target to the PBS are measured using Dihydrohodamine 123 (DHR, Thermo Fisher Scientific). DHR is a non-selective reactant, reacting with H_2O_2 and peroxynitrite ($ONOO^-$). It may react with other derivatives of molecular oxygen, including $^1O_2$. RONS are detected by the formation of fluorescent Rhodamine123 after the reaction with nonfluorescent DHR. In this experiment, we used DHR 123 5µM in PBS. The solution (420 µl) was distributed in each well of a 96 well plate and the 15mm×15mm agar gel targets with 2 mm thickness were placed over the top of the well so that the film contacted the PBS inside the well. Fifteen minutes after the treatment, the agar gel was removed and 200µl of the PBS containing the fluorescent probe were collected and transferred in a new 96 well plate. Fluorescence was measured using the microplate reader spectrofluorometer, excitation at 485nm and emission at 535nm.

3. Results

The waveforms of applied voltage and current of the plasma jet are presented in Fig. 2(a); it can be found that the peak applied voltage is 9 kV and the frequency is 2 kHz. Since the discharge power is applied in several short current pulses it is not a trivial task to calculate the power dissipated in the discharge. The method commonly employed for obtaining the discharge power is based on the charge–voltage Lissajous figure. The area of the closed Q-V loop for one period of the applied voltage is equal to the energy dissipated per one cycle. The mean discharge power is then simply the energy per cycle multiplied by the working frequency. A Q–V Lissajous figure of the plasma jet is shown in Fig. 2 (b) for tapered tube. It is obtained for applied voltage amplitude of 9 kV, 2 kHz signal frequency, gas flow of 0.5 slm and 10 mm, 15 mm and 20 mm distance between the tapered nozzle and the substrate. At these operating conditions, the area of the Lissajous figure for 10 mm, 15 mm and 20 mm gaps shown in Fig. 2 are 45 µJ, 32 µJ and 25 µJ giving a power of 90 mW, 64 mW and 50 mW, respectively. As expected, the amount of charge transferred to the agar target during a discharge cycle markedly decreased with increasing target gap. In addition, about the same value have been measured for straight tube.

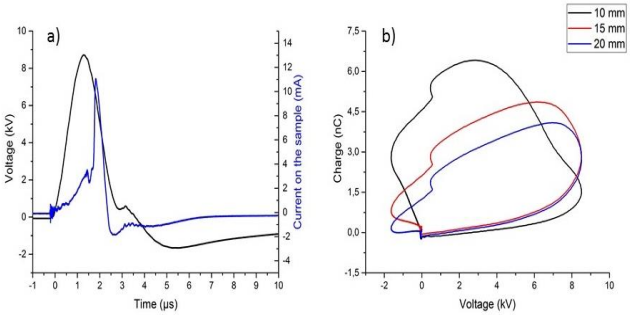

Fig. 2. a) Voltage pulse applied on the electrode and current time evolution measured across the target with a 100 Ω resistor for 10 mm target gap b) Q-V plots measured at different target gaps for tapered tube.

We used a KI-starch gel to show the oxidation reactions on the tissue surface [4]. As shown in Fig. 3, the color of the surface of the KI-starch gel changed from colorless to purple after plasma treatment. As expected, for different plasma conditions, an area with a strong purple color is observed in the center of the dish, which indicates that contact of the plasma with the tissue surface induces oxidation reactions caused by the generated ROS.

Images of the treated KI-starch gel dish were exported and processed in ImageJ (Fig. 4). Real dimension of the 8-Bit grey scale images were assigned in mm units using the

set scale function. The intensity and the width of the purple spot were measured by tracing a straight line, passing through the center of the treated area. Data from each plot profile was exported in Origin software for analysis.

Concerning the plasma/gap distance (Fig. 3 and 4), the central strong purple area exhibits a bigger radial expansion and a decrease in intensity when the gap is increased from 10 to 20mm. At small gap, the central point on the agar gel surface shows a less pronounced purple color. In a first approximation, we may think that this could be due to the hollow shape distribution of the ROS in the plasma plume [5], but this is not totally consistent with the diffusion of the ROS within the agar medium elsewhere. Additional study must be conducted to study the potential effect of ablated material in that area. Figures 3 and 4 also show the strong influence of the nozzle shape on the ROS distribution.

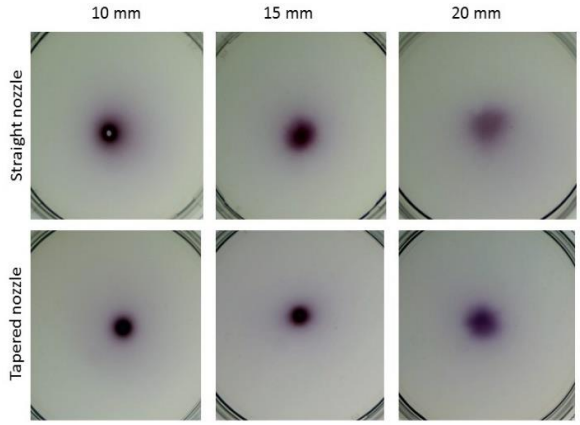

Fig. 3. Photographs of the KI-starch gel after plasma-jet treatment for 120 s with different nozzles and plasma-treatment distances.

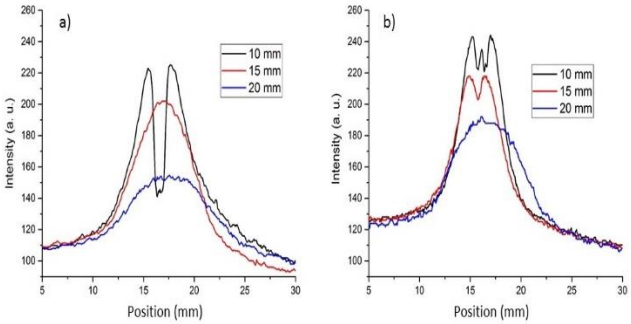

Fig. 4. Relative comparison of ROS concentration distributions on the KI-starch gel with different plasma treatment distances for a) straight nozzle and b) tapered nozzle.

The concentration of the reactive species that penetrate into the agar gel is of particular interest for a better understanding of the transport of RONS in the biological tissue. The concentration of H_2O_2 and RONS in PBS after penetration through the agar film under the different gaps and different nozzles are shown in Fig. 5. It is found that the concentration of H_2O_2 and RONS decreases with the increase in the gap to 20 mm. The treatment distance affects the energy deposition on the target and also the concentration of the RONS in the PBS. However, the change in concentrations of RONS is not in proportion to the energy deposition. It seems that the contribution of the humidity in production of the RONS is more than the charge deposition. A closer treatment distances result in a greater level of evaporation creating a more humid atmosphere that may affect the RONS concentration. In addition, significant higher concentrations are obtained for plasma treatment with tapered nozzle than the case of straight nozzle. In tapered tube, the He gas flow produces a more localized point of contact with the agar film, together with a higher gas velocity, which might result in a localized increase in RONS density at the gas-surface interface that may further facilitate the transport of RONS into the agar film. Such enhanced RONS penetration was measured to be more pronounced using DHR probe than with the H_2O_2 selective Amplex red assay.

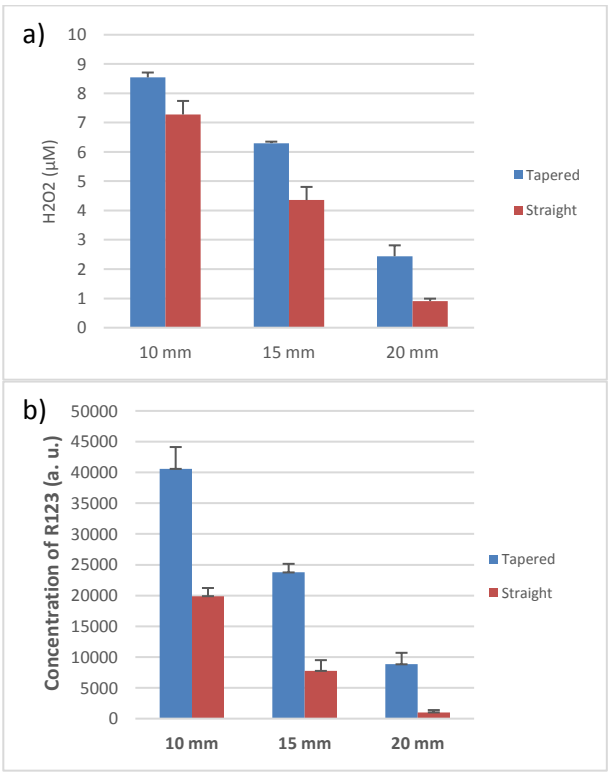

Fig. 5. The concentrations of (a) H_2O_2 and (b) RONS of the treated PBS samples after penetration through the agar gel under the different target gaps for tapered and straight tubes. V_p: 9 kV, f: 2 kHz, He flow: 0.5 slm.

4. Conclusions

The knowledge of plasma-tissue interaction is important for the development of cold plasma technology for biology, medicine and food industry. In this work, we used a tissue phantom using an agar gel that acts as a physical target and barrier to investigate how the RONS produced by a PG can

penetrate through the tissue. The concentration of RONS produced by a PG distributing on the surface and penetrating through the agar in different nozzle to target distance and nozzle shape were measured. As previously shown by other teams, we confirm that RONS have the ability to diffuse through few millimeter thickness of water containing medium. The produced RONS concentration is strongly linked to the application conditions such as distance to the target and shape of the plasma applicator inducing different conditions of air mixing with the helium flow. This emphasize on the fact that diagnostics of plasma plume and long lived species production must be performed in the closest condition to the real biological ones.

These results shows the potential of water containing tissue phantoms in determining an illustrative basis for specifying optimal and/or safe plasma conditions and provide consumer confidence in utilizing cold atmospheric plasma in medicine and cosmetics.

5. Acknowledgments

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6. References

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