Mass Spectrometry Analysis of the Real-Time Transport of Plasma-Generated Ionic Species Through an Agarose Tissue Model Target

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With ambient mass spectrometry, we followed the transport of neutral gas species and ionic species through a 3.2 mm thick agarose tissue model target during He non-thermal atmospheric pressure plasma (NT-APP) jet treatment. We found that the neutral gas species are unable to efficiently penetrate the agarose target. But both positively and negatively charged ionic species readily penetrate through the agarose target, following an initial time-lag period of several minutes. Interestingly, we also found that the ionic species are easily hydrated. The trends in the He NT-APP jet transport of ionic species observed in this study correlate well with the He NT-APP jet transport of reactive oxygen and nitrogen species (RONS) through agarose tissue model targets that was investigated in previous studies. Therefore, mass spectrometry might prove to be a useful tool in the future for analyzing the dosages of NT-APP-generated RONS in real biological tissues.

Keywords: Agarose, Ambient mass spectrometry, Ionic species, Plasma jet, RONS

1. Introduction

Non-thermal atmospheric pressure plasma (NT-APP) is being investigated for different biomedical applications such as wound decontamination [1,2], biomaterial surface treatment [3-5] and cancer therapy [6,7]. NT-APP jets typically operated with inert gases, such as helium and argon, generate a rich mixture of reactive oxygen species (ROS) and reactive nitrogen species (RNS), or collectively RONS, when the plasma effluent impinges upon air [8]. These RONS are generated when the primary plasma species (primary ions, electrons, metastable atoms, and photons) react with the ambient humid air. The biomedical effects of NT-APP are strongly linked to the NT-APP-generated RONS [9].

A rapidly emerging application of NT-APP is in cancer therapy termed Plasma oncology [10]. It was demonstrated that NT-APP treatment significantly reduces the size of solid cancerous tumors in the order of mm in thicknesses [6,11]. Initially, these results seem surprising because (i) the highly-reactive RONS, such as the hydroxyl radical (OH●) that induce significant cell damage, have relatively short lifetimes and diffusion distances [12], and (ii) the NT-APP treatment of solid organic materials is typically limited to the uppermost (first few nm) of the surface [13]. Therefore, initially it is difficult to reconcile how NT-APP treatment can penetrate into a solid tumor target of mm in thickness.

The ‘deeper’ effects of NT-APP into solid tumors of several mm in thickness can potentially be explained by cell-cell communication [12,14,15]. In this scenario, cells stimulated at the surface of tissue by the NT-APP treatment, transmit signals deeper
into the tumor through cellular signaling processes. In addition to NT-APP stimulating cells at the tissue’s surface, using synthetic models of tissue fluid and tissue, we have shown that NT-APP can potentially be used to deliver RONS ‘deep’ within tissue (to mm depths) [16-21]. We have proposed that a NT-APP jet delivers RONS deeper within tissue via a rapid transport process in the first few hundred μm below the target surface, after which the RONS are transported to mm depths via a slower molecular process [16,17]. A He NT-APP jet delivering RONS into a tissue target is illustrated in Scheme 1.

In this study, we have employed ambient mass spectrometry to study the transport of He NT-APP-generated RONS and ionic and neutral species through an agarose target (2% w/v and 3.2 mm in thickness) as a surrogate for tissue (Fig. 1). The purpose of this study is to obtain a better understanding of the phenomenon of He NT-APP jet transport of RONS into tissue for biomedical applications.

2. Experimental

2.1. He NT-APP jet

The He NT-APP jet consisted of a 150 mm long glass tube that was tapered from an inner diameter of 4 mm to 800 μm at the nozzle. The glass tube has a 15 mm long metallic external ring electrode wound onto the glass tube at a distance of 40 mm from the end of the nozzle. He gas was fed into the glass tube with a fixed gas flow rate of 0.5 slm. A capillary dielectric barrier discharge was generated using a sinusoidal high voltage of 10 kV (peak-to-peak) applied to the external electrode with a fixed frequency of 50 kHz. The driving voltage and discharge current were measured with a high voltage probe (Tektronix P6015A) and a conventional current monitor (Pearson 2877), respectively. More details on the current and voltage measurements can be found in a previous publication [16,22,23]. The He NT-APP jet was mounted onto an x-y-z stage for the molecular beam alignment.
2.2. Agarose tissue model target

A 2% (w/v) agarose target was prepared by dissolving 20 mg / mL agarose powder (Sigma-Aldrich, catalogue number A9539) in deionized (DI) water (18.2 MΩ.cm) purified through a Millipore Direct-Q system (model number ZRQ50PO30). The solution was heated (typically for 1–2 min) in a conventional microwave oven until all the powder was dissolved. The solution was cooled before pouring 20 mL of solution into an 85 mm diameter (plastic) petri dish. The plate was gently agitated to enable the solution to be evenly spread over the bottom of the dish, whilst taking precaution not to create air bubbles in the solution. The agarose was allowed to set at 4˚C for 12 hours before use. Square sections of approximately 2 × 2 cm² were cut using a scalpel and removed with a polytetrafluoroethylene tweezer, taking care not to tear the agarose. The agarose targets were stored in DI water at 4°C until use. The thickness of the agarose target was 3.2 mm, as measured with a vernier caliper. The agarose target is a hydrated barrier containing 98% of water.

2.3. Ambient mass spectrometry

Ambient mass spectrometry was used to identify neutral gas, and positively and negatively charged ionic species either directly in the output of the plasma plume or delivered through an agarose target by the He NT-APP jet. We show and discuss the species with mass-to-charge ratios (m/z) up to 100 amu. We used the HPR60-EQP300 system (Hiden Analytical Ltd.) [22-25]. It consists of a differential pumping system (HPR60) and the quadrupole based mass spectrometer (EQP300). The HPR60 has a three-stage differentially pumped inlet system separated by aligned skimmer cones and turbo molecular pumps. Eventually, the HPR60 provides a high vacuum condition below 10⁻⁷ torr for operation of the mass spectrometer (EQP300). The experimental setup of the plasma jet and the ambient mass spectrometry system is shown in Fig. 1 for measuring the species 1(a) directly generated by the He NT-APP jet and 1(b) the species transported by the He NT-APP jet through an agarose target.

2.4. Molecular beam alignment

Correct alignment of the molecular beam is critical in ambient mass spectrometry. The alignment of the molecular beam is optimized by internal mechanical structures using the aligned skimmer cones. In addition, it is necessary to correctly align the He NT-APP jet to the mass spectrometer to achieve a maximum signal intensity for the measurements. For the alignment, we simultaneously monitored the mass intensity change selected at a couple of mass numbers, i.e. He (4 amu) and N₂ (28 amu) of the He gas jet (only gas flow without plasma) in multi-ion detection (MID) mode associated with residual gas analysis (RGA) mode. Then, we decided that the optimal position of the He NT-APP jet was when a maximum signal of He was obtained with a minimum background signal of N₂ from ambient air. For the use of the 3.2 mm thick agarose targets, the distance between the plasma jet and sampling orifice of the mass spectrometer was fixed at 8 mm. The agarose target was placed on the sample holder at a fixed distance of 2 mm from the sampling orifice.

3. Results and discussion

3.1. Agarose barrier

It is necessary to confirm how the 2 cm × 2 cm agarose target can block the gas flow and affect the
intensity of mass signals. Here, we focused on the He NT-APP jet-generated ionic species on the underside of the agarose target (i.e. the opposite side to the surface treated with the He NT-APP jet). However, there is a 2 mm air-gap in-between the sampling orifice of the mass spectrometer and the agarose target, which needs consideration. Therefore, we needed to know the background level of mass intensities of He and ambient air species and we also needed to distinguish the ionic signals through the agarose target. Measurement time-points were (a) 1 min, (b) 10 min and (c) 20 min.

Figure 2 shows the time-resolved mass intensities of (a) \( \text{N}_2 \), (b) \( \text{O}_2 \), (c) \( \text{H}_2\text{O} \), and (d) He, respectively. In common, 43 scans were obtained for the time-resolved data including the first three scans and the last three scans where the agarose target was removed to confirm that the level of mass intensities had not changed. \( \text{N}_2 \), \( \text{O}_2 \), and \( \text{H}_2\text{O} \) are the molecules in humid ambient air. In the spectrum of positively charged ionic species in the plasma plume, \( \text{N}_2 \) is dominant over the other intensities under the fixed distance of 8 mm. For \( \text{H}_2\text{O} \), the intensity immediately increased when the agarose target was placed on the sample holder, which was set in front of the sampling orifice of the mass spectrometer with a distance of 2 mm. This is due to the vaporized water from the hydrated agarose target. The He signal intensity was similar to the \( \text{O}_2 \) intensity without the agarose target, but that suddenly decreased when the agarose target was placed in-between the He NT-APP jet and the mass spectrometer. However, a lower He signal intensity was still detected due to a small amount of the He gas flow penetrating through the agarose target during the \textit{in situ} mass spectrometry. Although a hole was not created in the agarose target during treatment, eventually the He signal intensity increased by an order of magnitude between the time-points of \( \sim 10 \) min to 17 min of He NT-APP jet treatment, before the signal stabilized.

We should note that the increase of He with agarose target was not observed with the He gas jet, which always remained at a constant lower signal intensity with the agarose target, as shown in Fig. 2(d). We speculate that the delayed increase of the

![Fig. 3. Mass spectra of positively charged ionic species generated by the He NT-APP jet measured by ambient mass spectrometry in positive SIMS mode either (a) directly from the He NT-APP jet (Without agarose) or (b and c) indirectly through an agarose target (With agarose). Measurement time-points were (a) 1 min, (b) 10 min and (c) 20 min.](image-url)

![Fig. 4. Time-dependent changes in the positive ions during He NT-APP jet treatment. The plots on the graph are for the atomic ion \( \text{O}^+ \) (16 amu), molecular ion \( \text{O}_2^+ \) (32 amu), and for the water cluster \( \text{H}^+(\text{H}_2\text{O})_4 \) (73 amu).](image-url)
He signal observed during He NT-APP jet treatment is the time taken for the neutralized He⁺ or metastable He⁺⁺ species to penetrate through the agarose target.

3.2. Positively charged ionic species

Secondary ion mass spectrometry (SIMS) mode was used to detect ionic species. In SIMS mode, the internal ionizer is not used. Therefore, the mass spectral signals are due to externally generated charged species. Fig. 3 shows the mass spectra of positively charged ionic species: 3(a) without the agarose target (free stream jet) at an early measurement time-point of 1 min; 3(b) with the agarose target at 10 min; and 3(c) with the agarose target at 20 min.

Figure 3(a) shows a typical mass spectrum of positive ions (O₂⁺, N₂⁺, H⁺N₂, NO⁺, etc.) generated in the plasma plume of the He NT-APP jet. As mentioned above, the distance of 8 mm was fixed for the set-up of the agarose target in-between the He NT-APP jet and the sampling orifice of the mass spectrometer. For the free-stream He NT-APP jet, the fixed distance and flow rate (0.5 slm) allows only the tip of the He NT-APP jet to touch the sampling orifice of the mass spectrometer. This allows us to measure the secondary ions generated by the reactions between effluent of the He NT-APP jet and ambient air. With these parameters, it is hard to detect the main plasma species (He⁺ ions). Indeed, we note that the He⁺ ions were observed at a closer sampling distance of ~ 3 mm (data not shown). In Figs. 3(b) and 3(c) with the agarose target (barrier), the ionic intensities were much lower than without the agarose target (Fig. 3(a)) in the plasma plume. But, interestingly the same ionic species, O₂⁺, N₂⁺, H⁺N₂, NO⁺, could be detected. A number of water cluster ions, e.g. (H₂O)ₙ⁺ and H⁺(H₂O)ₙ, could be measured. Especially H⁺(H₂O)ₙ clusters were created through hydration reactions with the water molecules present in the agarose target and/or in humid ambient air with the He NT-APP jet-generated species.

For investigation of time-dependent changes in the generation and transport of ionic species, we choose a couple of positive ions, O⁺ for the atomic ion, O₂⁺ for the molecular ion, and (H₂O)ₙ⁺ for the water cluster ion. The time-dependent ionic intensities clearly show a delay of several minutes with the agarose target (Fig. 4). We observed that the time-lag of O⁺ is longer compared to the other species. After the initial time-lag periods, the overall trends show that the signal intensities for O⁻ and O₂⁻ continuously increased as a function of the He NT-APP jet treatment time. However, the signal intensity for (H₂O)ₙ⁺ increased for about 5 min before decreasing again. We attribute the decrease in (H₂O)ₙ⁺ due to dehydration of agarose target during the exposure to the He NT-APP jet (or He gas jet).

3.3. Negatively charged ionic species

Figure 5 shows the mass spectra of the negatively charged ionic species either directly generated by the He NT-APP (Without agarose) or transported through the agarose target after different treatment times.

Figure 5(a) shows a typical mass spectrum of negative ions generated by the He NT-APP jet upon interaction with the ambient air. It is well known that the negative ions are produced by the dissociative electron attachment (e⁻ + AB → A⁻ + B) [26] and further attachment (A⁻ + M → A⁻ M) [22], where M denotes molecules in air. The
negative ions observed in Figs. 5(b) and 5(c) after He NT-APP jet treatment through the agarose target were thought to arise from the dissociative electron attachment of the water molecule (H$_2$O) and subsequent hydration during negative ion formation. Closer inspection of Fig. 5(c) shows that there are significant signal intensities for CO$_3^-$ and its related clusters [HCO$_3^-$, (OH)CO$_3^-$, CO$_3^-$(H$_2$O), and HCO$_3^-$(H$_2$O)]. The signal intensity for CO$_3^-$ is more than 30% higher than the intensity for O$_2^-$, which is the major negative ion in the plasma plume in ambient air (without agarose). We attribute the increase of CO$_3^-$ to the presence of agarose target. We speculate that the CO$_3^-$ is formed within the agarose target during He NT-APP jet treatment before being gradually transported through to the mass spectrometer.

Finally, the time-dependent changes of the selected negative ions, O$_2^-$, NO$_2^-$, CO$_3^-$, and O$_2^-$(H$_2$O)$_2$, were investigated. Figure 6 clearly shows the effect of agarose target: (i) a time-lag before the appearance of the negative ions; (ii) a significant increase of water clusters formation indicated by the appearance of O$_2^-$(H$_2$O)$_2$; and (iii) relatively large intensities for NO$_2^-$ and CO$_3^-$ with agarose target. The increase in the signal intensity for NO$_2^-$ with the agarose target is nearly double when compared to without agarose (direct measurement at the early and later time-points). For CO$_3^-$, the signal intensity with agarose increased by an order of magnitude compared to without agarose. When we consider the trends for the positive ions, only the hydrated water cluster ions, H$^+$(H$_2$O)$_n$ (n=1–5), showed a similar trend. (See Fig. 3). The intensity of hydrated water cluster positive ions increased at a time-point of 12 min, but decreased afterwards. To explain the above trends, we should consider that the agarose target dehydrates during treatment resulting in a decrease in water cluster positive ion [H$^+$(H$_2$O)$_n$] formation in Fig. 4 and also a decrease in the signal for the neutral water molecule (H$_2$O) in Fig. 2(c). The increase in the signal intensity for the negative ions (NO$_2^-$ and CO$_3^-$) at the later treatment time-point we attribute to the easier permeability of these negative ions within the agarose target. Whilst, the positive ions are not as easily transported through the agarose target. We speculate the reason for this is that the positive ions may easily form into water clusters and/or negative ions via multi-step dissociative electron attachment or negative ion attachment.

4. Conclusion
With mass spectrometry, we successfully demonstrated the transport of neutral gas species and He NT-APP jet-generated positively and negatively charged species in the gas phase through a 3.2 mm thick agarose tissue model target. We observed a time-lag (in the order of several minutes) before the appearance of the species through the agarose target. This may indicate an accumulation of the ionic species within the agarose film and a subsequent release behind the film. Notably, we found that the transported ionic species, generated by interaction between the emerging He NT-APP jet species (He$^+$, He*, and electrons) and ambient air, were easily hydrated within the agarose target.

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