22nd International Symposium on Plasma Chemistry
July 5-10, 2015; Antwerp, Belgium

Atomic oxygen – a potent precursor in RF plasma induced liquid chemistry

K. Wende[1,2], P. Williams[2], P. Lukes[3], W. Van Gaens[4], H. Aboubakr[5], Th. von Woedtke[1], A. Bogaerts[4], K. Masur[1] and P.J. Bruggeman[2]

[1] *Leibniz Institute for Plasma Research and Technology, Greifswald, Germany*
[2] *University of Minnesota, Department of Mechanical Engineering, Minneapolis, MN, U.S.A.*
[3] *Academy of Sciences of the Czech Republic, Institute of Plasma Physics, Prague, Czech Republic*
[4] *Research group PLASMANT, Department of Chemistry, University of Antwerp, Antwerpen-Wilrijk, Belgium*
[5] *University of Minnesota College of Veterinary Medicine, St. Paul, MN, U.S.A.*

Abstract: The major active species produced by two argon plasma jets (RF jet and MHz jet) in liquids were identified by biological, physical and chemical methods and complemented with chemical kinetics modelling. Argon plasma led to the creation of hydrogen peroxide dominated chemistry in both jets while in argon-oxygen plasma atomic oxygen appeared to be dominant in case of the RF jet. The data suggest that atomic O reacts with Cl^-, yielding $\bullet Cl_2^-$ or ClO^-.

Keywords: RF jet, kINPen, liquid chemistry, atomic oxygen, chlorine species

1. Introduction

Reactive oxygen and nitrogen species (ROS and RNS) generated in the active plasma, its afterglow, or at the liquid interface play a major role in creating biological effects of jet plasmas [1, 2]. Several species have been detected in the gas phase using spectroscopic methods [3, 4]. The liquid phase chemistry induced by the plasma is investigated by indirect methods which can disturb the system under investigation and/or are highly non-selective, posing a considerable challenge to unravel the chemical processes in the solution phase. Different plasma derived species have been suggested to be important for the interaction with biological systems, including H_2O_2 [5, 6], O_3 [7], peroxynitrite [8], NO_2/NO [1], O_2^- [9], and singlet oxygen [10]. This report seeks to identify the major active species produced by an RF jet under different conditions (for details see [11, 12]) and compare it with a MHz argon jet (kINPen). A detailed description of the experiment can be found in [13].

2. Methods

Plasma source: A non-equilibrium atmospheric plasma jet plasma source was used for the experiments (RF plasma jet) [14]. A gas flow of 1.5 slm argon is applied through the tube, if desired with admixtures of molecular gases (O_2, N_2, air) or water saturated argon. The discharge is ignited using a RF frequency of 13.7 MHz generated by a signal generator (Agilent 33220A) connected to a power amplifier (Amplifier Research). The plasma dissipated power was measured as described in [15]. The MHz jet kinpen features a powered inner needle electrode that is mounted in the center of a capillary with radius of 0.8 mm and a grounded outer electrode As feed gas argon at a mass flow rate of 3 slm is used. The inner electrode is driven at a frequency of 1 MHz and a peak-to-peak Voltage of about 2 kV [16]. In both cases, the visible plasma effluent measures about 12 mm in length.

Cell viability assay: 3500 cells were seeded into clear flat bottom 96-well plates. Directly before plasma treatment, medium was discarded, and replaced by 100 µL of phosphate buffered saline w/ Ca^{2+}/Mg^{2+} containing 1 g/L glucose. Plasma treatment applying the above mentioned conditions was achieved by the following routine: 3 mL of physiological liquid (phosphate buffered natural saline + 1 g/L D-glucose) was treated distance and time controlled. The gas flow prevented the visible plasma tip to come in direct contact with the solution. Immediately after treatment (< 1 min), the treated liquid was transferred onto the prepared 96 well cell culture plates and diluted serially within the plate as described before [17]. After addition of the treated buffer, cells were incubated for 1 h. Subsequently, buffer was replaced by fresh complete RPMI 1640 medium and cells were incubated for 70 h. Cell viability was determined by MTS compound conversion (Promega, Madison, WI, USA) according to the manufacturer's protocol. OD was measured at 490 nm using a VMax multiplate reader (Molecular Devices, Sunnyvale, CA, USA) one hour after substance addition.

Chemical assays: H_2O_2 was quantified by Ampex Red or Ti sulphate assay as described by suppliers. Griess assay was used to determinate NO_2^-/NO_3^-. Phenol (500µM) was treated with RF jet or kinpen in different buffer systems and products analyzed by reversed phase liquid chromatography.

3. Results

a) Argon RF plasma jet

If pure argon was the sole working gas, the impact on cell viability correlated with the H_2O_2 deposition in the solution (up to 250 µM, Fig. 1). If the plasma treatment and its dilution is plotted as H_2O_2 according to the

measured concentrations in the liquid the apparent IC_{50} was found to be around 10 ± 2.5 µM which is lower than for authentic H_2O_2 (15.5 µM) and indicates the presence of other ROS/RNS. A 100 % rescue of cell viability by catalase (10 U/mL) was observed for a broad treatment intensity range (Fig. 2).

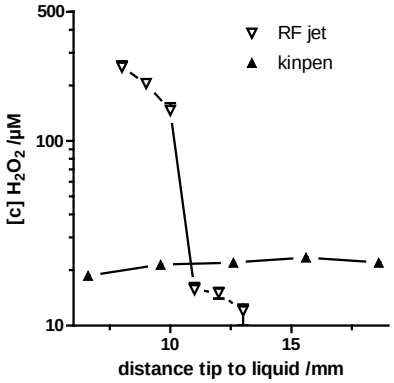

Fig. 1. H_2O_2 deposition by Argon RF plasma jet or kinpen [13].

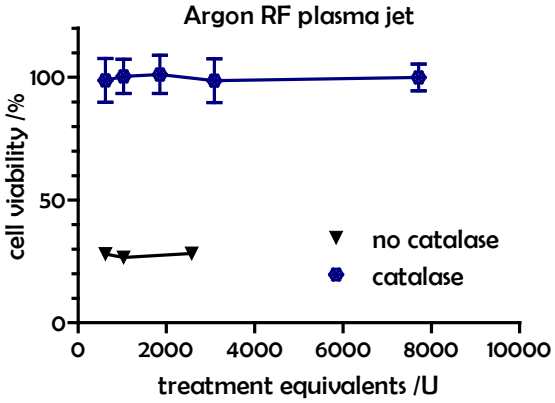

Fig. 2. H_2O_2 scavenger catalase modulates biological activity of the Argon RF plasma jet [13].

b) Argon-O_2 and Argon-Air RF plasma jet

Under oxygen presence in the working gas, the plasma chemistry changed significantly. H_2O_2 deposition in phosphate buffered saline was not observed (Amplex Red Assay or Ti-sulphate assay) although the cell proliferation rate was decreased (Fig. 2). Addition of catalase did not rescue the cell viability (Fig. 2). Interestingly, if a 200 µM H_2O_2 containing saline based buffer was treated for 40s the H_2O_2 concentration decreased to about 100 µM (see Fig. 4, column *PBS+spike*). A strong dependency of the biological impact on distance between nozzle-liquid was observed: at 12 mm no effect on the cells was observed while at 8 mm distance there was a strong impact. Additionally, an eminent effect of delay between plasma treatment of the liquid and addition of the treated liquid to the cells was measured. The half life time of the plasma triggered chemistry was 30 min in case of O_2 admixture while in case of pure argon plasma no such decrease was observed within 60 min.

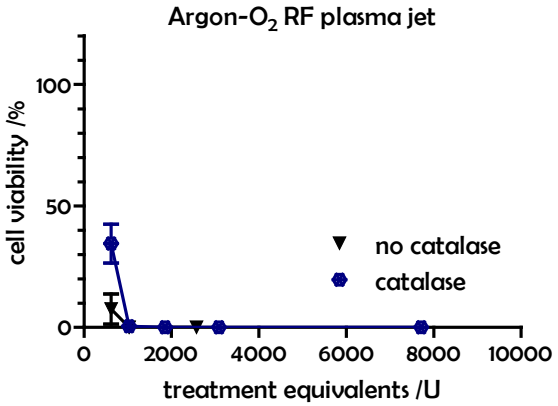

Fig. 3. H_2O_2 scavenger catalase has no impact on biological activity of the Argon-O_2 RF plasma jet [13].

c) Argon-O_2 RF plasma jet in chloride free buffer

In contrast, if a chloride free isotonic and isohydric buffer system was treated with the Ar-O_2 RF plasma jet, hydrogen peroxide deposition was observed (Fig. 4, column *non-ionic buffer* and). No H_2O_2 decay was detected (column *non-ionic buffer+spike*), but a gain in H_2O_2 concentration. The cell viability could be rescued by the addition of catalase.

d) Effect on phenol oxidation pattern

When phenol is treated by the different plasma jets, a time dependent oxidation takes place. Major products are polyhydroxylated phenols and quinones. Phenol conversion was most efficient using RF Ar/O2 plasma, with RF Ar plasma following up. Kinpen plasma was less effective and independent from working gas composition. Product spectra differed between RF Ar/O2 and RF Ar plasma. In case of RF Ar/O2 plasma, presence of chloride ions influenced the spectrum.

4. Discussion

The observations made in the case of argon RF plasma jet indicate the presence of H_2O_2 as a dominant ROS and NO_2^- as one highly prevalent RNS (data not shown) as was expected [1, 18-20]. Accordingly, a decrease in cell proliferation rate of mammalian cells is observed for longer treatment times. Tests using reactive species scavengers and delay experiments gave no hints on other chemical species deposited in the liquid. However, peroxynitrite chemistry could be expected. A similar correlation between H_2O_2 production and cell viability has been reported by Winter/Wende et al. for the kINPen [6].

In contrast, the addition of O_2 to the working gas led to the creation of a different chemistry in the liquid. Most striking features are i) missing activity of catalase (Fig.

2), a negative H_2O_2 quantification assay (Fig. 4), ii) a finite lifetime of the reactive species and a strong distance dependency (Fig. 3), and finally iii) the mandatory presence of chloride ions to create this H_2O_2-free chemistry (Fig. 4). A chemical kinetics model showed the significant amount of oxygen species produced by the plasma jet [13].

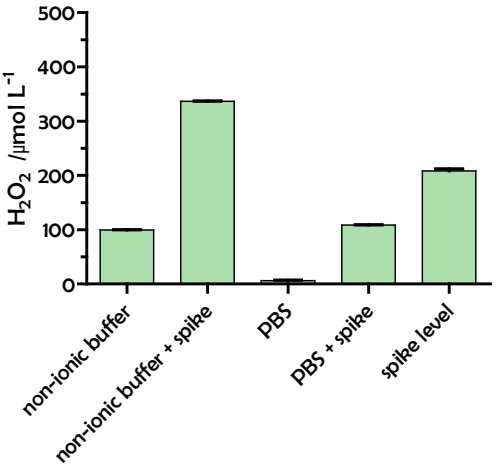

Fig. 4. Role of chloride presence on H_2O_2 destruction or production by 40 s of Ar-O_2 RF plasma jet. Spiked in H_2O_2 level is 200 µM [13].

Atomic oxygen is the only species (apart from ionic species) which seems to have significant distance dependence in the effluent consistent with the distance dependent effect observed in the cell viability study. Secondary chemistry induced by O in the liquid phase with a longer lifetime seems to be responsible for the effect. The following reaction mechanism is proposed:

$$Cl^- + O \rightarrow OCl^- \quad (1).$$

However, to the authors` knowledge reaction rates of this reaction are not reported in literature. It is similar to

$$Cl^- + \cdot OH \rightarrow HClO^-;\ k = 3 \times 10^9\ M^{-1}\ s^{-1} \quad (2) \quad [24]$$

which is a very fast reaction and an equivalent reaction is proposed in biology [25].
The ClO^- will react with H_2O_2 forming Cl^-, O_2 and H_2O as is as a standard performed as a dechlorination process (3) [26]:

$$OCl^- + H_2O_2 \rightarrow ClO^- + H_2O + O_2 \quad (3) \quad [27]$$
$$H^+ + ClO^- \leftrightarrow HOCl\ pKa = 7.8 \quad (4)$$

ClO^-, being in equilibrium with its corresponding acid (4) has an impact on cell viability if present at higher concentrations which can be reached in a normal treatment. ClO^- is thermodynamically instable and it is in equilibrium with Cl_2 through:

$$2\ H^+ + ClO^- + Cl^- \leftrightarrow Cl_2 + H_2O \quad (5)\ .$$

The formed chlorine gas (5) can be released from solution which could account for the limited lifetime of the reduction of cell viability found in Argon-O_2 RF plasma treatment. A similar observation has not been made for the kINPen due to the lower O density produced [28]. An alternate reaction mechanism might be centered around $\cdot Cl_2^-$.

5. Conclusion

Using modelling data, chemical detection, comparison with the kINPen, and literature, we propose that in case of Argon-O_2 RF plasma jet hydrogen peroxide plays no prominent role when standard physiologic buffer or body fluids are treated. Instead, the plasma chemistry is driven by atomic oxygen. Via reaction with chloride yielding $\bullet Cl_2^-$ or ClO^-, any hydrogen peroxide produced by the plasma itself is decomposed. These chlorine species have a limited lifetime under physiologic conditions and therefore show a strong time dependent biological activity. They interact with biological macromolecules, show an antimicrobial effect and could hence be attributed to be responsible for the strong effects of oxygen admixtures in the biological impact of many non atmospheric plasma sources. In addition, the results show clear differences in the biological action between two seemingly similar plasma jets, i.e., the RF plasma jet and the kINPen, which is attributed to strong differences in reactive species densities and different geometries. Hence, a distance dependency of species concentrations occurs in the RF jet while this is not found in the kINPen.

6. Acknowledgements

This work was in part founded by the German Federal Ministry of Education and Research (grant# 03Z2DN11), the Department of Energy Plasma Science Center through the US Department of Energy, office of Fusion Energy Sciences, Contract: DE-SC0001939 and the University of Minnesota W.V.G. and A.B. thank Professor Mark Kushner (University of Michigan) for providing the numerical code.

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