COST plasma jet: More than a reference standard

Y. Gorbanev,[1] A. Privat-Maldonado,[1,2] J. Van der Paal,[1] C. Verlackt,[1] S. Tinck,[1] E. Smits[2] and A. Bogaerts[1]

[1]*Research group PLASMANT, Department of Chemistry, University of Antwerp, Antwerp, Belgium*
[2]*Center for Oncological Research, University of Antwerp, Antwerp, Belgium*

Abstract: The COST jet was first introduced as a reference microplasma jet. Later, its potential in biomedical applications has been studied. We present a detailed investigation on the liquid phase chemistry and anti-cancer capabilities of the COST plasma jet. Using various analytical techniques coupled with computational studies, we determined the sources of reactive species found in aqueous solutions, both primary (originating in the gas phase plasma) and secondary species (arising from the reactions of the primary species with solutes).

Keywords: cold plasma, plasma-liquid interactions, cancer therapy, COST plasma jet

1. Introduction

Cold atmospheric plasma (CAP) research is an extremely burgeoning field. The COST radio frequency (RF) plasma jet was created as a result of the European COST action, and suggested as a reference microplasma jet set to establish a comparison standard in plasma research [1]. It has been used in several areas of applied research, such as nanomaterials synthesis or photoresist removal [2].

In this work, we studied the effects of the COST jet on glioblastoma spheroids as 3D tumour models *in vitro*, in aqueous solutions. We show that this plasma jet operated with water-saturated helium feed reduces the size of the cancer cell spheroids, impedes their growth, and decreases cell migration and proliferation. We also assessed the production of various reactive oxygen and nitrogen species (RONS) in solutions under various conditions, and identified the main RONS responsible for the anti-cancer effects. In addition, we determined the reactive compartments of the plasma-liquid system comprised of the COST jet and an aqueous solution. The experimental results were in good agreement with the data obtained from computational 3D fluid dynamics and 0D chemical kinetics models. Finally, we studied the generation of secondary reactive species, formed in reactions of the primary RONS generated by plasma, with the dissolved components of aqueous solutions exposed to CAP.

2. Reduction of human glioblastoma spheroids

We used 3D structures (spheroids) built of cancer cells, which closer represent the actual behaviour and survival rates of the *in vivo* cancers than e.g. cell monolayers.

The COST jet was operated with He with various degrees of H_2O-vapour saturation. The gas phase plasma was in contact with phosphate buffer saline (PBS), containing glioblastoma spheroids (Fig. 1). This medium was used to reduce the scavenging effects of macromolecules present in other, nutrient-rich, media [3]. Screening of various operation parameters revealed that the largest effects leading to the spheroids shrinkage and growth prevention were achieved with CAP ignited with He with 20% relative saturation of H_2O vapour. Various analytical techniques were used to assess cell migration, and proliferation in 3D structures.

The effects of plasma in biological milieu are largely defined by the reactive species generated by CAP [4]. The measurements of plasma-produced RONS showed that the main RONS created under these conditions are H_2O_2 and •OH radicals. We also used plasma-treated medium (PTM), where PBS was first pre-treated by CAP, and then used on spheroids. The comparison of the effects of the solutions of persistent RONS (H_2O_2, NO_2^-) with PTM showed nearly identical results. This clearly indicated that short-lived RONS, induced in the solution during plasma treatment, are crucial for the full-scale effectiveness of such anti-cancer treatment [5].

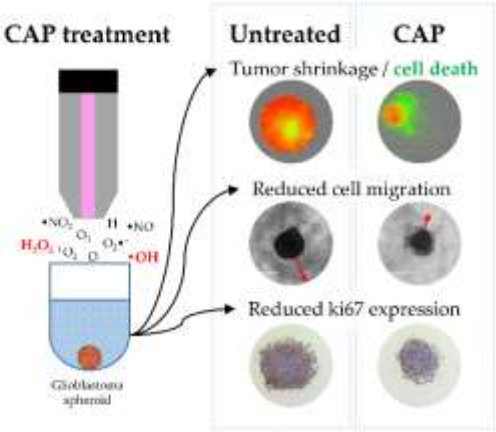

Fig. 1. COST plasma jet treatment of human glioblastoma spheroids *in vitro*.

3. Reactive compartments of a COST jet-liquid water system

In a chemical system consisting of a CAP jet and liquid H_2O, the reactive species induced in water can either be formed directly in water (e.g. by direct electron impact or reactions with high-energy neutral metastables) or be delivered into the liquid from the gas phase. We have previously demonstrated empirically that most of the short-lived species in the case of a parallel-field plasma jet are formed in the CAP effluent [6].

Here, we used a combined approach: experimental RONS measurements in liquid were compared with zero-dimensional chemical kinetics model data, which predicted concentrations of the same RONS in the gas phase. We also used isotopically labelled water to distinguish between H_2O molecules in the gas phase and D_2O molecules in the liquid, and vice versa. The use of a specifically designed reactor [7] allowed controlling the composition of the CAP effluent. A 3D fluid model predicted the gas phase dynamics inside the reactor, and agreed very well with the experimental data on effluent composition, as confirmed by the NMR analysis of H_2O content delivered by gas plasma to the liquid D_2O samples.

Short-lived RONS (radicals) were measured by electron paramagnetic resonance spectroscopy, and the long-lived H_2O_2 by UV-Vis spectrophotometry. The results clearly indicated that under all conditions, almost all of the reactive species detected in the liquid were created inside the COST jet, i.e. in the plasma ignition region. In the effluent, only the further reactions of the initially formed radicals occurred: recombination and reactions with air components [2] (Fig. 2). In other words, even the low levels of feed gas impurities had higher impact on the production of the •OH radical (a key reactive species in our plasma system) than an important ambient parameter such as air humidity.

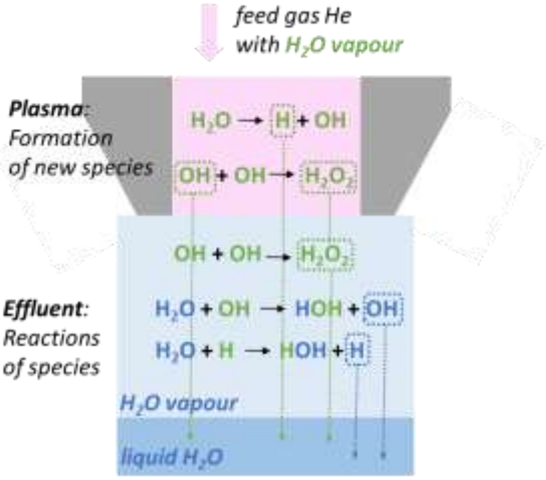

Fig. 2. Reactive compartments of the COST plasma jet-liquid water system.

4. Reactive species formed from solutes

Secondary RONS formed in the liquid can contribute to the biomedical effects of CAP. One of these reactive species is ClO^-, which can be formed upon plasma exposure of Cl^--containing aqueous solutions such as PBS. Recently, it has been suggested that ClO^- can be formed in a reaction with atomic O [8]. This a new, previously unreported chemical reaction. Generally, literature on the chemistry of atomic O in liquid phase is scarce due to the high reactivity of O and the absence of simple chemical systems for its generation.

To study the possibility of this reaction, we used the COST plasma jet as an efficient source of O atoms in liquid H_2O [9]. The CAP was ignited here with a He+O_2 mixture as feed gas.

We compared the generation of ClO^- in PBS with the trends of atomic O concentrations in the liquid (experimentally) and in the gas phase above the liquid (by modelling). For example, the net production of ClO^- was remarkably similar to the concentration trend of the produced O (Fig. 3). This, together with the virtual absence of any other RONS capable of creating ClO^-, clearly demonstrated that indeed ClO^- was formed in a reaction of Cl^- with atomic O.

We also studied the stability of ClO^- in PTM in the presence of other RONS [10]. ClO^- may potentially be produced by •OH, O_2•$^-$/•OOH, O, etc. in situ. However, we show that its persistence in PTM as well as its effects on biological substrates during the exposure will depend on the decay rates of ClO^- by other plasma-induced RONS.

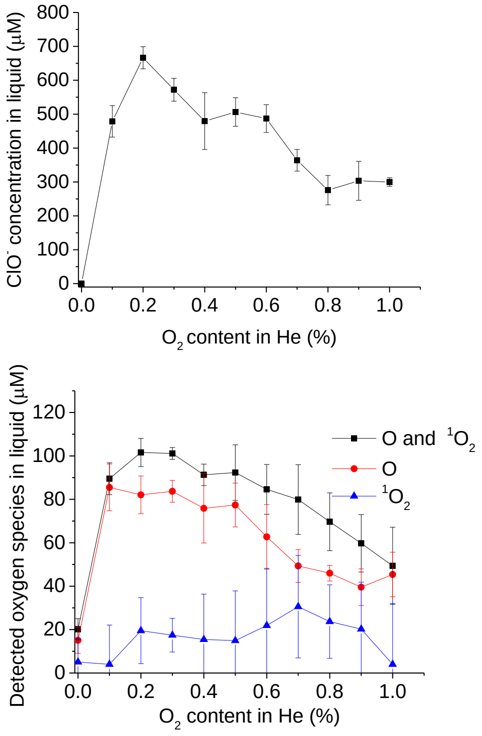

Fig. 3. The trends of concentrations of ClO^- (top) and various O species (bottom) detected in aqueous solutions exposed to the COST jet ignited with He+O_2.

5. Conclusions and outlook

Our results confirm and emphasise the ‘standard’ nature of the COST jet. The low effect of ambient parameters suggests that it indeed can be used in different environments as a reference.

Moreover, this jet has also shown great promise in the field of anti-cancer biomedical plasmas, as seen in our experimental results with 3D tumour models.

We show that the generation of RONS by the COST jet is highly tuneable, and several species can be produced with a very high selectivity (e.g. •OH radicals or O atoms). This makes the COST plasma jet an invaluable tool in chemistry research, as we also demonstrate by studying the reaction of Cl^- in liquid water with the CAP-induced O(aq) atoms.

This uniformity of performance regardless of many uncontrollable parameters together with the tuneability of the COST jet opens paths to many interesting applications, including radical reactions with the plasma-induced species for synthetic chemistry [11].

6. Acknowledgements

The authors thank K. Foubert, E. Tuenter (Department of Pharmaceutical Sciences, University of Antwerp), O. Voet, K. De Wael (Antwerp X-ray analysis Electrochemistry and Speciation, Department of Chemistry, University of Antwerp) for their help with experiments. We also thank P. Cos (Department of Pharmaceutical Sciences) and S. Dewilde (Department of Biomedical Sciences) for providing analytical equipment.

This work was funded by the European Marie Sklodowska-Curie Individual Fellowship within Horizon2020 (‘LTPAM’, grant no. 743151).

7. References

[1] J. Golda, J. Held, B. Redeker, M. Konkowski, P. Beijer, A. Sobota, G. Kroesen, N. S. J. Braithwaite, S. Reuter,M. M. Turner, Journal of Physics D: Applied Physics, **49**, 084003 (2016).

[2] Y. Gorbanev, C. C. W. Verlackt, S. Tinck, E. Tuenter, K. Foubert, P. Cos, A. Bogaerts, Physical Chemistry Chemical Physics, **20**, 2797 (2018).

[3] A. Privat-Maldonado, Y. Gorbanev, D. O’Connell, R. Vann, V. Chechik, M. W. van der Woude, IEEE Transactions on Radiation and Plasma Medical Sciences, **2**, 121 (2018).

[4] Y. Gorbanev, A. Privat-Maldonado, A. Bogaerts, Analytical Chemistry, **90**, 13151 (2018).

[5] A. Privat-Maldonado, Y. Gorbanev, S. Dewilde, E. Smits, A. Bogaerts, Cancers, **10**, 394 (2018).

[6] Y. Gorbanev, D. O’Connell, V. Chechik, Chemistry – A European Journal, **22**, 3496 (2016).

[7] Y. Gorbanev, R. Soriano, D. O’Connell, V. Chechik, Journal of Visualized Experiments, **117**, e54765 (2016).

[8] V. S. S. K. Kondeti, C. Q. Phan, K. Wende, H. Jablonowski, U. Gangal, J. L. Granick, R. C. Hunter, P. J. Bruggeman, Free Radical Biology and Medicine, **124**, 275 (2018).

[9] M. M. Hefny, C. Pattyn, P. Lukes, J. Benedikt, Journal of Physics D: Applied Physics, **49**, 404002 (2016).

[10] Y. Gorbanev, J. Van der Paal, S. Dewilde, A. Bogaerts, manuscript submitted for publication.

[11] Y. Gorbanev, D. Leifert, A. Studer, D. O’Connell, V. Chechik, Chemical Communications, **53**, 3685 (2017).