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Atmospheric pressure plasmas: Generation and delivery of reactive oxygen species for biomedical applications

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Reactive oxygen species (ROS) that can trigger biological responses are readily attainable in atmospheric pressure plasma sources. Admixtures of oxygen and water can act as precursors for the generation of these ROS and lead to the production of O, OH, O₃, ¹O₂, OOH and H₂O₂. The dynamics and chemistry in these discharges is complex and result in intricate spatiotemporal profiles of the species that cannot be accurately captured by zero dimensional analysis. Besides fluxes of neutral ROS, ionic fluxes including anions are also observed. The high reactivity of most of the ROS, however, limits their penetration into the treated sample and therefore encapsulation of the ROS and/or triggering of a secondary chemistry is required for the plasma treatment to reach beyond the first layers of biomolecules.

1. Introduction

One of the fastest growing fields in low-temperature plasmas is “plasma medicine”, an emerging scientific discipline that exploits the interaction of gas plasma with biological targets for therapeutical purposes. The number of potential applications in this field has risen in recent years, and expands from sterilisation of abiotic and biotic surfaces for health care and food processing industries to alternative approaches to wound healing, dentistry and cancer therapy.[1,2]

In the context of plasma medicine, the plasma is required to operate at atmospheric pressure and remain at low-temperature so that human tissue can be treated without inducing thermal damage. These requirements are typically achieved by using noble gases as a background carrier and adjusting the input power and the distance between the treated target and the plasma source.

Although the potential of plasma medicine has already been demonstrated by a growing number of research groups, the underlying mechanisms leading to the observed biological responses remain largely to be revealed. A large number of plasma sources and biological targets have been investigated and there is growing amount of literature that suggests that for low-temperature atmospheric-pressure plasmas the interaction with biological targets is mainly chemistry driven.[3,4,5] UV radiation and physical etching observed in low-pressure plasma treatments [6,7] do not seem to play a dominant role in atmospheric pressure plasmas. Indeed the flux of neutral ROS is orders of magnitude larger than that of ions and at atmospheric pressure the ion energy is small due to the large collisionality of the plasma.⁸ Nevertheless, charge accumulation from ions (and

electrons) may create electric fields that could lead to important biological responses such as electroporation,[9] and these could interplay synergistically with the ROS chemistry.

In particular atmospheric pressure plasmas can be engineered to produce large quantities of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are known to play important roles in biology.[10] In this contribution we concentrate on the generation of ROS in He rf plasmas with admixtures of oxygen and water.

2. ROS in He+O₂+H₂O rf plasmas

In applications where ROS are desired to induce oxidative stress in targeted microorganisms, small admixtures of oxygen are typically introduced in the discharge. In addition to the background gas and the oxygen admixture, water is also likely to be present in the discharge because biological targets are typically moist. The presence of water in the

Oxidant	Oxidation potential (V)
Fluorine	3.0
*Hydroxyl radical (OH)	2.8
*Atomic oxygen (O)	2.4
*Ozone (O ₃)	2.1
*Hydrogen peroxide (H ₂ O ₂)	1.8
Potassium permanganate	1.7
Chlorine	1.4
...	
*Oxygen (O ₂)	0.4

Table 1. List of oxidants and oxidation potentials. The star indicates that the oxidant is produced in He+O₂+H₂O plasmas.

discharge can interfere with the oxygen chemistry and a better understanding of the chemistry of that take place oxygen and water containing plasmas is currently required. Water can quench ROS generated in pure He+O₂ discharges but it can also produce additional ROS that complement those in He+O₂ plasmas. Therefore water can also be deliberately introduced as an additional precursor in the discharge. For example, the presence of water results in the generation of hydroxyl radicals (OH), which are not attainable in pure He+O₂ discharges. Table 1 lists the main ROS that are generated in He+O₂+H₂O plasmas ordered by their oxidation potential.

OH has special significance because it is a very reactive radical and has an oxidation potential (2.8V) that is larger than that of both atomic oxygen (2.4) and ozone (2.1), the main ROS created in O₂ plasmas. Due to its high reactivity, OH has been long studied by the free radical biology community and it is known to be able of directly oxidizing DNA, proteins and lipids. Contrary to endogenous OH that requires the presence of other chemical species to form (e.g. transition metals in Fenton-type reactions and superoxide ions in Harber-Weiss reactions), plasmas generate OH independently of the cell composition and therefore have the potential

to target different parts of the cell and trigger different cell responses than endogenous OH. Further highlighting the significance of OH in biological systems, recent studies have also linked bactericidal properties of antibiotics to OH production.

Although He+O₂+H₂O plasmas can generate the ROS listed in table 1, the chemistry of these discharges can be complex. Comprehensive global models of He+O₂ and He+H₂O plasmas with hundreds of reactions have been reported in the literature and used to identify key reactions and species.[11,12] Global models, however, do assume spatial uniformity, an assumption that works well in low-pressure non-local discharges but that does not capture accurately conditions encountered in most atmospheric pressure plasmas. For example, Fig. 1 shows the net generation rate, i.e. generation minus loss, of ROS in He+H₂O (0.3%) and He+O₂ (0.3%) discharges obtained with a 1-dimensional fluid simulation for an rf discharge (13.56MHz) across a gap of 1mm and an input power of 1W/cm². The 1 dimensional simulation model captures the spatial variations across the discharge gap and solves the continuity equation for each plasma species, the electron energy equation and Poisson's equation for

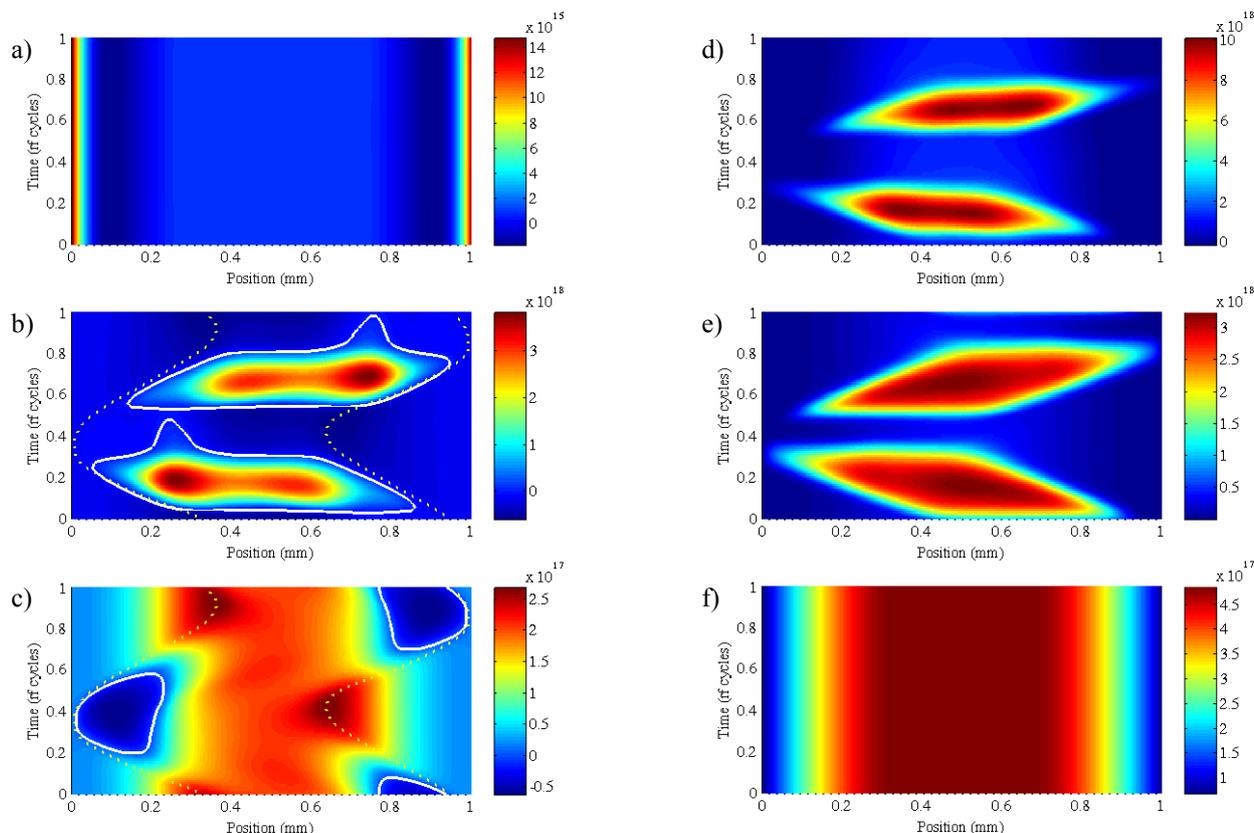


Fig. 1 - Spatio-temporal evolution of the generation of (a) OOH, (b) OH and (c) H₂O₂ in a He+H₂O (3000ppm) discharge and (d) O, (e) ¹O₂ and (f) O₃ in a He+O₂ discharge. White solid lines indicate the contour of zero net generation and the dotted yellow lines the spatio-temporal evolution of the electron ensemble.

the electric field calculation. Due to large collisionality of the plasma, the charged particles inertia is neglected and the drift-diffusion approximation is used to determine their mean velocity. The spatio-temporal inhomogeneity and different dynamics among ROS are evident in Fig. 1, and these reflect the non-uniformity of the density and electron energy profiles (not shown).

The most abundant ROS in the He+H₂O discharges is OH whereas O is the most abundant ROS in the He+O₂ plasma. By adjusting the ratio of H₂O to O₂ in the admixture, it is possible to combine ROS generated in He+H₂O and He+O₂ discharges. A maximum concentration of O and OH is obtained when the relative concentration of precursors is ~1, i.e. for [H₂O]=[O₂]. [13]

The presence of water in the discharge also leads to the hydration of anions and cations. [13,14] As a result the O₂⁻·(H₂O)_n and O₃⁻·(H₂O)_n instead of O₄⁻ and O₃⁻ become the dominant ions when the ration [H₂O]/[O] is <0.5, and H⁺·(H₂O)_n and OH·(H₂O)_n at higher water concentrations.

Besides neutral ROS, plasma also generates charged species that are of relevant for biomedical applications. In particular plasmas can produce superoxide (O₂⁻), a radical that plays an important role in biology not only because it can directly oxidize biomolecules but also because it can trigger the ‘remote’ formation of other ROS, including HO₂, OH, ¹O₂ and H₂O₂. [10] The transport of this anion from the plasma to the target is not well capture in global models as in those models it is customary to assume that anions are perfectly confined in the bulk

plasma by the ambipolar field. However, mass spectrometry experiments have detected fluxes of negative ions from atmospheric pressure rf discharges in He+H₂O [14], suggesting that some anions do indeed escape the discharge.

One dimensional simulations also predict fluxes of negative ions (see fig. 2). It is noted that this flux is weakly related to the mean anion density in the discharge volume as only anions created near the electrodes are able to escape. Studies of discharges sustained in different gap sizes show that decreasing the discharge gap enhances the anion flux to the electrodes even though the mean densities decreases.

In many plasma medicine applications, the plasma treatment is required to reach beyond the surface of the biological sample, often through an aqueous environment with organic content. The high reactivity of many of the ROS, however, precludes the penetration of these plasma species deep underneath the surface. For example, the lifetime of singlet oxygen (¹O₂) in the gas phase is on the order of tens of minutes. As a result its main loss is through diffusion out of the discharge gap. In aqueous environments, however, its lifetime reduces to ~μs and in biological samples where it can react with biomolecules its lifetime further reduces to ~40ns. [15,16] Therefore, assuming a typical diffusion constant (D) of ~5·10⁻⁵cm²/s, singlet oxygen diffuses sqrt(Dt)~10nm beyond the surface, i.e. a fraction of the size of a single cell. As a result, most reactive species will only directly affect the most outer layer of the sample being treated with O₃, O₂⁻ and H₂O₂ being the ROS that can further diffuse due to their lower reactivity. This simple estimation reflects the well-known superficial nature of plasma treatments and supports, for example, the rapid decrease of the bactericidal properties of plasmas when bacteria are piled up or buried in biofilms. [17,18] Nevertheless, there is also evidence of long range effects of plasma treatments, for example through liquid media, [2] which suggests that stable compounds of biological relevance capable of reaching far beyond the size of a cell are generated at the plasma-medium interface.

Potentially a way to deliver ROS species further inside a target would be to encapsulate them for example in a water droplet, thereby limiting their interaction with top layers of the biological material and extending their lifetime. Going back to the singlet oxygen example, its lifetime increases by ~2 orders of magnitude by entrapping it in water and more than 3 orders of magnitude if heavy water or other solvents are used. [19, 20,21]

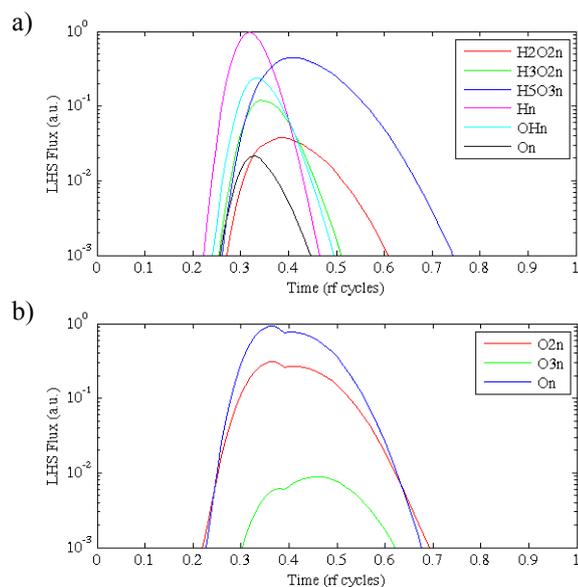


Fig. 2 – Anion flux to an electrode in a 1W/cm² (a) He+H₂O (3000ppm) discharge and (b) He+O₂ (3000ppm) discharge.

3. Conclusions

In conclusion, He+O₂+H₂O admixtures can be used to generate cocktails of ROS that contain all the main species typically encountered in He+O₂ and He+H₂O plasmas. The ratio of H₂O to O₂ can be used to control the actual composition of the cocktail of ROS and maximum simultaneous production of O and OH is accomplished when [H₂O]/[O₂]-1. Neutral and charged ROS are expected to interact with biological samples by reacting directly with outermost layer of biomolecules and by triggering a secondary chemistry that will transport the effects of the plasma treatment beyond the gas/sample interface. To enable the direct effect of plasma deeper inside the biological sample, a means of encapsulating ROS seem to be required.

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