Characterisation of plasma sources for biomedical applications

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Nowadays, non-equilibrium non-thermal atmospheric pressure plasmas are of great interest for several fields, especially for biomedical applications. A floating electrode dielectric barrier discharge device and a plasma gun have been developed and have shown to induce antitumor effect in vitro and in vivo.

Characterization of the two plasma sources had been achieved using UV-visible and FT-IR spectrographs. Air plasma is known to be an intense source of reactive species especially reactive oxygen species and reactive nitrogen species such as NOx, N₂O, OH, N₂⁺, nitric and nitrous acids. Evolution of their signals was followed by optical emission spectroscopy and Infrared absorption spectroscopy. Production of reactive species have been analysed parametrically and the study highlights the significant role of buffer gas and mixtures, flow rate and frequencies of the source. High resolution spectroscopy enabled obtaining rotational structures of nitrogen 2nd positive N₂ (C^3Π_u-B^3Π_g) bands which, compared to synthetic spectra, allowed the determination of a temperature around 330 ± 10K for the plasma gun working with Helium which is a little higher than skin felt temperature.

Several setup geometries and parameters were tested for in vitro and in vivo treatment. All these measurements allowed us to adapt experimental set-up to better treatment conditions.

Keywords: FTIR, OES, non thermal plasma, biomedical application

1. Introduction

In recent years the development of new sources of non-thermal plasmas (NTP) at atmospheric pressure has been a topical issue with the goal of providing more convenient, selective and specific devices for a large range of application. For biomedical applications especially, the sources should be adapted to the target. Thus a floating electrode dielectric barrier discharge (FE-DBD) first [1] and then a plasma gun [2] have been developed at GREMI. Both have shown to induce antitumor effect [3]. Plasma production of reactive species, ions and UVC radiation can induce pH modifications, burns and moreover chain reaction of biological processes. Several studies enlighten the crucial role of reactive oxygen (ROS) and reactive nitrogen (RNS) species during plasma-biological tissues and/or cells interactions controlled by ROS scavenger where the plasma effect was inhibited and by treating the media before the cell culture, so called indirect treatment. The latest may be as efficient as direct treatment for in vitro studies. This study reports on spectroscopic measurements of chemical species present in plasma for the two sources as a function of gas used and frequencies applied. Simulation of the gas temperature is confirming very moderate temperatures which are still a little bit above skin felt temperature.

2. Materials and methods

A floating electrode dielectric barrier discharge (FE-DBD) device [1] and a plasma gun [2] have been developed at GREMI and will be briefly described in the following paragraph.

FE-DBD is powered by a 25 kV generator delivering µs duration pulses in the kHz range (Figure 1). Air NTP is produced with FE-DBD when the reactor is positioned at 1 to 5 mm from the floating electrode, which can be a living tissue, Petri dish or cell culture well. Discharge in the plasma gun is ignited in the DBD reactor and
propagates in a dielectric tube of diverse forms which can reach few meters long. Gas flow which can be down to the order of tens of sccm are Helium, Neon, Argon or mixture of gases. In this study only plasmas with Neon, standard Helium and their mixture with dry air were studied with gas flow of hundreds of sccm.

Figure 1: Scheme and pictures of the plasma sources
A) DBD B) Plasma gun

Spectroscopic measurements were performed with VM504 Acton research 300gr/mm and 30cm focal length for qualitative estimation of chemical species and VM505 Acton research 3600gr/mm and 50 cm focal length for spectrally resolved spectra acquisition and temperature determination. Spectrometers were coupled with Princeton ICCD camera, 512 times 512 pixels. Sources were positioned similarly in regard to the optical spectroscopic instruments focused on the interface between the reactor for DBD, or end of the capillary for the plasma gun and potato on a transversal view. This setup was kept constant during all the measurements and enables a comparison between signal intensities.

A Fourier Transform InfraRed spectrometer FTIR (Magna-IR 550 series II) from Nicolet with a diagnostic range from 500 to 4000 cm⁻¹ and a resolution of 1 cm⁻¹ enabled long life time species measurements, complementary to UV-visible spectroscopy.

All the spectroscopic measurements were performed on a potato to mimic dielectric constant and shape of mice and produce a similar electric potential coupling. As a matter of fact the discharge is influenced by the material serving as floating electrode especially for the DBD source. Depending on the dielectric constant and nature of the target, the plasma can be more or less diffuse or constituted by streamers. This appears to be less critical in the case of plasma gun. Moreover the gap between the reactor and the target is critical for air species production and spatial distribution which leads to plasma action on the surface.

3. Results and discussion
3.1 Temperature

In non-equilibrium atmospheric plasmas, the gas temperature can be estimated by measuring rotational temperature. Rotational temperature was investigated by mean of optical spectroscopy focusing on rotational structures of nitrogen 2nd positive \( \text{N}_2 \left( \text{C}^3\Pi_u - \text{B}^3\Pi_g \right) \) bands and simulation of resultant spectra. Preliminary simulations had been performed using freewares such as Specair [4] and Spectral analyzer [5]. Good agreements were obtained with uncertainties on temperature around 50K.

Simulation presented (figure 2) has been performed by Dr. Nader Sadeghi and is described elsewhere [6]. A moderate temperature is extracted from the DBD source spectrum, 370 ±15 K whereas the plasma gun spectrum in Helium corresponds to 330 ±10K. Differences can be extracted from the comparison of these spectra (zoom of figure 2). Between 334.4 and 335.75nm, higher temperature of the DBD spectrum expresses fine rotational structure with greater intensity than for the plasma gun spectrum. This feature is due to the population of rotational levels with high J (rotational quantum number) which express a higher temperature.

By comparing the results, gas temperature of plasma gun working with Neon is estimated to be 350 ±30K. One can justify this difference of temperature between Helium plasma and DBD by the nature of the discharge it-self. In the DBD source, plasma is randomly distributed by streamers with a high temperature and diffuse plasma which is colder. OES makes an average of the two which leads of temperature higher than felt
on the skin. Such feature does not account with the plasma gun.

3.2 Influence of the parameters characterized by optical emission spectroscopy

Parameters such as gas nature and mixtures, flow rate and frequencies of the source modify reactive species production. Main differences are seen when changing the nature of the buffer gas, due to the reaction kinetics induced by the excited and ionic species, mainly metastables atoms and molecular ions. In both cases the main signal observed is coming from molecular nitrogen, $\text{N}_2^*$. Relative spectral intensities of the two plasma sources (figure 3) and with two gases, Helium and Neon shows that Helium plasma is producing more $\text{N}_2^*$ and NO* whereas Neon plasma produces more OH*. On the one hand, Ne ($^3\text{P}_2$) is mostly transferring on $\text{N}_2^*$ then on OH*. On other hand, excitation and charge transfer from He ($^2\text{S}$) and He$_2^+$ enhanced by impurities of the buffer gas, allow expression of $\text{N}_2^*+(\text{B}^2\Sigma^+u)$ bands and NO*.

**Figure 3**: Emission spectra of the DBD (Black), of plasma gun with Neon (red) and Helium (Blue).

Frequencies 10Hz, 50Hz, 100Hz and 200 Hz, influence not only the production of chemical species but also their proportion (Figure 4) thus resulting in changes of reaction kinetics chain. Reported measurements show the evolution of excited species in the observed volume versus frequency. Signals result from an average on 10000 discharges pulses with ICCD gate apertures of 50μs. With increasing frequencies, NO* increases when OH*, $\text{N}_2^*$ and $\text{N}_2^*$ stay quite stable in both buffer gases. The fact that NO* increases with frequency is due to NO lifetime much longer than any other present species such as OH* around 100μs [7]. At high discharge frequencies, there is possibility of NO accumulation in the plasma volume between two pulses. The ratio of signal 200Hz/10Hz is four times more in Helium than in Neon plasma. Impurities in Helium gas and hydrodynamic of the mixing with ambient air may in part explain the reasons of differences appearing between Helium and Neon plasmas.

![Figure 4: Evolution of the species OES signal intensity in function of frequency applied in Helium plasma.](image)

Short time kinetic leading to reactive species production in the reaction volume such as $\text{N}_2^*$, $\text{N}_2^*$, NO*, NO*, H$_\alpha$ and O* were followed by UV and UV-Visible spectroscopy. These species are producing chemicals such as $\text{N}_2\text{O}$, NO and HNO$_2$ which were observed by FTIR monitoring long time kinetic.

3.3 Fourier Transform Infrared spectroscopy

Fourier Transform Infrared spectroscopy has been performed by connecting the end of the dielectric tube of the plasma gun to a 1l container, 10m long path, spectroscopic instrument cell. Gas going through the plasma gun, 0.3l/min, is temporally analysed by scanning the tank each 5 min. RNS as well as nitrogen’s acids, CO$_2$ and CO have been observed (figure 5). Intensity of FTIR absorption depends on each species and it especially sensitive to CO$_2$ and CO. Among other hypothesis for the presence of CO$_2$ and CO such as impurities in the setup, the ablation of gas transport tube was of major concern for medical application. Usually the input gas tube used in our experiments is made of polyamide C$_n$O$_m$N$_x$ which could be a source of carbon. To figure out if the carbon seen in CO$_2$ and CO was coming from ablation in the plasma discharge region, the polyamide tube was replaced by PTFE, Fluor-based tube and by glass. Fluor compounds spectra fingerprints have not been observed neither the bands disappeared when using the glass tube.

CO$_2$ and CO are not frequency and gas composition dependent unlike RNS. In fact, good spectral resolution enabled the distinction between
rotational structures of CO (2040 to 2210 cm\(^{-1}\)) and N\(_2\)O (2155 to 2260 cm\(^{-1}\)). N\(_2\)O increased with frequency while the area under the CO signal stayed constant and CO\(_2\) was evolving randomly.

Product species dependence had been studied by ignited the discharge in Helium, 300 sccm, Ne 180 sccm or in a gas mixture of He-air and Ne-air. Measurements were performed in different buffer gas and proportion of N\(_2\)/O\(_2\) (80%/20%) admixture at 3%, 5% and 8%. 8% of N\(_2\)/O\(_2\) admixture was the maximum percentage used due to the overlap of the water and the reactive species absorptions between 1300 and 1900 cm\(^{-1}\).

Figure 5: FTIR spectrum of Helium plasma gun with 3% of N\(_2\)/O\(_2\) admixture and NOx production by Helium plasma versus frequency, with (5%) and without N\(_2\)/O\(_2\) admixture.

In standard He, few ppm of N\(_2\) and O\(_2\) are already present in the gas thus N\(_2\)O, NO, NO\(_2\) are created in small concentration even without air admixture by opposition to spectra acquire with Neon where nothing else than CO could be seen. When air was added to the noble gases, production of reactive species was enhanced proportionally until saturation. N\(_2\)O appeared first and its saturation was quickly achieved. Then NO\(_2\), NO, nitrous (HNO\(_2\)) and nitric (HNO\(_3\)) acids were formed. HNO\(_2\) (cis and trans ro-vibrational bands) was detectable only with Helium and HNO\(_3\) with Neon. In Neon, the formation of HNO\(_3\) seems to consume NO which is progressively decreasing whereas in Helium, HNO\(_3\) and NO were simultaneously increasing. For 8% N\(_2\)/O\(_2\) admixture in Neon; traces of ozone, centred on 1040 cm\(^{-1}\), were detected.

Evolution of the NOx in the Helium plasma was followed at different frequencies 40, 80 and 160 Hz (Figure 5). N\(_2\)O and NO\(_3\) increased almost linearly with the frequency while NO seems to reach a maximum and then decreases at higher frequency. When N\(_2\)/O\(_2\) mixture is added to Helium and saturation reached, NO\(_3\) is multiplied by a factor of 3, N\(_2\)O increases slightly whereas NO dropped down completely because NO is one of the major reactant to form HNO\(_3\) and NO\(_2\).

4. Conclusion
Spectrally resolved spectra have been simulated enabling a temperature determination of 370 ± 15K in the DBD case, 350 ± 30K and 330 ± 10K for plasma gun working with Neon and Helium respectively. Excitation and charge transfers on N\(_2\)+* and NO* are more efficient in case of plasma gun working with Helium than with Neon. Moreover, considering gases with 5% N\(_2\)/O\(_2\) admixture, NO\(_3\) increased by a factor of 3 in Helium mixture while decreased in Neon mixture plasma. Nitrogen-based acids which are of main interest for medical applications [8], are observed with more or less equivalent proportions in FTIR spectra in case of Neon (HNO\(_3\)) or Helium (HNO\(_2\)) plasmas. Parametric studies performed allowed adapting the sources setup and parameters to the biological target.

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References