Biomedical applications and diagnostics of atmospheric pressure plasma

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Abstract. Numerous applications of non-equilibrium (cold, low temperature) plasmas require those plasmas to operate at atmospheric pressure. Achieving non-equilibrium at atmospheric pressure is difficult since the ionization growth is very fast at such a high pressure. High degree of ionization on the other hand enables transfer of energy between electrons and ions and further heating of the background neutral gas through collisions between ions and neutrals. Thus, all schemes to produce non-equilibrium plasmas revolve around some form of control of ionization growth. Diagnostics of atmospheric pressure plasmas is difficult and some of the techniques cannot be employed at all. The difficulties stem mostly from the small size. Optical emission spectroscopy and laser absorption spectroscopy require very high resolution in order to resolve the anatomy of the discharges. Mass analysis is not normally applicable for atmospheric pressure plasmas, but recently systems with triple differential pumping have been developed that allow analysis of plasma chemistry at atmospheric pressures which is essential for numerous applications. Application of such systems is, however, not free from problems. Applications in biomedicine require minimum heating of the ambient air. The gas temperature should not exceed 40 °C to avoid thermal damage to the living tissues. Thus, plasmas should operate at very low powers and power control is essential. We developed unique derivative probes that allow control of power well below 1 W and studied four different sources, including dielectric barrier discharges, plasma needle, atmospheric pressure jet and micro atmospheric pressure jet. The jet operates in plasma bullet regime if proper conditions are met. Finally, we cover results on treatment of bacteria and human cells as well as treatment of plants by plasmas. Localized delivery of active species by plasmas may lead to a number of medical procedures that may also involve removal of bacteria, fungi and spores.

1. Introduction
The choice of the plasma system used for treatment is usually guided by the kind of samples that are treated and the effect these plasmas are intended to have on the samples. The desire to use plasma for in-vivo treatments have made it necessary that several requirements for plasma sources be met. Necessarily, plasmas have to operate at atmospheric pressure if they are to be used for medical treatment of living organisms. At the same time, one needs non-equilibrium plasmas in order to achieve separation of electrons on the one side, and ions and neutrals, on the other. It is an advantage that no expensive vacuum systems are needed, while, on the other hand, it is much more difficult to achieve non-equilibrium (non-thermal) mode of operation at higher pressures.

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The sensitivity to heat of biomedical samples narrows the choice of non-thermal plasmas. There are many types of plasmas that can be generated under ambient pressure and temperature conditions suitable for treatment of sensitive samples [1, 2, 3]. The motivation is to develop new medical techniques, as plasma offers some possibilities for inducing desired processes with minimum damage to the living tissue [1, 2, 3, 4]. While the first results seemed quite impressive, including effects on tumor cells and even active tumors [5], tooth decay treatment and tooth cleaning [6], wound healing [5], treatment of fungi and spores and even treatment of ulcers and blood vessels, one can still not rule out negative effects that have not been studied over a sufficiently long time scale. Th preliminary results, however, show a large degree of selectivity.

Some of the well-known small-size atmospheric-pressure plasma sources are: plasma needle [7, 4], µAPPJ [8], plasma bullet [9], plasma torch [10] and floating electrode dielectric barrier discharge plasma [11]. Their electrode configuration, voltages and excitation frequencies are very different; some of them work at microwave frequencies, some at 13.56 MHz and others at 5-120 kHz in sine or pulse regime. Yet, all is not understood about their physics and while models are being developed mainly based on low pressure plasmas, the reliable experimental data are limited due to the limited availability of diagnostic techniques that are suited for such plasmas.

Here we will present several diagnostics techniques suited for atmospheric pressure plasmas and the operation of several different plasma systems working at atmospheric pressure. We will also summarize our results in treating living organisms and give examples of results mainly on sterilization of bacteria and biofilms.

2. Atmospheric pressure discharges – different experimental set-ups

Achieving non-equilibrium at atmospheric pressure is difficult since the ionization growth is very fast at such a high pressure. The high degree of ionization on the other hand enables transfer of energy between electrons and ions through Coulomb collisions. Furthermore, heating of the background neutral gas is achieved through collisions between ions and neutrals. Thus, all schemes to produce non-equilibrium plasmas revolve around some form of control of the ionization growth. It can be achieved either by an inhomogeneous field as in corona or by employment of a dielectric barrier which turns the field off after a space charge is deposited on the dielectric. Ionization growth limiting may also be achieved by a time-varying field. Another approach is to operate at the pd value corresponding to the Paschen minimum, i.e. at microscopic dimensions and high pressures. In that case, the breakdown voltage is below the threshold for streamer development and thus a glow discharge may be achieved. If the electronegative nature of the gas is increasing the breakdown and operating voltages, one may mix in an inert gas. The discharge is thus initiated in the inert gas and then the atmospheric gas is mixed to produce chemically active radicals. In most cases, however, atmospheric pressure plasmas have small dimensions making it very difficult to perform standard diagnostics.

Some of the plasma devices designed for in-vivo treatments are the µ-APPJ and the plasma needle, which operate at 13.56 MHz at atmospheric pressure. A micro-atmospheric plasma jet was developed by Schultz van der Gathen and coworkers [12]; this plasma source is interesting both for applications as well as for the study of its basic properties.

The micro-atmospheric pressure plasma jet [µ-APPJ] consists of two symmetrical electrodes of equal length (34 mm) made of stainless steel. The distance between the electrodes can be adjusted with good precision from a few mm up to several hundred micrometers. In all our experiments, the distance between the powered and the grounded electrode was 1 mm. One of the electrodes was powered by a signal generator at 13.56 MHz while the other electrode was grounded. The measurements were made at powers of 40-80 W fed by a RF power supply.

Plasma is ignited along the entire length of the electrodes (figure 1); for certain combinations of power/gas-flow parameters, effluent of plasma coming out of the cuvette can be formed. The main advantage of this design is that both the discharge volume (plasma core) and effluent region are accessible for diagnostics, such as optical emission spectroscopy (OES) and two-photon absorption...
laser-induced fluorescence (TALIF) [8]. Also, the plane parallel geometry of the electrodes adds to the simplicity when it comes to modeling this type of discharge.

Another plasma source that meets all the necessary conditions for treatment of organic materials and living tissues is the plasma needle (figure 1, picture on the right-hand side). Most importantly, in such a discharge gas the heating is minimized, while the effects on the tissue and bacteria have been clearly shown to be significant. The needle consists of a central electrode made of tungsten insulated almost to the tip by a slightly larger ceramic tube, both being placed inside a glass tube. The needle body is made of Teflon. We used helium as a buffer gas at several different flow rates. The central electrode is powered by a 13.56 MHz signal generator through an amplifier and a matching network. Both for the plasma needle and for the μ-APPJ, we have derivative probes and a Hiden HPR60 mass/energy analyzer in order to determine the power applied to plasma and the composition of the discharge, respectively. In both of these systems, inert gas is used to reduce the breakdown voltage and achieve stable non-equilibrium plasma formation. Yet these plasmas have shown several modes of operation and further studies are required to fully understand their operation and make further optimizations.

Another type of atmospheric pressure plasma relies on mixing the inert gas carrying the plasma created by external electrodes with atmospheric gas mixture. It is the so-called plasma jet. The operating frequencies of plasma jets are in the region of several tens of kHz which are much lower frequencies than those used for the plasma needle and μ-APPJ (in MHz). It has been shown that micro jet plasma is not always continuous but often is formed by a train of fast travelling bullets which only appear to be continuous to the human eye. The atmospheric pressure plasma jet/bullet that we constructed was made of a Pyrex glass tube with the electrodes made of a thin copper foil wrapped around the glass tube. The distance between the powered and the grounded electrode was 13.5 mm and their width was 13 mm. One of the electrodes (the left electrode, see figure 2) was grounded. The other electrode, closer to the end of the glass tube, was the powered one (see figure 2). In all experiments the buffer gas was helium. We used a signal generator connected to the custom-made amplifier to power the micro jet. The highest voltages that we could obtain from the amplifier were up to 1 kV, which was not enough to ignite the plasma. In order to increase the applied voltages to more than 5-6 kV, we had to use an additional homemade transformer. The operating frequency was 80 kHz and the applied voltage was sinusoidal in the range of 6-10 kV peak-to-peak. Micro jets with plasma bullets have been constructed with a range of different

![Figure 1. μ-APPJ with formed plasma and plasma needle system set-up.](image1)

![Figure 2. Atmospheric plasma bullet.](image2)
geometries, frequencies and shapes of applied voltage. The effect of bullets seems to be quite universal for an optimum range of electrode sizes and flows, while the frequencies and voltage shapes vary a lot. Plasma bullets, however, still need to be fully understood and properly modelled. We will focus here on the plasma jet diagnostics, while at the same time showing some results on the sterilization achieved by a plasma needle.

3. Plasma diagnostics

It has been reported only quite recently that the plasma jets formed by the source operating at low excitation frequency is not continuous. Instead it consists of small plasma packages that are formed in positive and/or negative half cycle of the period [13]. Amazingly these little bullets are formed and travel outside the plasma jet where there is no applied electric field. The velocity of these packages are larger than the speed of the flowing feed gas by several orders of magnitude. Several theories of bullet formation have been proposed [13, 14, 15, 16, 17, 18] but to date a definite explanation of the physical mechanisms involved in creation and propagation of plasma bullet are still not fully understood.

For the current and voltage measurements, we used two commercial probes. The current and voltage waveforms when the plasma is formed and without discharge are shown in figure 3. When the plasma is off, the phase difference between the current and voltage is close to 90°. In this case, we have a capacitive impedance of several MΩ, corresponding to a capacitance of about 0.5 pF. On the other hand, when the plasma is formed, the current signal is larger, deformed and shifted in phase overlapping more with the voltage signal. The plasma ignition changes the slopes of the $V_{\text{RMS}}$–$I_{\text{RMS}}$ curves (see figure 4). The mean power calculated increases with the increase of the applied voltage; it was in the range from 1 to 8 W in all measurements.

Integral and time-resolved images of the plasma jet system were obtained by an ICCD camera. For exposure times larger than the cycle period (12.5 µs), the plasma appears to be continuous, like a plume (see figure 1 LHS picture). The length of the plasma plume is up to five centimeters, depending of the flow rate and the voltage applied. For the time-resolved images, we had to use integration on the chip because the light emission in a single shot is not sufficient to obtain clear images with gate widths less than 50 ns. This was facilitated by the high reproducibility of the pulses and the small jitter. Figure 5 shows the plasma bullet images obtained for several different flows of working gas. We can see that with the decrease in the He flow, the plasma bullet starts to be elongated, deformed and its intensity is much smaller. Eventually, bullets are not formed at the very small flows.

![Figure 3. Current and voltage waveforms for helium flow rate of 3 slm. The dashed lines represent the case when discharge is OFF, the solid lines, when discharge is ignited [19].](image)

![Figure 4. Current-voltage characteristics for three different flows of helium [19].](image)
Figure 5. Plasma jet for 1, 2, 3, 4 and 5 slm of He flow. Exposure time 2 ms, gate width 25 ns, gate delay 10.8 $\mu$s [19].

Figure 6 shows the development of the plasma over the entire period of applied voltage (12.5 $\mu$s). All images are scaled to the same maximum intensity and thus can be compared. One can see that when the current and voltage signals are close to zero, the plasma is not visible. In the negative part of the current and voltage waveforms, the plasma is confined between the electrodes. During the positive part of the waveforms, the plasma is first confined between the electrodes (rising slope) and then, near the maximum of the curves, it leaves the glass tube in the form of a bullet. The dimensions of the bullet are of the order of a few millimeters. We calculated the speed of the bullet at ~20 km/s, depending on the position away from the end of the glass tube. The plasma bullet is much faster than the speed of the buffer gas flow (1 to 7 m/s). Thus we can conclude that our plasma source was not continuous, it consisted of very small plasma packages that traveled at a high speed. By varying the plasma parameters, the length and intensity of the plasma coming out of the tube can be adjusted.

For the two plasma devices operating at a much
higher frequency (13.56 MHz), the diagnostics was made by using homemade derivative probes in order to determine the power transmitted to the plasma and the operation mode of the discharge. The derivative probes were very sensitive; a numerical procedure for subtracting the displacement current based on accurate calibration of the system was performed so that it was possible to measure powers of the order of 0.1 W or less even with displacement current a couple of orders of magnitude larger than the plasma current.

Besides derivative probes, we used mass spectrometry to analyze the plasma products formed by a \( \mu \)-APPJ [19] and by a plasma needle [20]. Several problems occurred during the setting up of the experiment; they are described in detail elsewhere [19, 20].

The analysis of the composition of neutrals and ions was motivated by the need to check which species are formed in the discharge. These results may be used as a test of plasma chemical models, to identify radicals and ions (that may be used after acceleration to induce damage to the tissue). The performance of the mass analyzer was tested and techniques were developed to produce data without the uncertainty induced by a contribution of the ionizer to possible dissociation. It was found that the predominant ions created by the plasma are \( \text{O}_2^+ \), \( \text{O}^+ \), \( \text{H}_3\text{O}^+ \), \( \text{N}_2^+ \), \( \text{N}^+ \), \( \text{NO}^+ \), \( \text{OH}^+ \) [19]. When it comes to plasma treatment of samples of biological origin, the chemically active species that are of interest are \( \text{O} \), metastables \( \text{O} \) and \( \text{O}_2 \), \( \text{OH} \), \( \text{N} \), \( \text{H}_2\text{O}_2 \) and \( \text{NO} \).

4. Plasma sterilization

The entry point for most groups dealing with plasma medicine is a study of sterilization. The effects on bacteria may be shown quickly, although it requires expertise in biomedicine. The benefit is that direct potential applications may be developed outside the realms of strict medical regulations. Yet, in situ sterilization, for example, treatment of wounds to prevent infection, would be a much more important goal. Following preliminary work on sterilization in microwave plasma, albeit at low pressure, we reinitiated the studies of plasma sterilization as a part of our plasma medical project. So far, a plasma needle has been used to induce killing of Streptococcus mutans and Escherichia coli bacteria in the form of planktonic samples. Also, we have the plasma interaction with normal, living cells; for these experiments we used human peripheral blood mesenchymal stem cells (hPB-MSC) as a model system to predict the degree of possible damage to the cell responses [21]. Many factors are responsible for bacterial inactivation. Direct exposure of the bacterial samples to the plasma appears to be more effective than remote exposure. Another factor that determines the efficiency of the specific treatment is the type [22] of bacteria, gram positive or gram negative. Very importantly, we studied the sterilization of bacteria in planktonic samples, where bacteria are dissolved in a small amount of liquid that would otherwise give it some protection from other agents. We showed that efficient sterilization of planktonic samples is not only possible, but may be efficient depending on the initial population [21].

One of the most serious problems in the hospital environment is bacterial contamination of surfaces with methicillin-resistant Staphylococcus aureus (MRSA) responsible for significant nosocomial infections. The pathogenic contaminants form biofilms, which are difficult to treat with routine biocides. The biofilm is not just a secured shelter

![Figure 7. Treatment of MRSA biofilms of Staphylococcus aureus (ATCC 25923) by using plasma needle. Untreated sample showed STRONG bacteria formation (control intensity 0.65 a.u.). Initial concentration of bacteria used was 10^6 CFU/ml.](image-url)
but a defense mechanism and a nutrition depot for pathogens. We show below the preliminary results of plasma treatment of the MRSA bacteria samples in the form of a biofilm.

In figure 7, we show the optical density of bacteria samples after plasma treatment for several different treatment times (10, 30, 60 and 120 s). The initial concentration of the samples was $10^6$ CFU/ml, which corresponds to a measured optical density of 0.65 a.u. The buffer gas flow was 0.5 slm, but studies were also made as a function of the flow rate. The treatment efficiency increases with the increase of the treatment time and the mean power deposited to the plasma. For the highest power and only for the shortest treatment time of 10 s, there was scarcely bacteria formation; for the longer treatment, no bacteria formation was observed after the plasma treatment and yet there was very little or no heating of the gas.

Conclusions
We reviewed shortly our studies of atmospheric pressure plasmas and their application in biomedicine. In particular, we covered new results obtained with a plasma jet showing formation of plasma bullets and their properties as a function of geometry of electrodes and gas flow. In addition, we showed some practical results of sterilization using a plasma needle. The treatment of biofilms is essential, as are the studies of treatment of fungi, spores, prions and viruses. At the same time, one needs to extend the studies to specific medical problems associated with treatment of living organisms, including humans. As far as plasma goes, some further optimization may be made for localized accurate treatment of cells or sterilization. With good knowledge of the power deposited into the plasma and control of the radicals that are produced, together with spatial emission profiles indicating changing of the regime of operation, a sufficient control over the reproducibility of the plasma needle operation was achieved. Other sources may be sought for more refined interaction with living cells. Different applications may seek more uniform sources extended over larger areas or even more localized treatment, which is all within the reach of the present day techniques.

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