Development, diagnostic and applications of radio-frequency plasma reactor

N. Puć
Institute of Physics, Pregrevica 118, 11080 Belgrade, Serbia
nevena@phy.bg.ac.yu

Abstract. In this paper we present volt-ampere-power characteristics of a plasma needle operating in the flow of helium at the atmospheric pressure. Also, we will present discharge images obtained by using ICCD camera. In addition, we show some examples of how such plasma affects plant tissues. In characterization of the plasma needle, current and voltage waveforms were recorded by two derivative probes. These two probes are similar to the probes previously used in measuring transmitted power in low pressure CCP rf discharge [1]. The instantaneous power was calculated from current and voltage waveforms and \( U-I \) characteristics of the discharge were determined. Regimes of operation with and without the grounding ring at the tip of the needle were considered. We have chosen two model systems to study the effect of plasma needle on plant cells and tissues: sweet fern gametophyte (prothallus) and calli produced \textit{in vitro}.

1. Introduction

Nonequilibrium plasmas proved to be able to produce chemically reactive species at a low gas temperature while maintaining highly uniform reaction rates over relatively large areas [2, 3]. The fact that vacuum systems are necessary equipment is just one part of the problem as many targets cannot be treated at all under the low pressure conditions. Treatment of organic materials and living tissues falls under that category.

Lately, non-thermal atmospheric pressure plasmas have drawn considerable attention due to their enormous potential for technological applications mostly in surface modifications especially for materials or living tissues that may not allow putting it into vacuum chambers. It was found that plasmas may be easily operated under atmospheric pressure at RF frequencies in a flow of rare gases which mix with the atmospheric gases in the region of the discharge [4]. Apart from this, many different configurations and applications of non-thermal atmospheric plasmas have been developed and studied [5].

The effects of these plasmas on living cells and tissues have been studied on numerous occasions in recent literature. Dependent on plasma conditions, several refined cell responses are induced in mammalian cells [6]. Furthermore, application of plasma treatment in dentistry and deactivation of bacteria have been intensively studied [7]. However, there are few data in the literature about plasma effects on plant cells and tissues [8]. So far, effect of low pressure plasmas on seeds was investigated. It was shown that short duration pretreatments by non equilibrium low temperature air plasma were stimulative in light induced germination of Paulownia tomentosa seeds [1, 9]. As membranes of plants have different properties to those of animals and as they show a wide range of properties, we have
tried to survey some of the effects of typical plasma which is envisaged to be used in biotechnological applications on plant cells.

In this paper we will also present properties of our plasma source that is very similar to the plasma needle as first described by Stoffels and colleagues [10]. This source operates at atmospheric pressure and meets all the necessary conditions for treatment of organic materials and living tissues. It is desired that the plasma should be non-aggressive, local, with small penetration depth and, at the same time, that it produces chemically active species at a low gas temperature. Plasma needle meets all those requirements.

For the purpose of successful treatment and comparison of different samples by plasma needle it is necessary to characterize the plasma itself the best way possible. The standard parameters for treatment of samples are duration of treatment, power transmitted to the plasma and distance of the sample to the tip of the needle. For the power measurements the derivative probes were used. The percentage of the total power and mean power distributed to plasma itself was determined from the recorded voltage and current waveforms. ICCD camera was used in order to obtain light emission from the discharge.

2. Experimental set-up
Plasma needle is atmospheric pressure plasma source powered by 13.56 MHz RF generator that operates in a mixture of air and helium. Its construction makes it very useful for treating biological samples like plant tissue, for bloodless surgery, dental cavity antibacterial treatment and many other applications. The needle consist of central tungsten wire (0.5 mm in diameter) placed into a ceramic tube with slightly larger diameter and both placed into the glass tube with 6 mm diameter (see figure 1).

![Figure 1. Schematic representation of the plasma needle setup: body of the plasma needle, stainless steel box with derivative probes, home-made voltage transformer, power divider (dummy load) and RF source with matching box.](image)

In addition to the standard configuration (see figure 1) of plasma needle we have used configuration with grounded copper ring placed at the tip of the glass tube. The central wire represents the powered electrode and the grounded electrode is the surface in the vicinity of the plasma needle tip. Helium flows between ceramic and glass tube with typical flow rate of few hundred standard cubic centimeters per minute.

The rest of the electrical circuit is the same that we have already used [8] and is shown in figure 1. Low-temperature RF discharge at 13.56 MHz is generated using a Dressler Caesar 1010 power supply, in combination with Variomatch matching network. In order to increase the peak-to-peak voltage, we have used a home-made transformer and inserted it between the plasma needle and the RF matching network. We have implemented a dummy load to make the discharge more stable and to be sure that only a small part of the power supplied by the RF power supply is transmitted to the discharge. In this configuration the dummy load serves as a ‘power divider’.
Instantaneous voltage and current are monitored using two derivative probes developed previously [1, 8] and they are somewhat different from the probes already proposed in the literature. Both probes were placed inside a stainless steel box opposite to each other. The box was placed as close as possible to the plasma needle. The output of the probes was connected to a digital oscilloscope (Tektronix TDS220) with the cables of equal length. All waveforms were collected by the computer for further manipulation.

In order to record emission intensity of the discharge we have used ICCD camera Andor iStar DH720-18U-0. Camera was positioned orthogonally to the needle tip so we could obtain side-on images of plasma. Images were obtained in integrated mode for exposure times of 5 ms and 100 ms. In case of ICCD and U-I diagnostics target was rubber box. Distances between tip of the plasma needle and target were 1 mm.

2.1. Plant material and methods
Plasma treatment of plant tissues is demonstrated on two months old prothalli of Polypodium vulgare L. (fam. Polypodiaceae). The mature fern gametophyte (prothallium) is a multicellular, autotrophic yet small (1-2 mm) structure. As an experimental organism, the fern gametophyte is ideal for a study given that it can be cultured to maturity in a Petri dish and all aspects of its growth and development may be observed and manipulated in a nondestructive way [11].

The plant samples were directly exposed to the plasma needle. The duration of treatment and power of the plasma varied from 15 s to 2 min and 0.01 W to 0.25 W, respectively (of the power supplied to the plasma). Just after plasma treatment, plants were submerged in 1 ml of 0.1% aqueous Evans blue for 1 hour. Evans blue stain was used for microscopic determination of cell death. This led to nonpermeating or exclusion dye leak through ruptured membranes and stained the content of the dead cells [12]. Stained plants were observed using light microscopy.

In order to determine the plasma influence on the fresh weight of the plant tissue, we have used parts of the calli Fritillaria imperialis (fam. Lilliaceae) (about 5 mm diameter in size). It was grown on Murashige and Skoog (MS)-solid medium [13]. Callus is a distinctively organized mass of proliferating cells, with specific morphology and anatomy, and may be obtained from almost any type of plant.

3. Results and discussion

3.1. Electrical measurements
Plasma was generated for a range of powers of the RF power supply from 30W to 100W. Derivative probes were used to measure current and voltage waveforms. After collecting, current and voltage signals were transferred from time domain to frequency domain by using Fast Fourier Transform. While in frequency domain signals were adjusted according to the calibration curves for both probes. Calibration of the probes gives their sensitivity and has been determined by using a network analyzer.

Transition to the frequency domain is performed for several reasons. The first one is that in the frequency domain we can clearly see from the harmonics some basic properties of the discharge. The second reason is that all calculations could be performed more efficiently in the frequency domain. It is important to note that only a single harmonic was found to be significant for both configurations (with and without grounded copper ring) which give an indication of the symmetry of the plasma and its regime of operation.

Current and voltage waveforms were, after adjusting according to the calibration curves, transferred back to the time domain by using an Inverse Fast Fourier Transform. Current peak-to-peak values lie between 0.5-1.2 A and for voltage these values are between 300-600 V (see figure 2). Above the breakdown, the voltage changed very little with increasing the power given by the generator, but the current (and consequently the power consumed by the plasma discharge) changed almost linearly.

One should note that here we have not subtracted the reactive component of the current. The current observed in figure 2 is mainly the reactive component due to the capacitance of the cable.
between the probes and the tip of the needle (the distance is 30 cm). The reactive component is much
greater than the active component (the capacitance of 18 pF corresponds to an impedance of about
670 Ω versus the impedance of the plasma of about 10 kΩ). We have not subtracted the reactive
component of the current (although it is doable) because its influence is cancelled out in determination
of the integral of the power.

After returning voltage and current signals to the time domain we were able to obtain instantaneous
power (see figure 3). Mean values of power were also calculated by using 6T (T=7.375×10^8 s) and
they are shown in figure 4. By using a ‘power divider’ we have insured that most of the power feed
goes to the dummy load. Less than 10% of power given by RF power supply went to the plasma
needle branch and only less than 1% into the discharge. We can see that more power is transmitted to
the plasma needle in the case when we added grounded ring at the tip and, also, discharge could be
ignited for lower power given by RF power supply.

![Figure 2. Current and voltage waveforms after processing with FFT procedure and corrected
according to the calibration of the probes. Note that the reactive current has not been subtracted from
the current waveforms [8].](image)
In order to treat sensitive samples of biological origin power transmitted to the discharge should be less than 1 W and both configurations, with and without grounded ring, which we have used satisfy this condition. In all our treatments we have used configuration without grounded ring and power transmitted to the discharge did not exceed 0.5 W.

3.2. Light emission
Plasma needle can operate in two different modes [14, 15]. This was also confirmed in simulations done by Sakiyama and coworkers [16, 17]. In both cases plasma appears as a glow on the tip of the needle. It can work in unipolar mode, when there is no surface in the vicinity of the tip of the needle and discharge is localized at the tip of the needle. If it is brought in the vicinity of surface of the treated sample, plasma switches to a bipolar mode, where the glow spreads over the object's surface.

We have used ICCD camera in order to record integrated light emission from the discharge. In figure 5 light emissions of the discharge for the different powers given by RF power supply are shown. Exposure time was 5 ms, flow of helium was 110 sccm and configuration with the grounded ring was used. Plasma is ignited for the power of 60 W given by RF power supply and intensity of the discharge rises with the increase of the power. When we increase power above 80 W we can see significant increase of the light emission. This increase of the light emission is in correspondence with the increase of the power transmitted to the plasma needle (see figure 5, with grounded ring). Also, sheath is formed at the treated surface and it can be observed with bare eye.

We can see from figure 5 that formed discharge is not completely symmetrical and it has shape of the bean. This indicates that tip of the needle is not completely flat and that even that smallest irregularity of the needle surface influences the shape of the discharge.
4. Plasma treatment of plant tissues

We have chosen two model systems to study the effect of plasma needle on plant cells and tissues: sweet fern gametophyte (prothallus) and calli produced in vitro. These systems are chosen because their characteristics are determined in great detail. Both systems were treated with plasma needle for different powers (less than 1 W), different treatment times (15 to 120 s) and flow of working gas of 110 sccm. Flow of working gas was kept constant for all treatments.

We have demonstrated that cell death of the Polypodium prothallium occurred after a high dose of plasma (t=120 s). The untreated control plant is shown in figure 6 along with negative control plants treated with absolute ethyl alcohol for 12 h (inserts (a) and (b)). One should be careful when choosing right conditions for treatment of sensitive samples. In figure 7 samples treated for two different treatment times (P_r=80 W) are shown. One can observe that smaller sample, in not so advanced growth stadium, was completely destroyed while the bigger sample is only partially damaged.

Figure 5. Discharge emission obtained by ICCD camera. Exposure time was 5 ms and distance between needle tip and target was 1 mm.

Figure 6. Untreated (control) plant. Inserts (a, b): negative control plants treated with absolute ethyl alcohol for 12 hours. [8]

Figure 7. Plasma treatment of sweet fern gametophyte. Smaller picture: one month old gametophyte.
When sweet fern prothaliai were exposed over shorter periods to the plasma needle, with appropriate power, cell injury and necrosis were minimized and plants continued their growth. Detailed cytological and histological analysis of the referred gametophyte could elucidate the delicate alteration (if any) induced by the plasma treatment. Again, in order to determine the plasma influence on the fresh weight of the plant tissue, the plant samples were directly exposed to plasma. The time and power of the plasma varied from 15 s to 2 min and 0.1 W to 0.2 W, respectively (of the power supplied to the plasma). After the plasma treatment, calli were transferred to solid medium and fresh weight of the samples was measured every 7 days.

![Graph showing fresh weight increase](image)

**Figure 8.** Increase of the fresh weight (FW) of *Fritillaria imperialis* calli as a function of plasma treatment. The calli (about 5 mm diameter in size) were exposed to plasma for the indicated time, and then transferred to the fresh medium. Fresh weight increase was recorded for three weeks and is given by \((\text{FW}_t - \text{FW}_0) / \text{FW}_0\) ratio. \(\text{FW}_t\) – fresh weight measured at the indicated time (x-axes); \(\text{FW}_0\) – initial fresh weight. [8]

Plasma needle treatment causes an increase in the fresh weight of compact calli (figure 8). Moreover, values of the measured parameter were doubled for all treated calli compared with the untreated sample, even for longer exposure times. Increase in the fresh weight is an obvious implication of calli growth—an irreversible increase in size, accomplished by a combination of cell division and cell enlargement.

### 5. Conclusion

We have developed a plasma needle similar to that of Stoffels and coworkers [6, 10]. The power supply in our case is somewhat different, with a transformer and a dummy load. In order to obtain voltage-current-power characteristics we have added voltage and current probes. Typically we have achieved a transfer of power below 1 W to the plasma needle which is the condition to reduce thermal transfer to the surrounding gas and target living tissue. We have analyzed operation of plasma needle for two different configurations, with and without grounded copper ring placed at the tip of the needle. It seems that in case with additional ring more power is transmitted to the discharge and discharge is more stable, but for the effect of the treatment the proximity of the target surface will remain as a critical parameter. Apart from derivative probes we have used ICCD camera in order to obtain light emission of the discharge created at the tip of the needle.

Concerning plasma treatment of the plant tissues, we have analyzed interaction of the plasma needle with gametophytes and calli as representatives of small multicellular plant organisms that may be grown and analyzed in a controlled fashion. We have demonstrated that cell death (necrosis) of the
Polypodium prothalium occurred after high doses (power-treatment time) of plasma. As for calli, it was shown that plasma treatment induces the weight gain for monocotyledonous. Further histological and cytological analysis is required to elucidate the mechanisms of interaction, but one may expect that the effects are triggered by abundant radicals and reactive molecules produced by the plasma needle [14].

Acknowledgments
This work was performed as a part of requirement for a PhD thesis which was completed under supervision of Dr. Zoran Lj. Petrović. Also I would like to express gratitude to my co-supervisor Dr. Gordana Malović; Dr. Antonije Đorđević, Saša Lazović and colleagues from Biological institute “Siniša Stanković”. This research has been supported by the Ministry of Science Serbia under the contract number 141025 of the project Physics Fundamentals of Applications of Non-Equilibrium Plasmas in Nanotechnologies and Treatment of Materials.

References
[1] Puač N, Petrović Z LJ, Živković S, Giba Z, Grubišić D and Đorđević A 2005 Plasma Processes and Polymers ed. R d’Agostino, P Favia, C Oehr, M R Wertheimer (Wiley-VCH:Berlin) p193
[2] Makabe T and Petrović Z LJ 2006 Plasma Electronics (Taylor and Francis:New York); Lieberman M A and Lichtenberg A J 2005 Principles of Plasma Discharge and Materials Processing, (Wiley:Hoboken);
[3] Roth J R 1995 Industrial Plasma Engineering (Institute of Physics:Bristol, UK); Kelly-Wintenberg K, Hodge A, Montie T C, Deleanu L, Sherman D, Roth J R, Tsai P and Wadsworth L 1999 J. Vac. Sci. Technol. A 17 p 1539
[4] Park J, Henins I, Herrmann H W, Selwyn G S and Hicks RF 2001 J. Appl. Phys. 89 p 15
[5] Kunhert E E 2000 IEEE Trans. Plasma Sci. 28 p 189
[6] Stoffels E, Kieft I E, Broers J L V, Ramaekers F C S and Slaaf D W 2003 Proc. ISPC 16 International Symposium on Plasma Chemistry, 22-27 jun 2003, Taormina, Book of Abstract, Eds. R. d’Agostino, P. Favia, F. Fracassi and F. Palumbo, CD [ISPC-884]
[7] Sladek R E J and Stoffels E 2005 J. Phys. D: Appl. Phys., 38, 1716
[8] Puač N, Petrović Z LJ, Malović G, Đorđević A, Živković S, Giba Z and Grubišić D 2006 J. Phys. D: Appl. Phys. 39 p 3514
[9] Živković S, Puač N, Giba Z, Grubišić D and Petrović Z LJ 2004 Seed Science and Technology 32 p 693
[10] Stoffels E, Flikweert A J, Stoffels W W and Kroesen G M W, 2002 Plasma Sources Sci. Technol. 11 p 383
[11] Banks J A 1999 Annual Review of Plant Physiology and Plant Molecular Biology 50 p 163
[12] Baker C J and Mock N M 1994 Plant Cell Tissue and Organ Culture, 39, p 7
[13] Murashige T and Skoog F 1962 Physiologia Plantarum 15 p 473
[14] Stoffels E, Aranda-Gonzalvo Y, Whitmore T D, Seymour D L and Rees J A 2006 Plasma Sources Sci. Technol. 15 p 501
[15] Stoffels E, Kieft I E, Sladek R E J, van den Bedem L J M, van der Laan E P, Steinbuch M 2006 Plasma Sources Sci. Technol. 15 p S169
[16] Sakiyama Y and Graves D B 2006 Journal of Physics D: Applied Physics 39 p 3451
[17] Sakiyama Y, Graves D B and Stoffels E 2008 Journal of Physics D: Applied Physics 41 p 095204