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Experimental Study of a DBD Plasma Jet for Rapid Healing and Sterilization of Chronic Wounds

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Abstract

Non-equilibrium plasmas can be used for rapid wound healing and sterilization. The work presented in this paper highlights the underlying processes involved in plasma and living cells interaction. A detailed summary of the findings by various researchers is included. Different types of plasma devices previously developed for this application are discussed. To optimize the process of wound healing and sterilization, plasma jet devices with the option to vary the plasma jet characteristics are required. One such design with multiple electrodes is developed and characterized. Exposure of this DBD plasma jet to the blood shows a rapid blood coagulation. This was confirmed by taking real time images of the blood surface when the blood drop was exposed to the plasma jet under a microscope. To conduct the emission spectroscopy of the plasma jet, an optical fiber-based spectrometer was used. The problems and limitations to analyze the emission spectrum are discussed.

Keywords: DBD, Non-equilibrium plasma, Wound healing and sterilization, Coagulation, spectroscopy

1. Introduction and Background

Non thermal, non-equilibrium dielectric barrier discharge plasma jets find applications both in medical and engineering related fields, Figure 1.

These applications include wound healing and sterilization [1-8], dental [9], surface decontamination [10], surface treatment [11], and surgical equipment sterilization [12]. Plasma jets generated in open air makes it easier to expose the target at any appropriate distance from the plasma generator and does not require any special enclosure. As the plasma stream is delivered to the target in a non-contact manner, the risk of tissue adhering to the plasma surgical equipment is eliminated. Almost three decades ago, researchers began to investigate the possibilities of exposing living cells to low-temperature plasmas and because of these efforts, plasma surgery has now emerged as a powerful tool to control blood coagulation and wound sterilization. Rearch papers by Llyod [13] and Fridman [14] on plasma medicine contains 108 and 141 references respectively on this topic. Another paper on air plasmas for medical applications by Kuo [15] includes 77 references on this topic. The current global wound care market reflects the commercial importance of plasma based medical treatment technology. It is anticipated that due to the diverse plasma medical applications, commercial market in other areas including dentistry and cosmetic surgery will also grow exponentially in coming years.

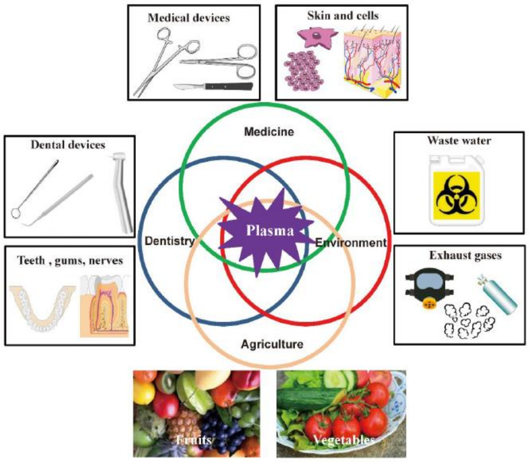

Figure 1. Plasma industrial Applications - Ref [1]

2. Plasma Interaction with Living Tissues: A complex Phenomenon

How and to what extent a low-temperature plasma plume interacts with living cells is a question that has initiated a great debate among researchers. While it has been experimentally confirmed that plasma can be tuned to achieve the desired medical effects, especially in medical sterilization and treatment of different kind of skin diseases, the mechanism that dominates the plasma interaction process is still under investigation. It has been argued that the presence of various gases along with the moisture in the air produce several chemically reactive species in the plasma plume that reacts with the target to provide the required results. Fridman and Chen's work [16, 17] shows that the plasma effluent of the plume carries an abundance of reactive atomic oxygen (RAO), which is the catalyst for plasma medical effects. As RAO reacts with H_2O in the blood, it produces H_2O_2; part of H_2O_2 is decomposed to oxygen, which dissolves into tissue to increase the oxygen tension. H_2O_2 also triggers Fibroblast Growth Factor, Platelet-Derived Growth Factor and other factors to induce reactions such as inflammation and angiogenesis. As a result, the healing process is improved, and the healing time is reduced [17]. Other hypothesis is that the radicals in the plasma support the endogenous radical-mediated defenses and healing mechanisms of tissue and drive the formation of cell mediators such as nitric oxide [18]. Laroussi et al [19] while investigating the inactivation of bacterial cells by air plasmas evaluated important inactivation factors that included UV radiation and reactive species. Their work showed that for non-equilibrium, atmospheric pressure air plasmas, it was the oxygen-based and nitrogen-based reactive species that played the most important role in the bacterial inactivation process [19]. According to Soffels et al [20,21], plasma releases controllable amounts of short-lived reactive oxygen (ROS) and nitrogen (RNS) species that address only the target areas in the tissue. Each of these species has a different physiological function: antibacterial, pro-apoptotic, and por-inflammatroy (ROS), or anti-inflammatory and pro-apoptotic (RNS). External administration of ROS or RNS by plasma locally reinforces the natural physiological processes. A recent study guide published by Plasma Surgical, Inc., highlights the use of plasma surgery on a variety of tissues to coagulate, cut and prevent fluid loss [22]. According to their study, the thin, flexible layer created by plasma surgery comprised of two distinct layers of eschar. On the surface, there was a spongy necrotic layer (SNL) which formed instantly and acted as a shield against diffusion of the thermal energy. Underneath this layer appeared a compact necrotic layer (CNL) that was much denser and more elastic. This layer was attached tightly to the underlying viable tissues. Risks of re-bleeding following sudden detachment of the eschar were minimized [22]. In another study conducted by Isbary et al [23], a use of 2 min cold atmospheric argon plasma in chronic wounds was tested on 24 patients with infected wounds. These wounds were treated in a prospective randomized controlled phase II study with 2 minutes of cold atmospheric argon plasma every day. Analysis of 70 treatments in 14 patients revealed a significant reduction (40%, $P<0.016$ with MicroPlaSter alpha device) in bacterial load in plasma-treated wounds, regardless of the species of bacteria. Analysis of 137 treatments in 10 patients showed a highly significant reduction (23.5%, $P<0.008$ with MicroPlaSter beta device) in bacterial load [23].

As the living cells/wounds are exposed to a low-temperature plasma plume, following observations have been reported.

- **a.** Floating-electrode dielectric barrier discharge plasma is shown electrically safe to human subjects. It is shown that during the exposure of plasma (on minutes time scale), no gross (visual) or histological (microscopic) damage to skin samples is observed. Complete tissue serialization from skin flora was observed in seconds after the exposure of the plasma plume. Similarly, blood clot formation was also reported in seconds of plasma treatment [16].
- **b.** Chen developed a plasma plume and conducted *in vitro* and *in vivo* blood clotting experiments using pigs as animal models [17]. Microscopy and cell count of smeared blood samples were used to explore the dependencies of erythrocyte and platelet counts on the exposure time and distance. It was reported that the degree of blood clotting increases and the platelet count reduced as a results of decreasing exposure distance or increasing the exposure time. It was observed by exposing the wound to plasma plume, the bleeding time reduced from minutes to seconds (~ from 3 min to 18 sec). Instead of continuous exposure, intermittent plasma exposure approach was found more effective on the bleeding control [17].
- **c.** The work of stoffels [20] includes the monitoring of delayed effects of cold atmospheric plasma on vascular cells. After an exposure of 6-10 hrs, a total (irreversible) cell inactivation without necrosis (cell damage) was observed in endothelial cells. The experiment brought new insights in the mechanism of cell detachment, which was ascribed to electrostatic interactions of plasma with cells. Results revealed that atmospheric plasma was capable of non-inflammatory treatment of arterial walls [20,21].
- **d.** Bactericidal effects of non-thermal argon plasma in biofilms and in the animal model of infected wounds was studied by Ermolaeva eta l [24]. It was found that Gram-negative bacteria were more susceptible to plasma treatment than Gram-positive bacteria. For the Gram-negative bacteria *Pseudomonas aeruginosa, Burkholderia cenocepacia and Escherichia coli*, there were no survivors among the initial 10^5 c.f.u after a 5 min plasma treatment. Fridman et al [25,26] also performed an investigation on comparison of direct and indirect effects of non-thermal atmospheric pressure plasma on bacteria. Their study shows a difference of a few times up to few orders of magnitude improvement in bacteria inactivation rates under direct plasma treatment as compared to a jet without plasma.
- **e.** Hoffmann et al [27] introduced the use of col-plasma coagulation (CPC) for tumor destruction at the pericardium and the diaphragm. In their work the technique was limited to superficial layers and accomplished a predictable depth and area of necrosis. CPC was done as part of a multimodal therapy in stage III mesothelioma patients. Histological examinations of pleura excisates after CPC were performed. The patients were followed up in three-month interval. Neither parenchymal fistulas, nor cordiotoxic effects were observed. The histological examination of the pleural excisates showed complete predicable necrosis. No relapse of the disease was observed after one year of this treatment. The results were preliminary and were subjected to further research for complete understanding.
- **f.** Dielectric Barrier Discharge plasma was used by Fridman et al [25,26] to promote apoptotic behavior in Melanoma skin cancer cell lines. Higashimori et al used plasma to treat Mesh Skin Grafted Scars [28] and for this purpose four Asian patients with mesh skin grafted scars were enrolled. The patients were also evaluated for any side effects from the treatment. All patients showed more than 50% improvement. The average pain score on a 10-point was 6.9 ±1.2 and all patients tolerated the treatment.

3. Atmospheric Pressure Plasma Jet Devices

Several designs of plasma jet have been tested and incorporated in various medical research projects. Few initial designs are listed below. Each design has its own limitations and is suited for a target application.

In general, to produce a non-thermal dielectric barrier discharge (DBD), two electrodes must be used with a layer of dielectric between them. On applying a high voltage between the electrodes will cause the plasma to appear on the surface of the dielectric material. Figure 2 shows a design scheme developed by Fridman et. al. [14] where a dielectric barrier discharge is created between two electrodes.

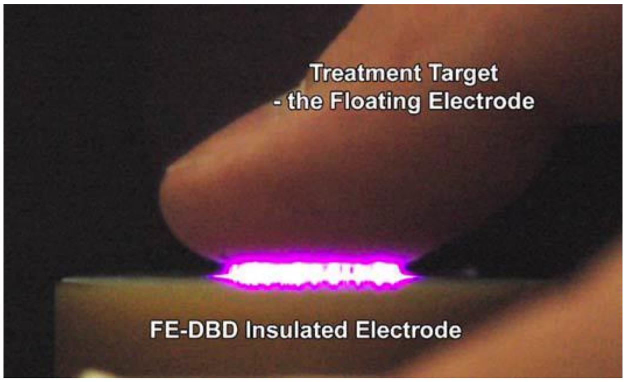

Figure 2. DBD plasma exposed to a living tissue that serves as an active electrode as well [14].

The exposed tissue in this case serves as an active electrode. In this setup, no voltage was applied directly to the body and most of the power was deposited in the discharge itself, leaving the exposed tissue unharmed.

The plasma PenJet was developed by Tushifuji et. al. [29] for surface treatment of various metals and polymer surfaces, Figure 3.

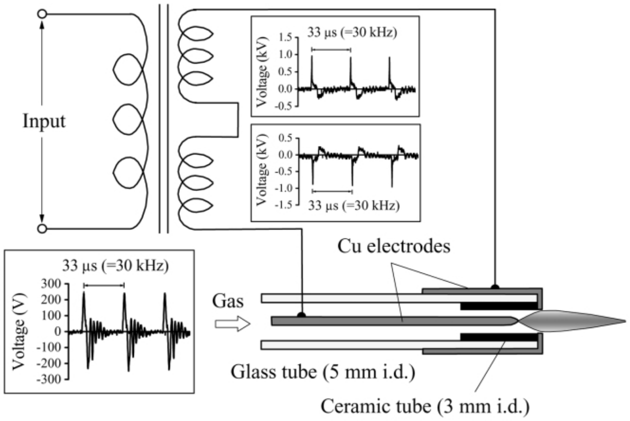

Figure 3. Plasma Jet Penjet [29]

An arc was created at the exit of the nozzle where the plasma plume was measured for its temperature and length as a function of applied frequency (10-30 kHz). Maximum jet length was approximately 15 mm at 30 kHz and maximum temperature at 5 mm from the electrode cap was around 250 degree centigrade. PenJet system was operated on dry air, nitrogen, and oxygen for its application of surface treatment. Due to higher temperature plasma plume, this may not be usable for medical applications.

Laroussi et. al. [30] gave another design of plasma (pencil) jet shown in Figure 4.

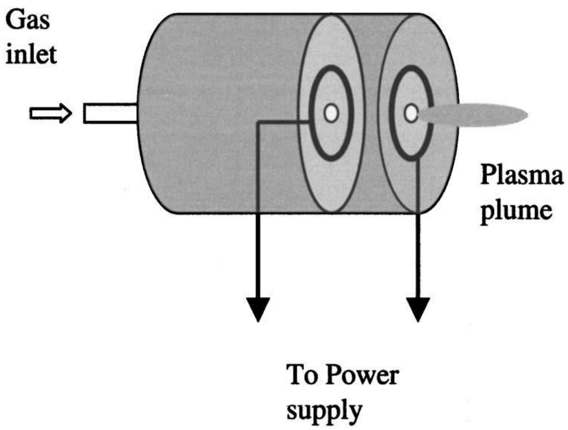

Figure 4. Plasma pencil [30]

The plasma pencil was designed to operate at near room temperature (~ 290 K) and was free from arcing reducing the risk for the patients to get affected by the high voltages involved. The narrow voltage pulses (submicrosecond) at repetition rate in the 1-10 kHz range were applied to couple the energy to the plasma plume. In another such system designed by Karakas and Laroussi [31], the plasma jet was generated by a unipolar square high voltage pulse [4.0-7.5 kV] that was applied to the electrodes with a pulse width of around 200 ns to several microseconds with a repetition rate up to 10kHz. Helium with a flow rate between 1.0 and 7.0 l/min was used as a carrier gas. The system set up is shown in Figure 5.

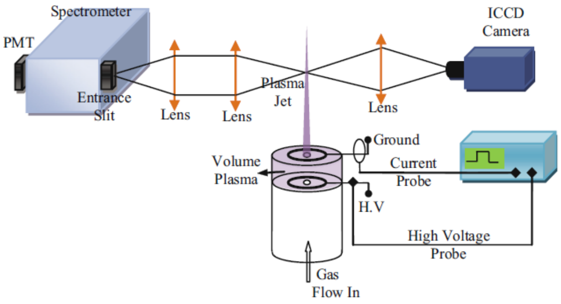

Figure 5. Plasma Jet by Larrousi [31]

Pei et. al. [32] designed a battery –operated air plasma jet device shown in Figure 6. The plasma flashlight device was used to inactivate a biofilm using room-temperature air plasma. The plasma was produced in self-repetitive nanosecond discharge wit current pulses of ~ 100 ns duration, current peak amplitude of ~ 6mA and repetition rate of ~ 20 kHz. It was shown that the reactive plasma species penetrated to the bottom layer of a 25.5 micron thick layer *Enterococcus faecalis* biofilm and produced a strong bactericidal effect.

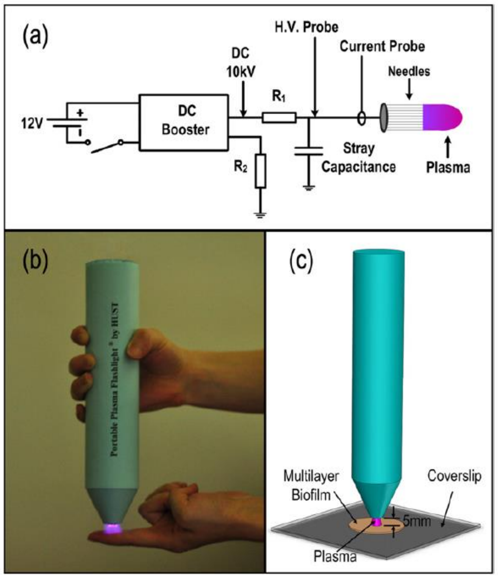

Figure 6. Portable Plasma Device [32]

Stoffels et. al. [20] developed a standard plasma needle that was operated by a 13.56 MHz, 10 W generator in combination with a matching network. The probe consisted of a 0.3 mm metal alloy pin, confined in a 5 mm Perspex tube, which was flushed with helium at a rate of 2l/min, Figure 7. Under these conditions the air content in the plasma was 0.5% and the ROS density was about $10^{19}/m^3$. The plasma source was operated at power levels of about 100 and 190 mW and was used to investigate the long-term behavior of vascular cells.

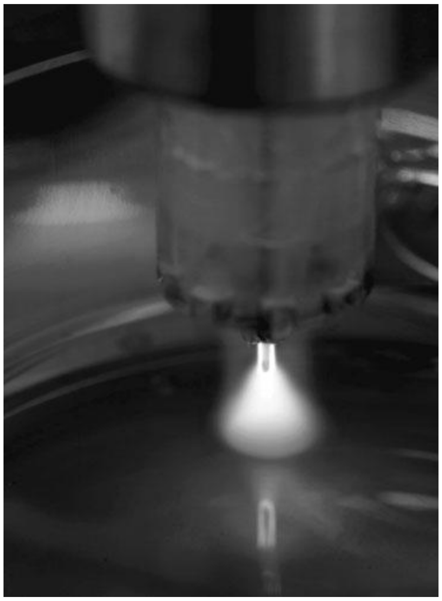

Figure 7. Plasma device by Stoffels et. al [20].

Plasma device developed by Shashurin et. al. [33] is shown in Figure 8. The operating voltage was around 5kV and the gas (helium) flow rate was around 17 l/min [30]. The plasma jet was around 5 cm long with a diameter of 1.5-2 mm in the ambient air. The interaction of the plasma jet with fibroblast cells was studied. The treatment of the cells with the plasma jet resulted in decreasing of cell migration rate, cell detachment, and appearance of “frozen cells” while treatment with helium flow without plasma resulted in appearance of frozen cells only.

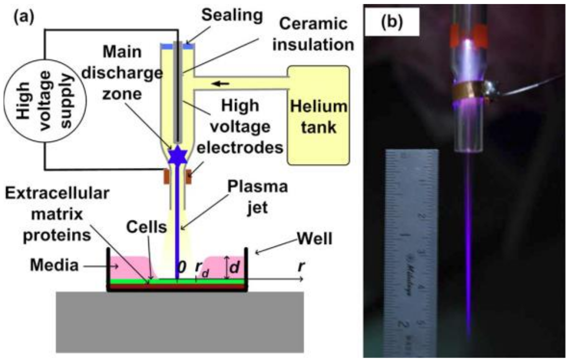

Figure 8. Plasma Jet designed by Shashurin et. al. [33]

Concept of creating and characterizing a plasma bullet drive was proposed by Ohyama et. al. [34]. In this design two electrodes separated by one mm distance were used to create a DBD plasma bullets, Figure 9.

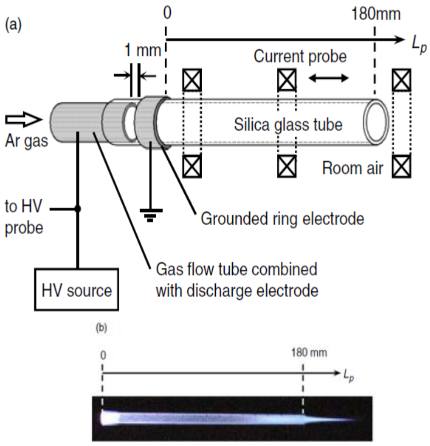

Figure 9. Experimental setup and optical image of plasma stream, Ohyama et. al. [34].

One of the electrodes was inside a dielectric tube whereas the second electrode was mounted outside the tube. A pulse power generator was used to generate ~15 kV pulses under 25 kHz frequency range. Plasma density was evaluated from the propagation velocity of the plasma bullet along with the current magnitude. The plasma density was measured as a function of the applied voltage and the length of the growth. It was found that the plasma density was about of the order of 10^{16} m^{-3} and the propagation velocity was of the order of 105 m/s. These values were found like those of weakly ionized non-thermal plasma jets. The system was not used in any of the medical applications discussed earlier in this document.

Brehmer et al. [35] used direct Plasma (DBD, Corona discharge) and indirect plasma (plasma torch, plasma jet) for treatment of chronic venous leg ulcers. DBD generated a low temperature plasma under atmospheric pressure and thus was suitable for a non-destructive treatment of biological material. The PlasmaDerm UV-2010 device developed in [35] was a non-invasive active medical intervention with no direct skin contact. One electrode of this system was incorporated in the device whereas the biological tissue itself acted as the second electrode. Authors suggested that the device may have additional plasma treatment potential to facilitate wound healing by disinfection, stimulation of tissue regeneration and microcirculation as well as acidification of the wound environment. A clinical trial with the PlasmaDerm VU-2010 were conducted to assess safety, applicability, and efficacy of chronic venous leg ulcer plasma treatment.

Figure 10 shows various slides of a KHz atmospheric pressure plasma jet developed by Graham et. al., [36] at Queens university of Belfast. In this design two tubular electrodes were used to surround a glass tube of around 4 mm diameter and wall thickness of 1 mm. The system was operated with helium and a high voltage pulser was used to produce pulses at KHz rate. Figure 10 shows an image of the plasma bullets that were captured at a nano-second time scale at an operating frequency of 40 KHz. The images of the plasma bullets were captured at 20 ns camera gate with a delay of about 40 ns.

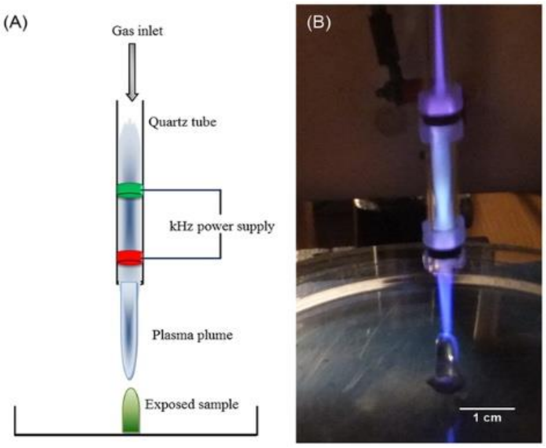

Figure 10. KHz atmospheric pressure plasma jet, Graham et. al [36]

4. Development of a Multi Electrode Plasma Jet Device

It is important to note that plasma medical applications still need full characterization of the plasma jets to understand their impact on wound rapid healing and sterilization. Plasma parameters including electron temperature and electron number density at various operating conditions are required for optimized wound healing and sterilization process. This can be achieved by designing a plasma torch that may have an option to change plasma parameters during its operation. This design was developed and tested by Zaidi et. al. [37]. In this design, a DBD plasma jet was produced using a dielectric tube in which the plasma was generated between the central electrode and a movable outer electrode ring. The plasma characteristics (temperature and plasma jet length) were changed by adjusting the outer ring during the torch operation. To make this design fully passive, the plasma jet device was further modified by incorporating three outer electrodes with one central electrode as shown in Figure 11. The plasma characteristics were modified by selecting any one of the outer electrodes at the same operating conditions e.g. gas flow rates and applied voltage/frequency. Plasma jet lengths were measured by capturing the plasma jet images along with a ruler. Plasma gas temperatures were measured using a k-type thermocouple that was scanned along plasma jet length. The results are shown in Figure 12. Plasma jet generated in Figure 12 was created by using an AC voltage source (~10 kV, 30 kHz) where the helium flow rate was set around 16.5 l/m. Figure 12 results show that the plasma jet length and its temperatures were dependent on the choice of electrode in the plasma system.

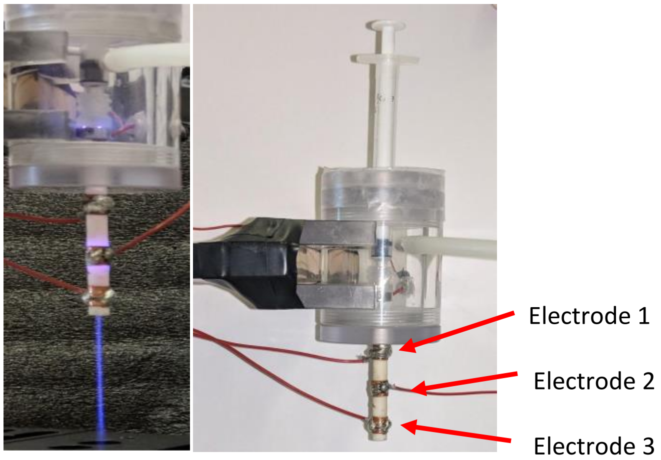

Figure 11. Multiple electrodes plasma jet system

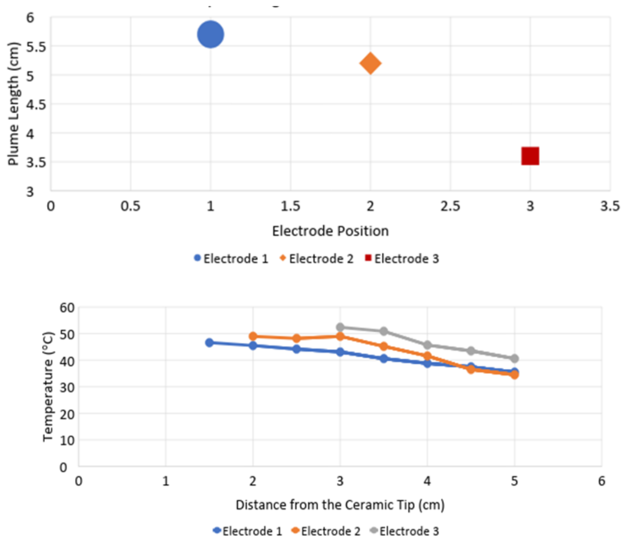

Figure 12. Plasma jet temperatures and plasma jet lengths as a function of electrodes

For electrode 3, plasma jet appeared at a higher temperature (~50 C) with a shorter plume length (~3.6 cm) whereas plasma jet from electrode 1 was found at lower temperatures (45 C and below) with a longer plume length (~5.7 cm). These observations will play an important role in the optimization of wound healing and sterilization process. Plasma impact on wound healing was investigated by exposing a blood drop to the plasma jet. Coagulation of the blood drop with and without plasma was observed by capturing blood surface images using a camera that was mounted on a microscope. As explained in reference [38], blood coagulation occurs as the clotting factor prothrombin activator and calcium ions are shuttled to the site of the cut. The prothrombin activator then converts prothrombin to thrombin. Thrombin is then used to cleave fibrinogen to form fibrin. The platelets then begin to release fibrin and start to close the cut in the epithelium. Fibrin is an insoluble protein that forms a fibrous mesh, which disrupts the flow of blood. Next, the fibrin webs trap red blood cells to complete sealing the wound. Figure 13 shows the coagulated images of the red blood cells in the fibrin network. Both images display a healthy coagulation.

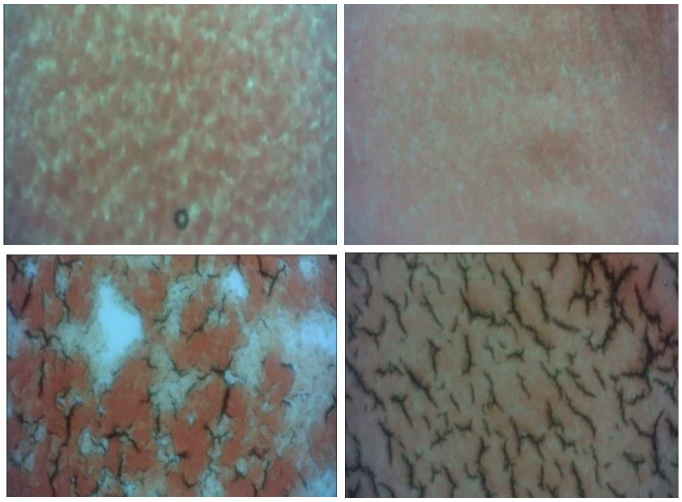

Figure 13. (Top to Bottom Left: Natural coagulation of the blood drop at time 0 and 15 minuted respectively) and (Top to Bottom right: Blood coagulation with plasma at time 0 and 60 seconds respectively).

Both clotting images look visually similar, indicating that the plasma did not harm the red blood cells. The increase in coagulation rates may be because of the plasma affecting the calcium in the blood. Ionized calcium is needed in various steps of coagulation and the plasma may be ionizing blood calcium, making it readily available to use in coagulation. Further testing will involve the use of plasma on larger wounds.

5. Emission Spectroscopy of the Plasma Jet

In order to optimize the wound healing and sterilization process, it is necessary to vary the plasma jet properties. Plasma temperatures especially electron and gas temperatures play a vital role in this regard. Measurement of electron temperatures and electron number density will be required to investigate it impact of wound healing and sterilization. Spectroscopy is an experimental tool that can be used for this purpose [39]. To conduct spectroscopic analysis of the plasma jet developed for this study, emission spectrum of the plasma jet was captured. An optical fiber-based Ocean Optics Spectrometer was used for this purpose. Figure 14 shows a typical emission spectrum that was captured. Helium was used as the working gas and the plasma device was operated at 10 kV and 30 kHz.

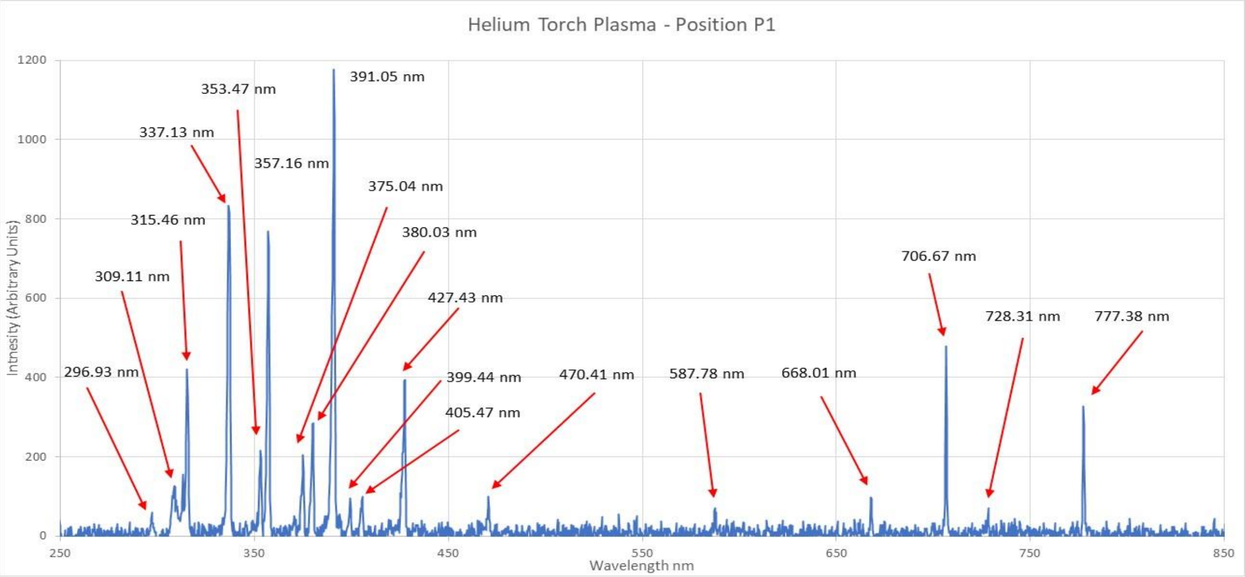

Figure 14. Plasma jet emission spectrum. An Ocean Optics Spectrometer was employed to capture the spectrum.

Various nitrogen and helium lines (587 nm to 728.31 nm) in the spectrum were identified. Few important observations were made. First, the resolution of the spectrometer was not enough to capture vibrational structures at 337 nm nitrogen band that may be used to find out rotational and excitation temperatures. Second, two observed peaks appearing at 391 nm and 375 nm can be used to obtain the ratio (391/375) to extract information on E/n but it needs system response function that was not measured. To fully conduct the spectroscopic study of this plasma jet, future work includes the measurement of the system response function along with the use of a high-resolution spectrometer that can resolve vibrational bands in the spectrum. Specair software is now obtained to analyze the emission spectrum. This would lead to meaning results on vibrational and excitation temperatures that will be required to optimize the process of wound healing and sterilization as a function of plasma parameters.

6. Conclusions

A multiple-electrode DBD plasma jet device is designed and characterized. The plasma jet was operated at atmospheric pressure with 10kV and 30 kHz AC power supply. Helium was used as the working gas. Plasma jet temperatures and lengths for various operating conditions were measured by using a k-type thermocouple and jet imaging respectively. It was found that the selection of the outer electrode did impact the plasma temperatures and its length. Blood coagulation process was investigated by exposing a blood drop to the plasma jet. Experiments show a rapid increase in blood coagulation under the plasma exposure that may accelerate the wound healing process. To conduct the spectroscopic analysis of the plasma emission spectrum, an Ocean Optics spectrometer was used. Few limitations of the spectrometer were identified. Suggestions are made for the future work that will fully analyze the emission spectrum to obtain information on the plasma excitation and vibration temperatures.

7. Acknowledgements

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