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Effect of Cold Atmospheric Pressure Argon Plasma Jet on Wound Healing

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Abstract:

Atmospheric pressure cold plasma is expected to be an effective tool for wound healing and treating skin diseases. In this paper, we generated an atmospheric pressure plasma jet [APPJ] in argon gas with an applied voltage of 3.5 kV operating at a frequency of 27 kHz. Low electron temperature of 0.572 eV was calculated from analysis of optical emission spectra of the jet and plume temperature in the range $(42\text{-}26)^0$C was measured by using laser gun thermometer, which demonstrated that it is suitable for treating animal body. In vivo study was performed to demonstrate that APPJ is an effective tool for wound healing in rats. Based on morphological changes in the wound, APPJ treatment for 120 sec showed complete wound healing at day 5 in comparison with the control (without treatment and wound treated with antiseptics). The wounds treated with single doses were slower in healing compared to the wound treated with multiple doses (i.e., twice a day). It was concluded that an appropriate dose of APPJ could inactivate bacteria around the wound to promote the healing process. This healing effect may be related to the potential killing of bacteria in wound due to presence of reactive oxygen and nitrogen species (ROS and RNS) in APPJ.

Keywords: APPJ, wound healing, optical emission Spectra

1 Introduction

Cold atmospheric pressure plasma jets (APPJs) are known as weakly ionized gases that are produced by electric discharges with different work gases (air, argon, helium, etc.). Plasma consists of many active components, including radicals (reactive species), ground-state and excited atoms, ions, electrons, and photons [1, 2]. APPJs can be generated at atmospheric pressure in ambient air and the temperature of all species does not exceed body temperature, allowing its application to animal body. The application of APPJ in biomedical research has increased during the last fifteen years in several domains such as infection-related disease in dermatology,[3] skin wounds, [4-7] blood coagulation,[8,9] cancer cell treatment,[10-11] inactivation of several microorganisms, [13-15] and decontamination of medical devices and surfaces in hospitals,[16,17]. Positive result have been obtained in these fields in vitro and in vivo, and some clinical trials have been conducted that produces successful treatment of different types of skin injuries including acute wounds, [18, 19] burns, [20] chronic leg ulcers, [21,22] Other clinical trials have proven that APPJ treatment is safe and causes no toxic effect, non-allergic, or pain and contact free.

Therefore, the aim of this study was to investigate the efficacy of applying indigenously built atmospheric pressure plasma jets using argon gas on acute wounds of rats. The APPJ was characterized by measuring electron temperature, gas temperature, electron density and relative intensity in the jet region of APPJ with the help of optical emission spectroscopy (OES). Consequently, APPJ was applied to treat wounds on rats and compared the wound healing speed with a control group (no treatment and treatment with antiseptics.

2 Experimental setup

Plasma jet concerned in this study was produced in the physics lab of Kathmandu University. Plasma is generated in a glass capillary tube with an inner diameter of 3.0 mm and an outer diameter of 4 mm. The electrodes, 1.0 cm wide, are made of aluminum foil wrapping the capillary tube and the distance between the inner edges of two electrodes is 15 cm. The ground electrode is on the upstream side; the active electrode is on the downstream side and 0.5 cm apart from the tube orifice. Argon gas was used as the working gas and the flow rate of the argon gas was maintained at 2 standards liters per minute (slm) so that the flow velocity would not exceed the limit for a laminar argon flow. In the present study, the jet in argon gases is generated by applying a sinusoidal voltage with a fixed frequency 27 kHz and a voltage 3.5 kV for the excitation and sustaining the discharges

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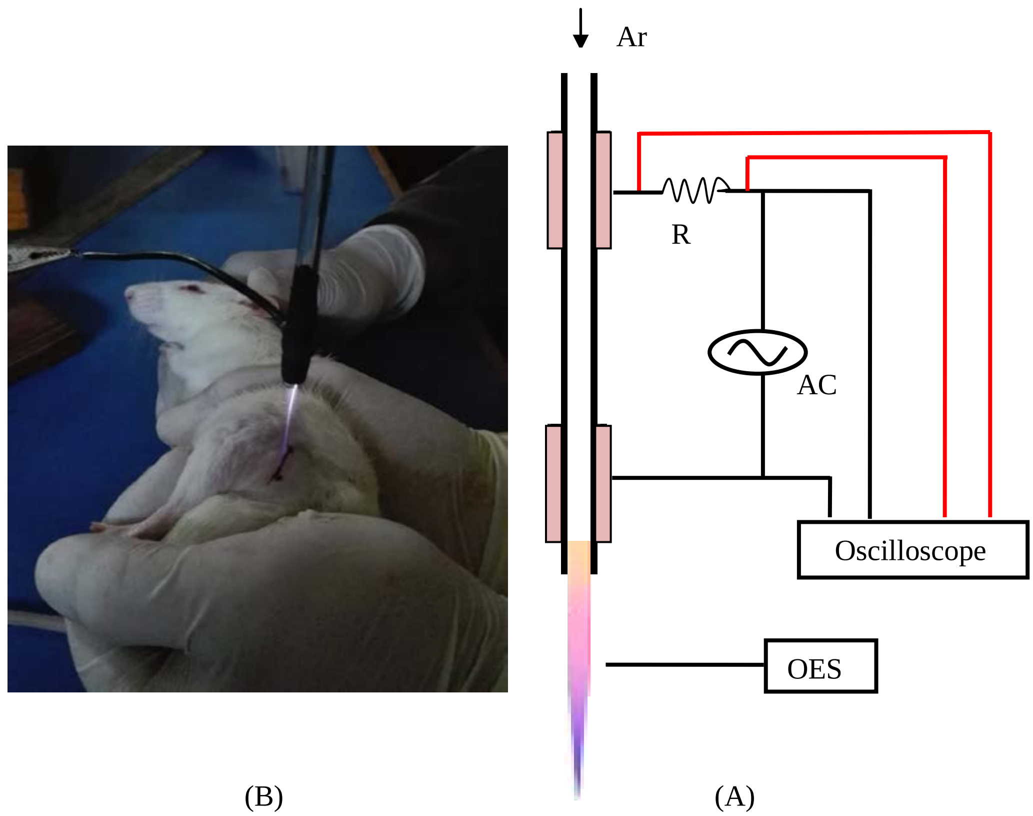

(B) (A)

Figure 1: Schematic representation of the APPJ system (A) and Photograph of APPJ designed and constructed at K.U. under operation (B).

The applied voltage was recorded by digital oscilloscope (Tektronix TDS2000) through high-voltage probe (Tektronix P6015A, 1000:1) voltage dividing ratio and the current was measured via inductance free resistor of 10kΩ. Optical emission spectra (OES) were collected perpendicular to the jet using an optical spectrometer (Ocean Optics USB2000) with a spectral range of 180–1100 nm and a resolution of 0.2nm full width at half-maximum. OES data were compiled using a personal computer equipped with relevant software for both driving and acquisition. We were thus able to obtain the relative irradiance of the active species in the plasma. During OES measurement, exposure time was 100 ms. Emission intensities of the active species were collected at a position of the plasma jet (3.5 cm from the end of the nozzle) through an optical fiber with a diameter of 100 µm.

3 Wound inductions in rats for in vivo study

The team carried an in-vivo experiment in seven Wister rat models. Skin surface of the rats were anesthetized with xylocaine and the acute cutaneous wound of length 10 mm were induced by veterinary professional in the upper part of the left lower limb of each rat. Two of the animal models were taken as positive control and were treated with antiseptic ointment whereas one of the models was taken as negative control and left untreated. The remaining four models were treated with different exposures and timings of Cold Atmospheric Plasma (APPJ) jet. During the whole procedure, the team was aware of the animal rights regulations and they were strictly followed.

4 Plasma treatment:

After the induction of wound, animal models were exposed to cold APPJ in different treatment time, which are categorized as follow:

1. Single short plasma dose for 2 mins
2. Multiple short plasma dose for 2 mins

Two of the animal models were taken as positive control and were treated with antiseptic ointment whereas one of the models was taken as negative control and left untreated. The remaining four models were treated with different exposures and timings of cold APPJ. Two rats were given 2 min multiple dose (two times per day) treatment, two were given 2 min single dose treatment. The wound was left under observation for 5 days.

5 Analysis of wound after APPJ treatment

Healing process of the wound treated with APPJ was analyzed based on the morphological changes of the wound for 5 consecutive days by measuring the length of the wound. Furthermore, the tissue obtained from the wound was subjected to histological analysis after 5 days. The tissue under observation and study was treated with 10% Formaldehyde solution for one week for fixation. Then the tissues were treated with 50%, 60%, 70%, 80%, 90% and absolute alcohol for 2 hours each completing dehydration. Then for clearing, tissues were dipped in two changes of Xylene for 1 hour each. The tissues treated with Xylene solutions were treated with 3 changes of Paraffin for 2 hours each for embedding. The tissue sections were put in paraffin blocks for blocking and then sectioning was done to get the section of the desired tissue sections. Then sectioned tissues were stained with Hematoxylin and Eosin dye and studied under a microscope for histological analysis.

6 Results and Discussions

6.1 Characterization of Cold Atmospheric Pressure Plasma Jet

The Optical emission spectra of atmospheric pressure plasma jet in Argon at discharge frequency 27 kHz and applied voltage 3.5 kV in the wavelength range of 180-1100nm were recorded and the identified lines are shown in Figure 2.

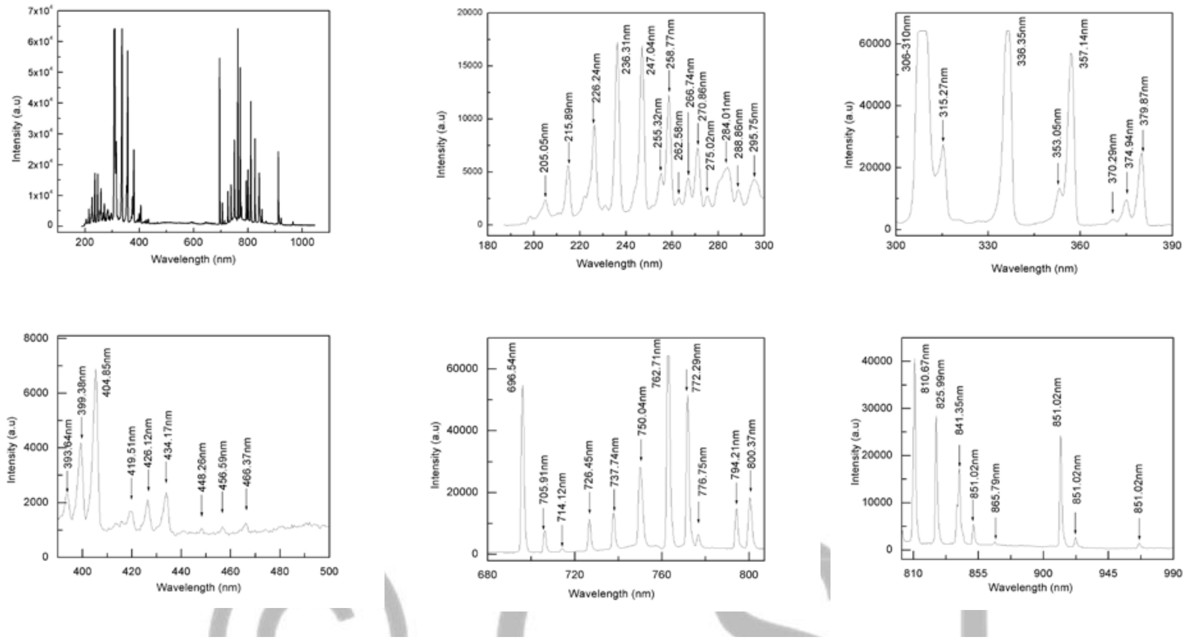

Fig.2. Optical emission lines of the plasma jet in Argon at discharge frequency 27 kHz and applied voltage 3.5 kV

The most intense emission lines of the discharge were the argon (Ar I) emission (4p→4s) lines in the spectral region 690–860 nm and the transitions of the OH band between 306–309 nm. Peaks corresponding to nitrogen and NO were measured between 330 and 400 nm and were presumably present due to mixing of the feed gas argon with the surrounding ambient air along the plasma jet. Atomic oxygen lines (OI), which are located at 777.4 and 794nm, respectively were also observed. The detected OH band between 306-309 nm indicates the presence of the water vapor in the afterglow. We assume that the source of the water is the ambient air, which penetrates by the diffusion into the working gas. Atomic oxygen and OH radical are highly reactive radicals that could play important roles in the potential biomedical applications of plasmas such as wound healing and bacteria inactivation. Furthermore, reactive oxygen and nitrogen species (ROS and RNS) can influence the intracellular environment, possibly by diffusing into cells or by inducing new species within the cells.

Based on the Ref. [23, 24], the electron temperature of plasma jet can be calculated by Boltzmann plot with the equation:

$$\ln\left(\frac{I_{ki}\lambda_{ki}}{g_k A_{ki}}\right) = -\frac{E_k}{k_B T_e} + C................(1)$$

Where A_{ki} is the transition probability, T_e is the electron temperature, λ_{ki} is the wavelength, E_k and g_k are the excitation energy and the statistical weight of the upper energy state respectively. I is the intensity of the spectral lines, k_B is the Boltzmann constant and C is a constant for all selected spectral lines

Now, plotting Eq. (3) with E_k in the horizontal axis and ln $(I_{ki}\lambda_{ki}/g_kA_{ki})$ in the vertical axis will result in a straight line, and the electron temperature can be determined from the slope of the straight line. In our experimental spectra, six ArI lines with wavelengths 801.47nm, 772.37nm, 714.704nm, 706.72nm, 696.54nm and 415.25nm respectively were chosen which has similar lower energy level (E_i) with different upper energy level (E_k) to calculate electron temperature of the plasma jet by the Boltzmann plot method. The atomic data of six ArI lines are displayed in the table1 given below.

Table 1: Parameters of the six selected lines taken from NIST Atomic data

λnm	Intensity (a.u)	A_{ki}	E_i	E_k	g_k
801.47	18821	9.3×10^6	11.548	13.094	5
772.37	54906	5.2×10^6	11.548	13.153	3
714.704	2120	6.3×10^5	11.548	13.282	3
706.72	8691	3.8×10^6	11.548	13.302	5
696.54	57111	6.40×10^6	11.548	13.327	3
415.25	1336	1.40×10^6	11.548	14.528	3

By substituting the values of E_k, A_{ki}, and g_k for six ArI in equation (1) and plotting the term ln $(I_{ki}\lambda_{ki}/g_kA_{ki})$ in vertical axis and E_k in horizontal axis [Fig: 3]. the electron temperature can be determined from the experimental data using a linear fit to calculate the slope.

The reciprocal of the slope gives the electron temperature in eV. Electron temperature of the plasma jet for applied voltage 3.5kV with frequency 27 kHz is 0.572eV.

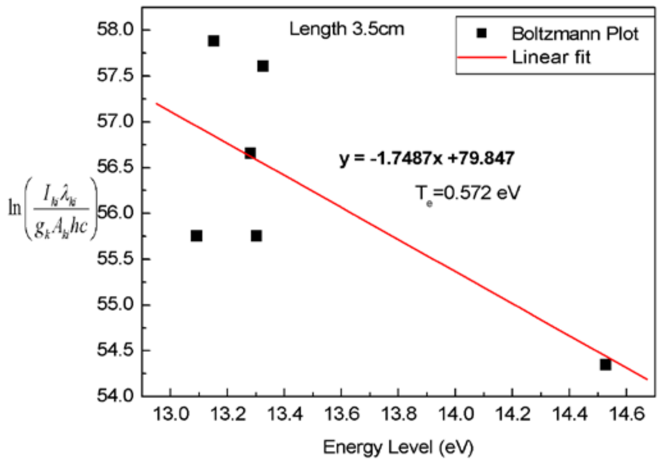

Fig: 3. Boltzmann plot fitting for estimation of electron excitation temperature at main discharge at 35mm from the glass nozzle

The electron density was measured by the stark broadening of ArI (696.54nm). The broadening due to collision of charged species in the primary mechanism influencing the width of the ArI emission lines. The stark broadening function is assumed to have the Lorentz profile. The electron density (n_e) related to the full width half maxima (FWHF) of the stark broadening line is given by the expression [24,25].

$$n_e = \left[\frac{\Delta\lambda_{stark}}{2\times10^{-11}}\right]^{\frac{3}{2}} \quad \text{.............(2)}$$

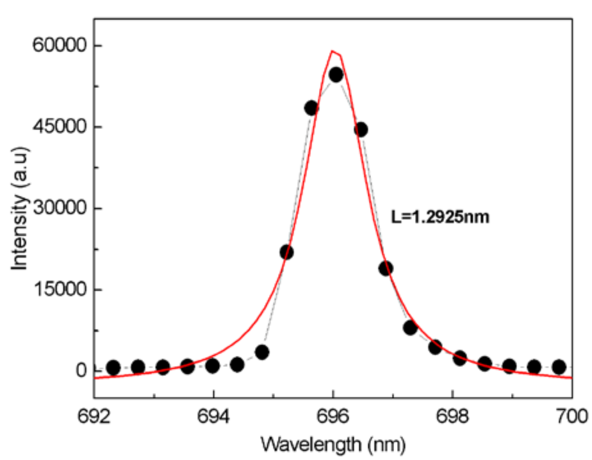

Fig: 4. Spectra for determination of full width at half maxima FWHM

For $\Delta\lambda_{\text{Stark}}$ = 1.2925nm the shape of broadening line is Lorentzian (Fig: 4) and the calculated value of electron density (n_e) is $1.643\times10^{16}cm^{-3}$.

6.2 Estimation of plasma plume temperature

Touchable atmospheric pressure plasma jet (APPJ) is important in biomedical application. Hence laser gun thermometer is used for measurement of plasma plume temperature for a continuous operation of 5minutes and is $(42\text{-}26)^0C$ in the range along the plume length which is shown in fig 5. This indicates the plasma jet can be used for several biomedical applications without causing thermal damage. The plasma temperature at the plume length 3.5cm is close to 31^0C after 5minutes of operation, which is still very safe for several biomedical applications having direct contact with animal body.

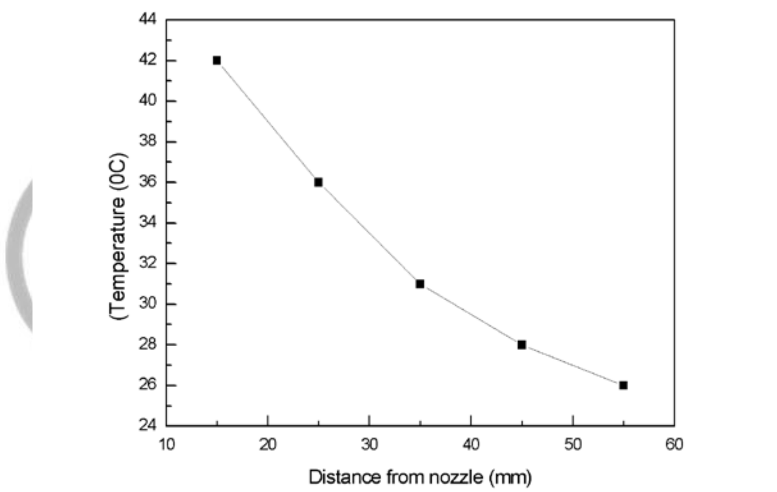

Fig.5: Variation of plasma plume temperature along the length of the plume measured by laser gun thermometer

6.3 Electrical measurements

Fig. 6 shows a typical waveform of applied voltage and discharge current profile during plasma plume formation in the ambient air of length 5.5cm. The discharge occurs in each half cycle of the applied voltage, which can be observed from the discharge peak current in current waveform. The current leads the applied voltage slightly less than 90^0, which is very similar to typical capacitively coupled plasma. The applied voltage in this experiment was 3.5kV and the discharge current obtained is 12mA.

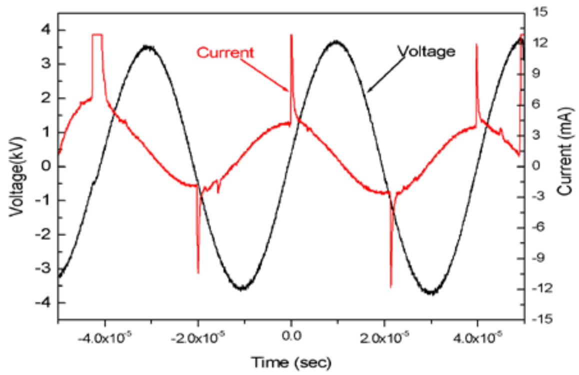

Fig. 6: Typical applied voltage and discharge current wave form for 5.5cm plasma plume formation

6.4 APPJ treatment enhanced wound healing in rats:

It was observed that the length of the wound did not decrease in case of the control subject. But in case of APPJ treatment, the bleeding immediately stopped, and clot was formed as well as the two flaps of the cutaneous layer got attached within 10-15 seconds of exposure. In the case of 2 minutes single plasma dose, the length of the wound decreased significantly. Further, the length of the wound for multiple 2 minutes plasma dose decreased even more significantly compared to single 2-minute dose. Added to that, positive control subject was treated with an antiseptic Povidone-Iodine ointment. It was observed that the subject treated with the ointment showed comparatively less recovery rate than that of APPJ treatment. It was also observed that as the wound healed, there was a significant amount of scar formation in the subject treated with ointment compared to the subjects treated with APPJ concluding that APPJ treatment didn't form much scar after the wound healed. The control subjects were observed for longer period of time as the healing process was slow. It was observed that the length of wound did not decrease in control subjects as compared to other subjects. Also, the control wounds became more chronic with more pus cells during healing process. Hence, APPJ was seen much more effective than antiseptics. The progress images after the treatment compared to the control is shown in the figure below;

Wound-healing process observation on Day 0 and Day 5 without receiving any treatment,

Fig.7: Wound induction

Fig.8: Control Wound after 5days (7mm)

Measurement of length of wound on different days in various conditions

Fig.9: Wound treated with plasma for 2 mins single dose per day after 2days

Fig. 10: Wound treated with plasma for 2 mins single dose per day after 4days

Fig.11: Wound treated with plasma for 2 mins multiple doses (twice per day) after 2days

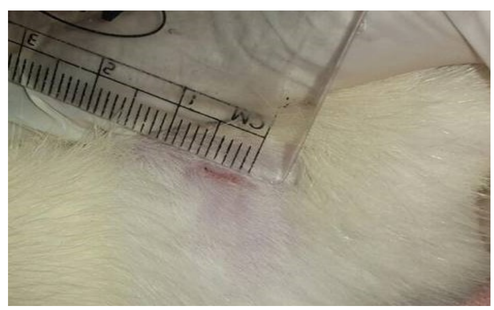

Fig.12: Wound treated with plasma for 2 mins multiple doses (twice per day) after 4days hours

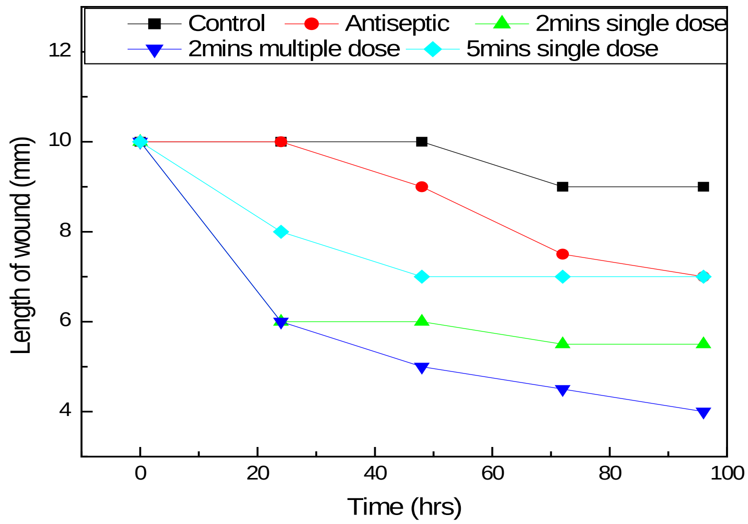

Fig.13: Graph showing the decrease in the length of wound after different treatments

The histological slides as in fig. 14 could not demonstrate significant difference between the treated and control tissue as collagen deposition are similar and wound was almost completely healed. But a work done by S. Kuvinova et.al showed decreased deposition of collagen in case of initial inflammatory phase of healing in plasma treated than that of control wound [26].

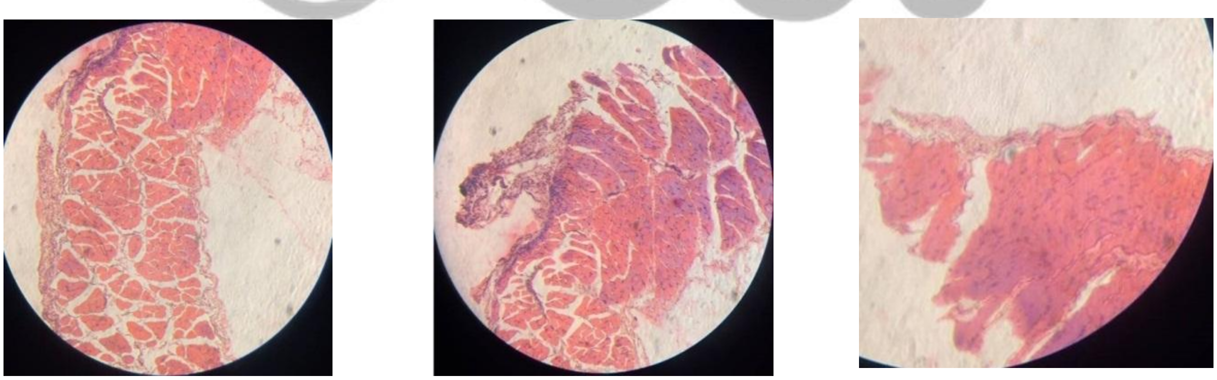

Fig.14: Microscopic view of rat's tissue after APPJ treatment, a) 2 min single dose, b) 2 min multiple doses, and c) Control (no treatment). Magnification 10X

7 Conclusions

In this study, we have presented non thermal atmospheric pressure plasma plume characterization for biomedical applications and investigated the efficacy of the wound healing process by treating acute cutaneous wounds directly on rat skin. By using high voltage (3.5KV and 27 kHz frequency) source, a low temperature atmospheric pressure plasma plumb of length 5.5cm has been produced. The temperature measurement showed that the plasma plume at the length 3.5cm in nearly 31°C with 5 minutes of continuous operation which is very safe for biomedical application.

Optical emission spectroscopy measurement indicates the present of active species (OH, O, N_2^+ etc) in the plasma plume. The electron excitation temperature of APPJ is found to be 0.572eV and electron density $1.643*10^{16}cm^{-3}$. We have shown the wound healing process for typical cases using Hematoxylin and Eosin staining results. The result show that the wounds healed fastest by using APPJ Compared with the traditional ointment treatment. The trend of wound healing is especially pronounced for the two minutes multiple doses (i.e twice per day) than two minutes single dose .The studies on mechanism of the wound healing process by plasma jet treatment are currently in progress and will be published. This work demonstrated that plasma jet is emerging as promising technique in the medical field for developing countries like Nepal because of cost effective and easy application procedure.

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