Chapter 28

Variable Frequency Atmospheric Pressure Cold Plasma Jets for Biomedical Applications

- V. C. Misra, N. Tiwari & S. Ghorui

28.1 Introduction

The atmospheric pressure plasma jets (APPJs) have received a lot of interest in the last decade for biological and material functionalization applications. Plasma sources that function at atmospheric pressure and a low gas temperature can be maintained are known as atmospheric pressure plasma jets. These plasmas have a lot of biomedical potential. Plasma medicine is based on the application of an electrical discharge to a biological target, either directly or indirectly. When an atmospheric pressure plasma jet source is used in an ambient environment, it generates reactive oxygen and nitrogen species (ROS and RNS), which are referred to as RONS [265].

A molecule containing single or a greater number of unpaired electrons in the outermost shell is termed as a free radical [266]. Free radicals are created when a chemical bond is broken and each fragment maintains one electron, when a radical is cleaved to produce another radical, and when redox reactions occur [266]. Hydroxyl (OH^*) radical, superoxide ($O_2{}^{*-}$) anion, nitric oxide (NO^*) radical, nitrogen dioxide ($NO_2{}^*$) radical, peroxyl (ROO^*) radical, and lipid peroxyl (LOO^*) radical are examples of free radicals. Despite the fact that, ozone (O_3), dinitrogen trioxide (N_2O_3), singlet oxygen ($^1O_2$), nitrous acid (HNO_2), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) ion, and lipid peroxide (LOOH) are not free radicals, yet they can lead to reactions like the free radicals in the animals [267]. As a result, free radicals in biology are electron rich and extremely unstable species that can destroy the structure of lipids, proteins and DNA.

Simply stated, reactive species that comprise of oxygen as a constituent is referred to as reactive oxygen species (ROS) as discussed above [268]. Similar to ROS, the term reactive nitrogen species (RNS) has been typically used to refer to nitric oxide (NO), nitrogen dioxide radical (NO_2), peroxynitrite ($ONOO^-$) ion, and further oxides of nitrogen or reactive nitrogen species. RNS are categorised as ROS by definition since they are almost exclusively oxygen-containing species [268].

RONS are either endogenously or exogenously produced. Several factors in the organism may be held as responsible for the production of endogenous free radicals. Some of these are medication, radiation, inflammation, immune cell stimulation, psychological stress, extreme exercise, some type of infection, cancer as well as ageing. Cigarette smoking, Air pollution, water pollution, alcohol, heavy metals like Fe, Cd, Pb, As and Fe may be called as the agents responsible for generation of exogenous RONS [269]. These exogenous chemicals are converted into free radicals after entering the body through variety of mechanisms.

Useful effects of free radicals and oxidants

Moderate amounts of RONS are essential. These radicals are needed as weapons for the host defence system for an organism. Undeniably, as the component of the body's defence system to combat infections, phagocytes (monocytes, macrophages, and neutrophils) generate these free radicals to destroy invading infective germs [266] and therefore fight infections. The physiological roles of the radicals in the purpose of several cellular signalling systems are also beneficial implications [268]. Nitric oxide radical (NO^*), for example, is the intercellular messenger that regulates blood clot, blood flow and brain function. The activation of a mitogenic response is another advantageous property of these free radicals [268]. Hence, these radicals are extremely useful.

Harmful effects of free radicals and oxidants

When free radicals are released in excess, they cause oxidative stress, a detrimental process that disrupts cell membranes and several other components like lipids, proteins and deoxyribonucleic acid (DNA) [269]. When cells are unable to efficiently remove the excess free radicals released, oxidative stress can occur. As the body has its own balancing mechanism, the oxidative stress is instigated due to an imbalance in the creation and elimination of these species. The plasma may induce an imbalance and can lead to deleterious effects observed after plasma treatment. Surplus hydroxyl radicals and peroxynitrite ions for example, can promote lipid peroxidation, which damages cell membranes and lipoproteins, can ROS/RNS can potentially harm proteins, causing structural alterations and the loss of enzyme activity and can also lead to DNA damage which can induce mutations [267]. APPJs have a low gas temperature to treat biological targets and a high radical density to generate more ROS. As a result, these plasma sources have the potential to be utilised in a variety of biomedical applications. This unique plasma use is being investigated all across the world for various applications as listed below.

1) **Plasma-aided wound healing [266]:** Cold atmospheric plasma (CAP) treatment results in faster wound healing. It was discovered that cold plasma might inactivate germs around the wound and could activate fibroblast growth to aid wound healing.
2) **Plasma dentistry:** Because of the existence of the OH radical, which takes an electron from the environment and destroys the pigment structure, improved tooth bleaching was observed. Due to its remarkable efficacy at deactivating bio-films generated by bacteria S. mutans, another prospective application of CAP in dentistry is the disinfection of dental cavities [270].
3) **Cancer applications or "Plasma Oncology":** Researchers have observed different mechanisms of cell death viz. cell detachment, reduction of the cell migration, apoptosis, necrosis on numerous different types of cancer cells depending on the plasma

power and the different time of plasma exposure [271]. In addition, two basic CAP techniques are being researched. One option is to use the plasma jet to treat the cells sown in a petri dish directly. Another technique is to employ plasma treated media or plasma activated medium (PAM) to stop cancer cells from growing during the regular cell culture procedure or to stop tumour tissues from growing by injecting PAM into the tumour tissues of an organism [272].

4) **Plasma treatment of implants for biocompatibility:** Atmospheric pressure plasma processing enhances the hydrophilicity of the material surface and therefore lead to better biocompatibility.

5) **Blood Coagulation:** Since isolated platelets were highly stimulated following exposure to plasma, APPJ allows for effective blood coagulation without any heat effects. When cold plasma is used after surgery, the blood coagulates without causing any apparent tissue damage [273].

28.2 Variable Frequency Plasma Configuration

A typical VFAPPJ (Variable Frequency Atmospheric Pressure Plasma Jet) device has the ability to tune the application temperature and radical density. This tunability makes this device extremely important for biomedical applications where just a change in driving frequency may alter the plasma parameters. An in-house developed VFAPPJ is shown in Fig. 28.1. It has coaxial cylindrical configuration with central tungsten pin and stainless-steel outer housing which acts as the ground. A power source with continuously variable frequency capable of operating in the range of 10 kHz to 100 kHz is used in generation of the plasma. The basics of the power source have recently been published elsewhere [274, 275]. These might be referred by the readers for further information.

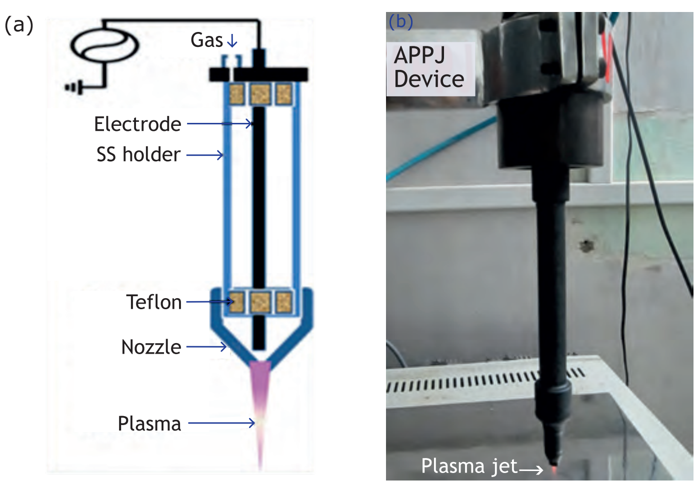

Figure 28.1: (a) Schematic of the experimental setup, and (b) The device with generated plasma.

A mixture of 18.0 LPM Argon and 0.8 LPM of Nitrogen is used to generate plasma at different frequencies. This combination showed the best results in terms of intensity. To elaborate a variable frequency response, plasma generated at 17 kHz and 53 kHz are compared for species temperature and radical density. The results of the experiments are discussed in the subsequent sub-sections.

28.2.1 Species identification

Emission spectra originating from plasma were captured using optical emission spectroscopy setup. The essential spectroscopic setup makes use of an optical bench including an optical fiber and a spectrograph with the data acquisition and the control system. It can be mentioned right here that the light emitted is collected through the fiber tip at 2 mm distance from the exit of the nozzle, positioned and aligned with the jet. The light is taken to the access port of a diffraction grating to capture the spectra with the wavelength resolution of 0.1 nm. Furthermore, sixteen representative atomic emissions of Ar, few for atomic nitrogen and only two for atomic oxygen (777.5 nm, 844.6 nm) were observed. Figure 28.2 represents the spectra recorded at 17 kHz from 250 nm to 310 nm. Presence of NO and OH band is observed. The nitric oxide radicals and hydroxyl radical are found to be extremely significant in DNA damage and therefore killing of the cancer cells. Presence of these bands make the device imperative for the treatment of cancer.

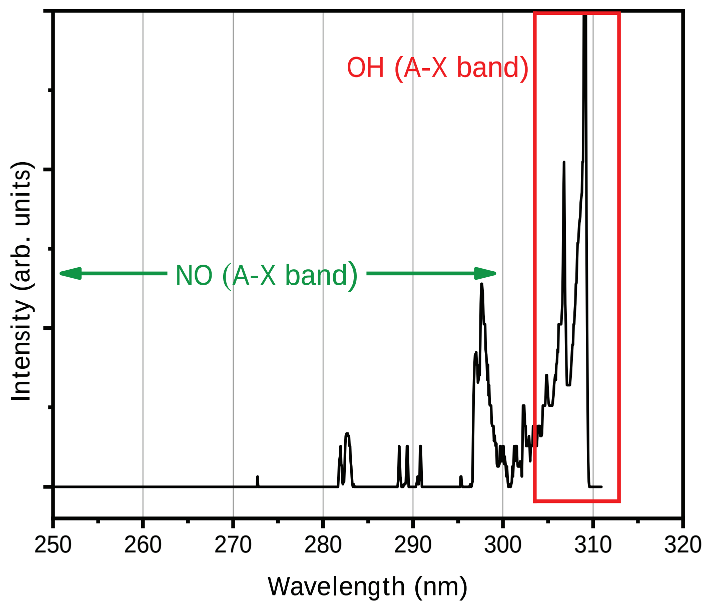

Figure 28.2: OH and NO bands observed at 17 kHz.

28.2.2 Determination of excitation temperature

The excitation temperature gives the energy of the electrons. Cold atmospheric pressure plasmas are expected to have much higher excitation temperature as compared to heavy species temperature. Higher electron energy is responsible for the generation of reactive species, which in turn is extremely important for biomedical applications. Spectral feature of the emission from the plasma is presented in Fig. 28.3a. Although the plasma under consideration is obviously non-thermal plasma with extremely low degree of ionization, experimental evidences [see Fig. 28.3b] indicate existence of some excitation equilibrium among the atoms excited in the process.

Following Griem [276] that the intensity (I_{ui}) of radiation with the wavelength (λ_{ui}) due to transition from level 'u' to level 'i' follows the following relation:

$$ln\left(\frac{I_{ui}\lambda_{ui}}{g_u A_{ui}}\right) = -\frac{E_u}{k_B T} + C \tag{28.1}$$

where k_B is the Boltzmann constant; A_{ui} is the 'transition probability' for the definite transition; E_u and g_u are the excitation energy and statistical weight factors of the upper level 'u' respectively. Plot of Eq. (28.1), results in a straight line when excitation equilibrium

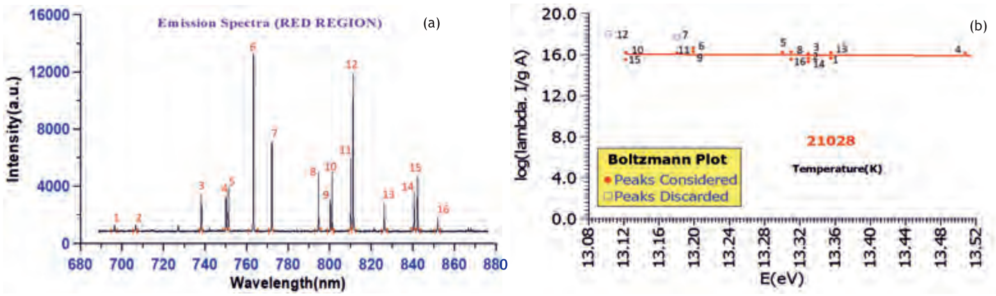

Figure 28.3: (a) Emission spectra with peaks considered for the measurement are marked in red, and (b) Boltzmann plot obtained from the emission data.

prevails among the states in the excited atoms. This is very much observed in Fig. 28.3b. Excitation temperature may be obtained from the plot (Boltzmann plot) from the slope of the straight line [277]. The temperature shown in Fig. 28.3b is ∼1.8 eV. It is worthy to mention that the excitation temperatures measured at 17 kHz and 53 kHz show similar values. Estimated excitation temperatures fall in the range of 1-2 eV.

28.2.3 Gas temperature measurement

The cold atmospheric plasma's most important prerequisite for treating biological tissues is a low gas temperature. A mercury thermometer was used to measure the temperature of the gas at various distances from the plasma source. It was discovered that adjusting the frequency and distance might change the temperature. The temperature of the plasma gas was just slightly higher than that of the surrounding air. As a result, these sources can be used to treat biological targets. Figure 28.4c shows the integrated intensity at various frequencies, determined using a photodiode. The intensity of emission lines measured at 17 kHz, 30 kHz, and 53 kHz is shown. The frequency 17 kHz has the highest intensity of all the frequencies considered. The plasma jet image obtained at 17 kHz (Fig. 28.4a) shows the similar result. Because the ions are unable to respond to the high frequency, they are unable to gain energy. Furthermore, the displacement amplitude is also expected to be greater at lower frequencies, implying a wider plasma volume. Because electrons are light and mobile, they retain a constant excitation temperature even when the kHz frequency changes. It's worth noting that the treatment temperature can be readily adjusted by altering the frequency and distance from the nozzle exit.

28.2.4 H_2O_2 detection in plasma activated water

Since the effect of plasma on cancer cells seem to be mediated by the reactive species [278], it is essential to determine the concentration of H_2O_2 in plasma treated water. A higher level of reactive oxygen species (ROS) results in a larger level of hydrogen peroxide. The amount of hydrogen peroxide caused by plasma treatment in 20 ml DI water were measured using Quantofix peroxide-25 test sticks. For each test, one stick is taken and dipped for

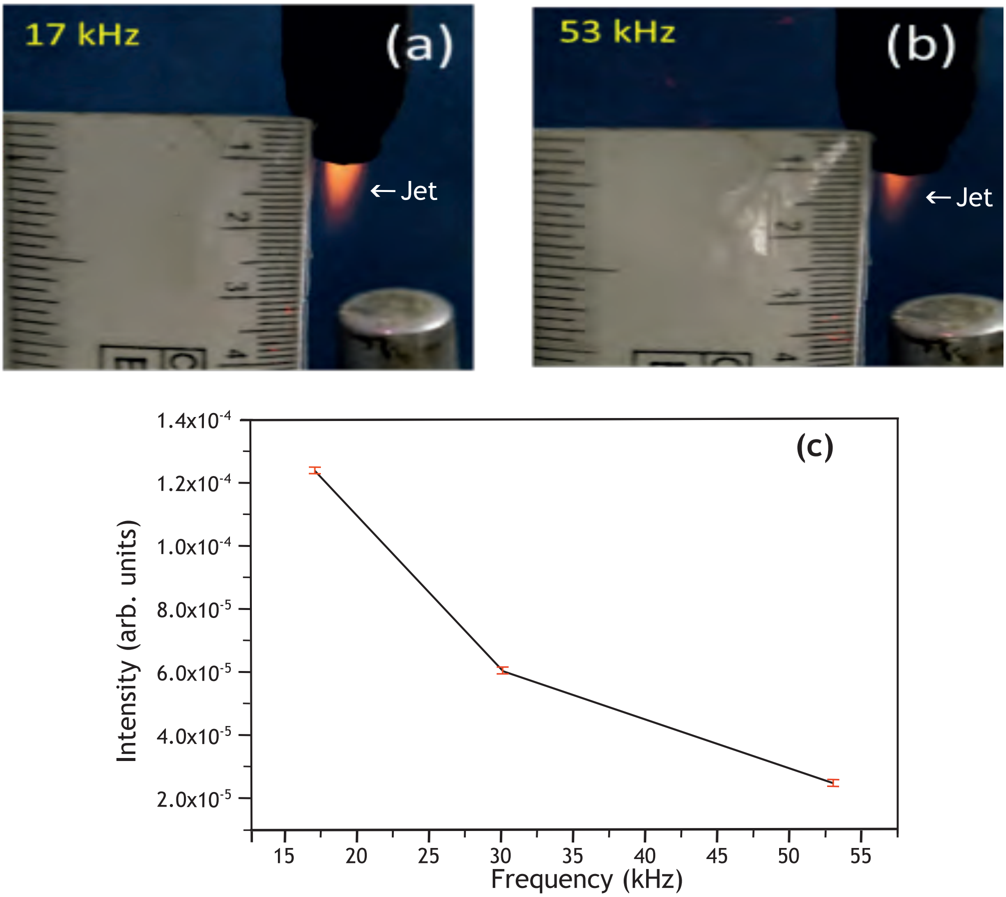

Figure 28.4: Jet volume captured at (a) 17 kHz, (b) 53 kHz, and (c) Intensity measured using photodiode.

one second in plasma-treated water. The colour shift of the stick is matched to the colour indication written on the bottle after 15 seconds. When plasma interacts with water, many direct reactions occur at the plasma-water contact, as well as indirect cascade events that form long-lived species in the water. It has been observed that electron dissociation of water produces OH in all atmospheric pressure low temperature plasmas with electron temperatures larger than 2 eV, as stated in Eq. (28.2) [279].

$$e^- + H_2O \rightarrow \; OH + H + e^- \tag{28.2}$$

Figures 28.5b and 28.5c show the H_2O_2 variation with frequency. The observed difference in H_2O_2 generation is attributed to the change of plasma properties with frequency. It was found that the treated water may be used within 48 hours for plasma treatment.

28.3 Conclusions

In order to increase control over radical intensity and application temperature, a variable frequency atmospheric pressure plasma jet system is developed by L&PTD. The system is

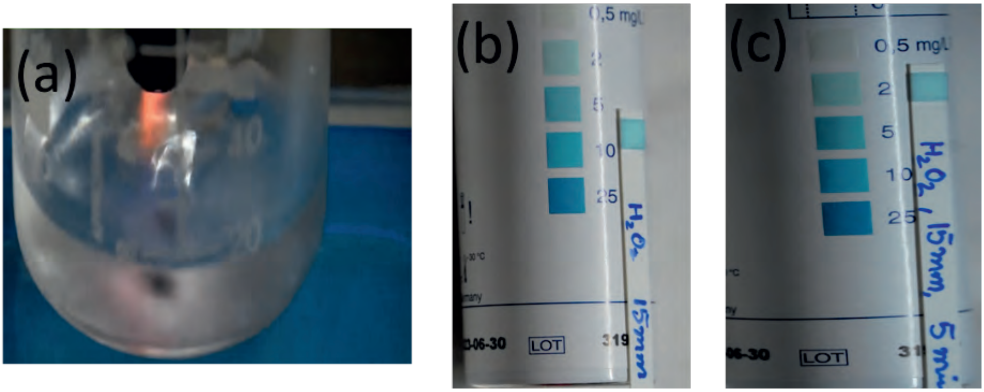

Figure 28.5: (a) Experimental set-up for water treatment at 5 mm from jet, (b) H_2O_2 generation at 17 kHz after 5 min treatment, and (c) H_2O_2 generation at 53 kHz after 5 min treatment.

characterised at different frequencies. It is discovered that altering the frequency simply regulates the radical intensity and gas temperature. Because only a little amount of energy is required to heat plasma gas, the greater ratio of electron temperature to gas temperature demonstrates a high chemical reaction efficiency. In contrast to MHz plasma, the developed device produces less reactive species and also has a lower treatment temperature, making it ideal for treating biological tissues. The technology invented also allows us to adjust the density of oxidative species based on demand.

Frequently Asked Questions

Q1. Why atmospheric pressure plasma jets find application in the field of biomedicine?

Q2. How can one determine the excitation temperature in atmospheric plasmas?

Q3. How the radicals generated by attack the cancer cells?

Q4. What is RONS? Give examples.

Q5. How does the human body generate endogenous ROS?

Q6. How is oxidative stress caused?

Q7. Why do you think a cold atmospheric pressure plasma jets are important in wound healing?

Q8. How is APPJ different from thermal plasma sources?

Q9. Why are the reactive species considered to be useful but sometimes detrimental?

Q10. How is H_2O_2 produced when water is treated with plasma?

Q11. Why is the study of kHz frequency generated plasma important?