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Chapter

Cold Atmospheric Pressure Plasma Technology for Biomedical Application

Rakesh Ruchel Khanikar and Heremba Bailung

Abstract

Cold plasma generated in an open environment with a temperature nearly around room temperature has recently been a topic of great importance. It has unlocked the door of plasma application in a new direction: biomedical applications. Cold atmospheric pressure (CAP) plasma comprises various neutral and charged reactive species, UV radiations, electric current/fields etc., which have several impactful effects on biological matter. Some of the significant biological effects of CAP plasma are inactivation of microorganism, stimulation of cell proliferation and tissue regeneration, destruction of cells by initializing apoptosis etc. Although the detailed mechanism of action of plasma on biomaterials is still not completely understood, some basic principles are known. Studies have indicated that the reactive oxygen species and nitrogen species (ROS, RNS) play a crucial role in the observed biological effects. In this perspective, this chapter first provides a brief discussion on the fundamentals of CAP plasma and its generation methods. Then a discussion on the optical diagnostics methods to characterize the plasma is provided. Optical emission spectroscopy (OES) is used to identify the reactive species and to measure their relative concentration. Other important plasma parameters such as gas temperature, electron/excitation temperature and electron density measurement methods using OES have also been discussed. Then a discussion on the application of CAP plasma in biomedical field is provided. A thorough understanding of biochemical reaction mechanisms involving highly reactive plasma species will further improve and extend CAP plasma technology in biomedical applications.

Keywords: Cold atmospheric plasma, plasma jet, dielectric barrier discharge, reactive oxygen and nitrogen species, plasma medicine, wound healing, cancer therapy

1. Introduction

In physical sciences, the term "plasma" often refers to the fourth state of matter consisting of electrons, ions and neutral particles; while in biology, it refers to the yellowish non-cellular liquid portion of the blood. The Nobel prize-winning American chemist Irving Langmuir first used the term plasma in 1927 to an ionized gas - an electrified fluid carrying electrons and ions, in analogy to the biological plasma carrying blood corpuscles, germs etc. Despite this historical connection, there had been no real correlation between the two plasmas until the emergence of the plasma medicine field recently [1].

Plasma can exist in a variety of forms and can be created in several ways. More than 99% of the visible universe is considered to be in the plasma state. The twinkling of stars, nebulas, auroras in the night sky are some examples of plasma that we observe away from us, and so is our Sun. On the other hand, flashes of lightning, fluorescent tubes, neon signs along our city streets are some other plasma examples that we encounter in our everyday life. Plasma can be classified in different ways based on thermodynamic equilibrium, ionization degree, density etc. Based on the thermal equilibrium between the electrons and the heavy particles, plasma can be categorized into thermal or high temperature plasma and nonthermal or cold plasma [2]. The distinction between thermal and nonthermal plasmas is very important in the context of this chapter. In all plasmas produced by applying an external electric field, the energy transfer to the electrons is much faster than that to the heavier ions. Due to their very high mobility, electrons have the opportunity to heat up to several thousands of degrees of Kelvin before the surrounding environment heats up or even without heating them at all. In thermal plasma, the electrons and heavy particles (neutrals and ions) reach a local thermodynamically equilibrium state, i.e., energy transfer from electrons to the heavy particles equilibrates the energy transfer from heavy particles to the environment, and all the species in the environment remain at almost the same temperature. Because of this, this type of plasma is also called equilibrium plasma. Thermal plasma can reach a temperature up to 10^8 K, as found in the solar core. On the other hand, in nonthermal plasma, cooling of heavier particles is more efficient than the energy transfer from electrons to them, and the gas temperature remains low. Therefore nonthermal plasma is also called non-equilibrium plasma or cold plasma.

With the advent of atmospheric pressure plasma discharges in the early 1990s, various industrial and environmental applications that do not require low pressure operating conditions became possible [3]. Among these, the use of low-temperature atmospheric pressure plasma for biomedical application took center stage. The ability of these cold atmospheric pressure (CAP) plasma discharges to produce enhanced gas phase chemistry at low gas temperature has led to their widespread application in fields that require low temperatures, such as biomedical applications and material processing [4]. In recent years, different devices have been designed to generate cold plasma in atmospheric pressure and have been investigated for their ability to use in biomedical applications [5]. It is demonstrated that CAP plasma could interact with organic substances without causing any electrical/thermal damage. The early results have indicated the great potential of these CAP plasma devices for biomedical applications. These devices can produce plasma at nearly room temperature (less than 40°C) at the contact zone, which is essential for direct application on the human or animal body as well as for sterilization of some medical devices [6]. **Figure 1** shows a typical photograph of a cold plasma jet extending out of a 7 mm quartz tube.

In the mid-1990s, a few researchers, for the very first time, demonstrated the efficient bactericidal property of the CAP plasma. This has opened up a new field of research in science and technology, combining plasma physics and biology called plasma medicine. Since then, CAP plasma devices have been successfully utilized in various applications ranging from sterilization to wound healing to killing cancer cells [3, 7]. From the very beginning, it was expected that the reactive species generated by the CAP plasma play a crucial role in the observed biological effects. Even if many details regarding the mechanism of interaction of plasma with biological matter are still not clear, some basic principles are known, and our depth of knowledge is growing very fast in this field. By the beginning of the second decade of the 2000s, clinical trials on patients started with some success [8]. Several applications have reached the clinical trial stage, and some of the CAP plasma devices have already been certified as medical devices. Woedkte et al. list three clinical trials
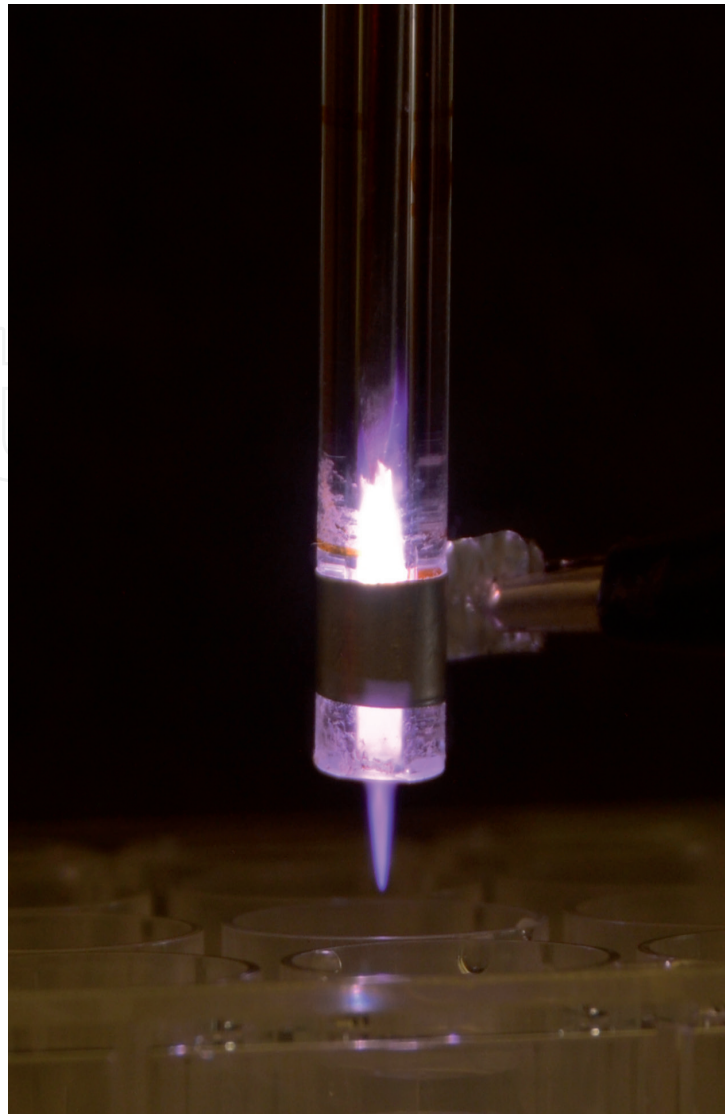
Figure 1.
Photograph of a CAP plasma jet.

using CAP plasma sources conducted in Germany, and two devices got CE marking as medical devices in 2013 [9]. Another device named SteriPlas (Adtec Ltd., London, UK) is the latest one to be certified for use as a medical device [10]. Several other devices have been tested under experimental and laboratory environments and are expecting possible clinical use certification. In 2019, the Food & Drug Administration (FDA) in the USA also approved for their first clinical trials of CAP plasma for cancer treatment [11].

It should be pointed out here that the early studies on plasma application in the biomedical field were concentrated on the thermal effects of plasma [12]. One such successful application was argon plasma coagulation (APC). It has been used to cut tissue in endoscopic applications. These devices operate by heating the tissue using electric current. Therefore their effects are mainly thermal. On the other hand, cold plasma transfers little heat, and its effects are primarily nonthermal.

These remarkable achievements tents of CAP plasma applications took only about 25 years from the initial discovery to the fundamental scientific investigation stage and finally to applications on actual patients.

In this chapter, we shall concentrate mainly on the nonthermal or cold plasmas produced at atmospheric pressure, their production methods, diagnostics, and their various applications in the biomedical field. After the introductory portion in Section 1, the fundamentals of nonthermal plasma is discussed in Section 2.

In Section 3, various CAP plasma generation methods will be discussed. These include dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ) and corona discharge. Then in Section 4, we shall discuss the diagnostics methods of CAP plasma. Due to the small size of the CAP plasma, generally passive, non-contact diagnostic methods are utilized for characterization. Optical emission spectroscopy (OES) is one such very popular non-invasive diagnostic tool for CAP plasma characterization. Then in Section 5, we shall discuss the interaction of CAP plasma with biomaterials and their biological effects. Section 6 discusses various significant biomedical applications of CAP plasma ranging from sterilization to wound healing to killing cancers. The final section then summarizes the application of CAP plasma technology in biomedical applications and their future outlook.

2. Fundamentals of nonthermal plasma

The term nonthermal plasma refers to a plasma that is not in thermodynamic equilibrium, meaning that the temperature of electrons, ions and neutrals are not equal. In this type of plasma, the electrons remain at a very high temperature (up to a few eV, 1 eV ≈ 11,600 K), whereas the temperature of heavy particles is quite low. Because of this reason, they are also termed as non-equilibrium or cold plasma. The high energetic electrons provide the unique reaction chemistry of the cold plasma by facilitating excitation, ionization and chemical dissociation of atoms and molecules at a very low gas temperature. The cold plasma generated at atmospheric pressure produces a myriad of reactive and charged species, including electrons, ions, free radicals, neutral or excited atoms, UV photons etc. These exciting properties of cold plasma have led to their extensive use in various technological fields such as material processing, environmental remediation, nanomaterial synthesis, textile industry, food processing and biomedical applications etc. [13].

Plasmas can be generated by supplying electrical energy to a gas in the form of an electric field. When the applied electric field between the two electrodes is high enough to initiate a breakdown, plasma is formed. Electrons can rapidly gain energy from the applied electric field because of their tiny mass and high mobility. Then they transmit the energy to the neutral atoms and molecules through collisions, providing energy for ionization, excitation, dissociation and other chemical processes. Two types of collisions occur in plasmas [14]:

Elastic collisions: These type of collisions raise the kinetic energy of the neutral species but do not change their internal energies. They increase the temperature of the heavy particles.

Inelastic collisions: These type of collisions between electrons and heavy particles are excitative or ionizing. They modify the electronic structure of the neutral species. When the electronic energy is high enough, it can create excited species or ions. Most of the excited species of plasma have a very short lifetime. They come down to the ground state by emitting a photon. The metastable species are also excited states, but they can decay only by energy transfer through collisions as there are no allowed transitions. Hence, they have a longer lifetime. These collisions do not raise the temperature of heavy particles.

2.1 Paschen's law

The voltage necessary to start a discharge in a gas between two electrodes is given by Paschen's law. It is named after Freidrich Paschen, who discovered it

empirically in 1889. The breakdown voltage depends on the electrode spacing d and the pressure p and is given by the formula [15]:

$$V_b = \frac{B(p.d)}{\ln\left[A(p.d)\right] - \ln\left[\ln\left(1 + \frac{1}{\gamma_{se}}\right)\right]}$$

Here, A and B are constants determined experimentally, and γ_{se} is the secondary electron emission coefficient of the cathode. **Figure 2** shows the dependence of breakdown voltage of various gases on the product of electrode spacing and pressure. It is seen that for a constant electrode spacing, the voltage required to ionize a gas is high towards higher pressure, which implies that a narrow gap is necessary to have a reasonable breakdown voltage at atmospheric pressure.

2.2 Current-Voltage characteristics

The plasma behavior inside a discharge is determined by the values of current and voltage between the electrodes. A typical figure that almost every plasma physics textbook discusses is the current–voltage characteristic of a low-pressure (~ 1 mTorr) DC discharge, which describes different gas discharge regimes as shown in **Figure 3**. Arc discharged is characterized by a very high current and a low voltage between the anode and the cathode. Glow discharge occurs at a low current (typically in mA range) and a high voltage. The corona discharge is characterized by a very low current (few μA) and a very high voltage. For low-temperature atmospheric pressure applications, arc discharge is not acceptable as it produces a very high gas temperature. Therefore, a special setup is necessary to create a cold plasma and keep the plasma current low so that discharge remains in glow and corona regime.
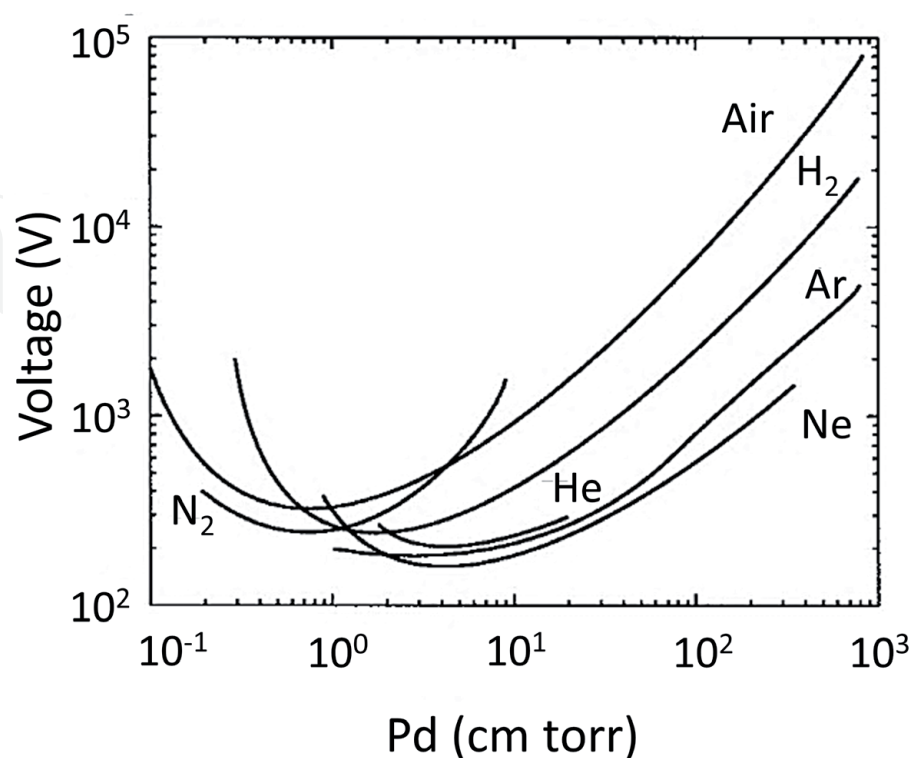

Figure 2.
Breakdown voltage in various gases as a function of the product of pressure, P and gap distance, d for plane parallel electrode.
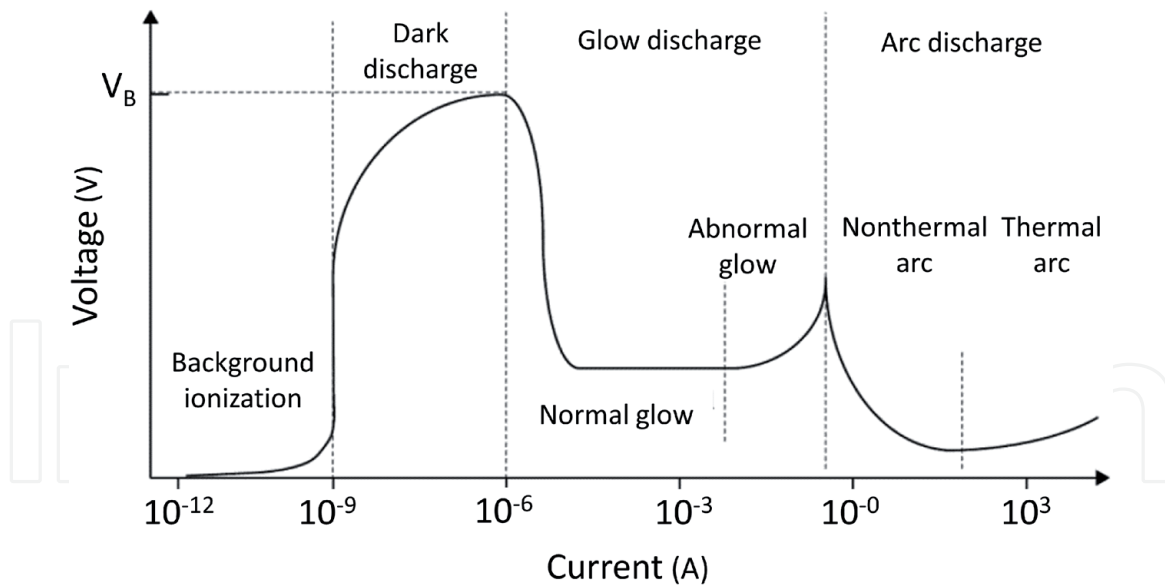
Figure 3.
Current–voltage characteristics of a low pressure DC discharge showing transitions from Townsend to glow and arc discharge.

The necessary condition for a plasma to be suitable for biomedical application is that the plasma has to be produced at atmospheric pressure and the gas temperature has to be near room temperature to avoid thermal damage of biomaterials (tissue etc.) at the contact zone. For this purpose, the plasma needs to be near glow mode. However, glow discharge is generally produced at low pressure. At higher pressure, glow discharge is unstable, and a glow to arc transition can always occur. Therefore, a special electrode arrangement is required to maintain the discharge near glow and corona regime at atmospheric pressure. One general method of producing CAP plasma is to place a dielectric barrier between the two electrodes, and the resulting plasma is known as dielectric barrier discharge (DBD). The role of the dielectric is to limit the discharge current and thus keeps the plasma temperature low. The different types of CAP plasma generation methods are discussed in the next section.

3. Methods of CAP plasma production

Production of atmospheric pressure nonthermal plasma is quite challenging due to high electron-neutral collision frequency, and low applied electric field. Fortunately, several methods have been developed over the years to overcome these challenges. Different production methods have been reported to produce cold plasma in the open environment. These include Dielectric Barrier Discharge (DBD), Atmospheric Pressure Plasma Jet (APPJ), corona discharge etc. Several different working gases such as Helium, Argon, Nitrogen, Heliox (a mix of helium and oxygen), air etc., are used to produce CAP plasma. This section gives a brief overview of the commonly used CAP plasma generation techniques.

3.1 Dielectric barrier discharge (DBD)

One of the most widely used techniques for generating CAP plasma is the dielectric barrier discharge (DBD) using alternating or pulsed electric field. As the name itself suggests, a dielectric cover is used at least at one of the two electrodes for producing the discharge. The function of the dielectric layer is to suppress the spark or arc transition by limiting the discharge current. DBDs are also called

"silent" discharges as it produces no sound during discharge. Typical electrode gap distance in a DBD varies from 0.1 mm to several centimeters. Different dielectric materials such as glass, quartz, ceramics and polymers etc., are used in DBDs. To avoid a spark or arc transition, sufficient breakdown strength of the dielectric layer is necessary for insulation of discharge current. But a thicker layer requires a higher voltage, so a compromise must be made here. The electrode arrangement is generally enclosed in a chamber to introduce various gas mixtures between the two electrodes [16]. High voltages sources with frequencies in the kHz range generally drive DBDs. There are many different configurations of DBD are available, but the concept behind them all is the same. These include planar, parallel plates separated by a dielectric or a cylinder, or coaxial plates with a dielectric tube between them. Some basic DBD electrode configurations are shown in **Figure 4**.

More recently, Fridman et al. developed a floating electrode DBD (FE-DBD) [17]. It is similar to the original DBD and consists of two electrodes: an insulated high voltage electrode and an active electrode. The difference between FE-DBD and DBD is that the second electrode is active, meaning it is not grounded. The second electrode can be human skin, a sample, or any other target. Here, the powered electrode needs to be close to the surface of the second electrode to create the discharge.

The discharge in a DBD at atmospheric pressure is generally non-uniform filamentary type which can result in non-uniform treatment of the sample. The dynamic distribution of these filaments determines the appearance of the discharge. Although DBDs usually produce filamentary plasmas, under certain conditions, homogeneous diffuse plasma can also be created. Several groups have reported successful production of diffuse homogeneous atmospheric pressure glow DBD plasmas [18–21]. The mechanism of generating a glow DBD is to initiate a Townsend breakdown instead of a streamer breakdown [22]. To form an avalanche under a lower electric field and avoid growing a large number of positive space charges, sufficient initial seed electrons should exist in the gap before breakdown.
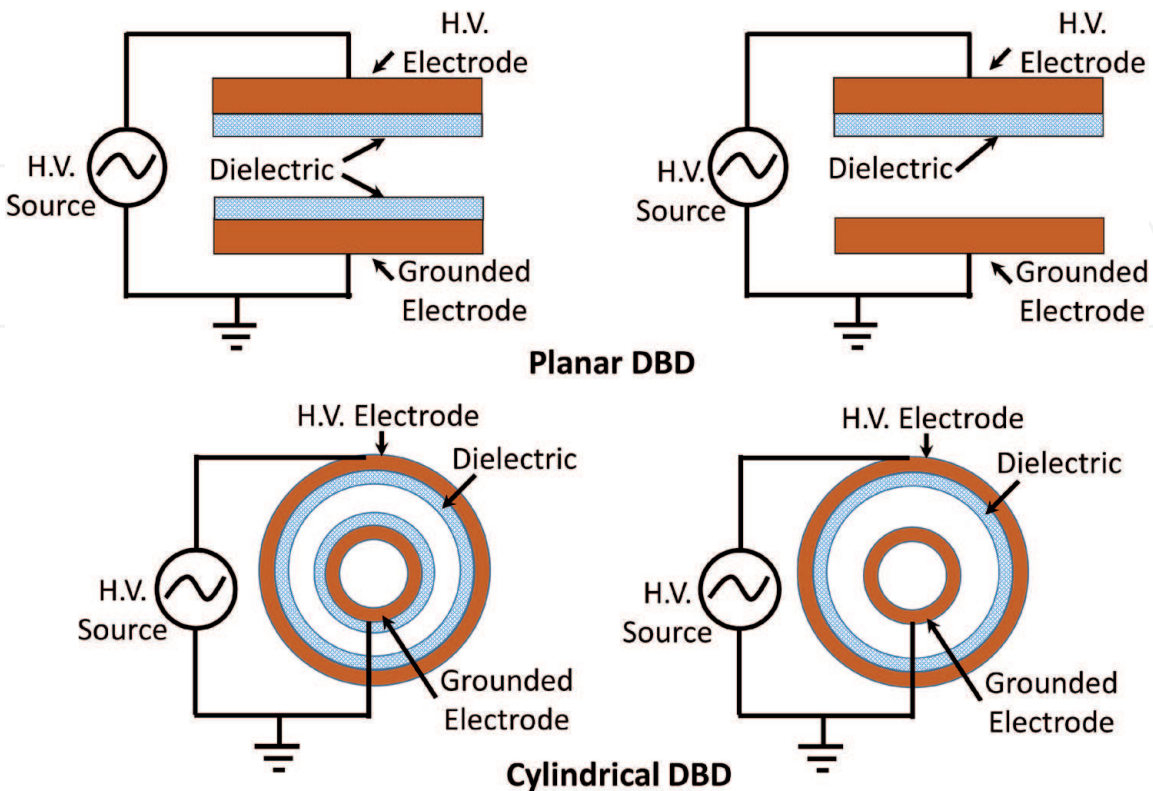

Figure 4.
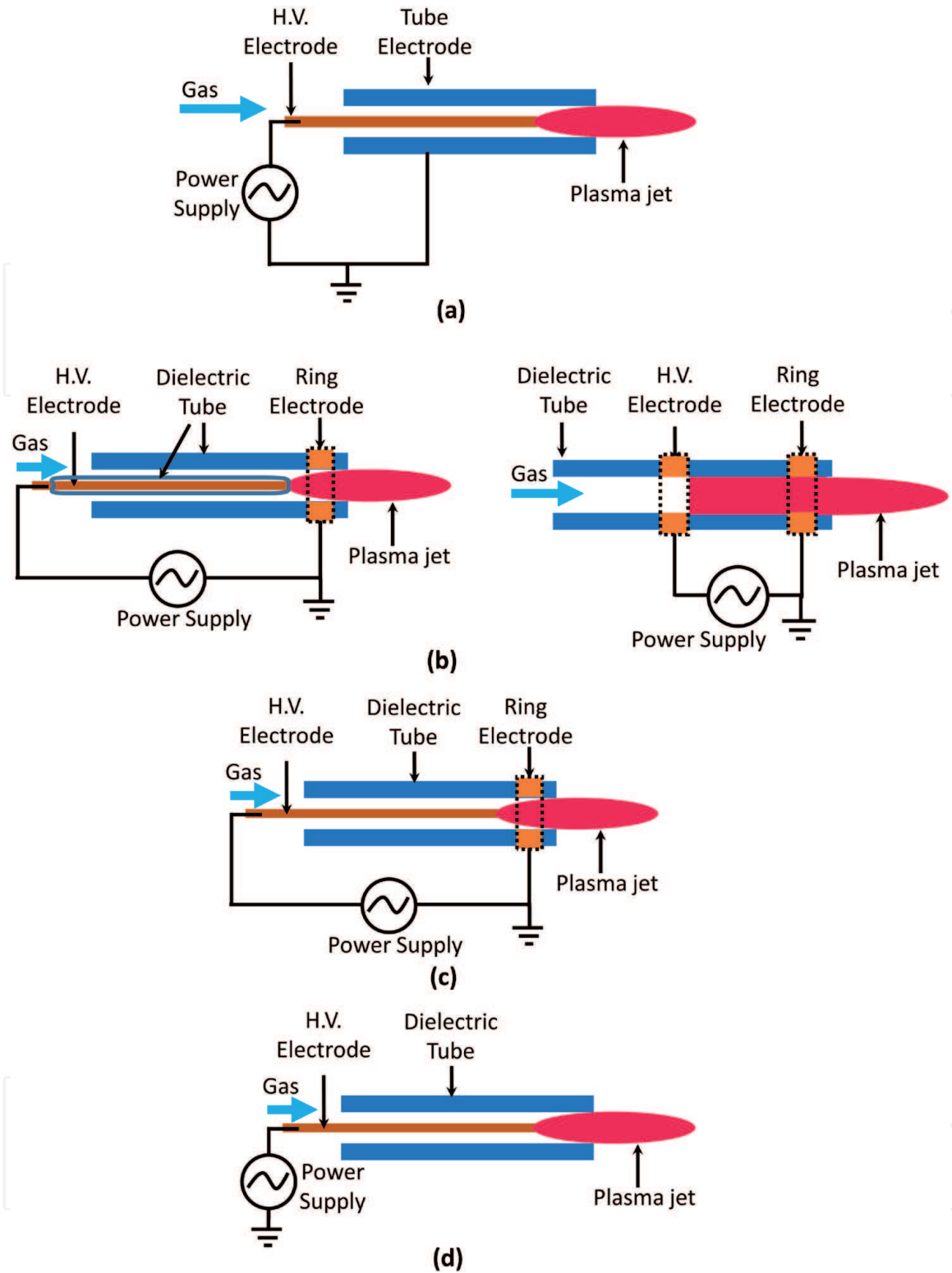*Schematics of DBD with different electrode configurations.*

Figure 5.
Typical electrode configurations of (a) DFE jet, (b) DBD jet, (c) DBD-like jet and (d) SE jet. H.V. = high voltage.

In DBDs, residual species from the previous half period of the applied voltage provide the seed electrons or enhance the initial field for the next discharge cycle. This is the so-called memory effect [19].

3.2 Atmospheric pressure plasma jet

One of the most versatile techniques for the generation of CAP plasma for biomedical application is the nonthermal atmospheric pressure plasma jets. Because of their practical capability to produce plasmas that are not spatially bound or

confined by electrodes, they can be used for direct treatment on any object irrespective of their shape and size. As a result, they can deliver the plasma generated essential short lifetime active radicals and charged particles to the sample to be treated. Many plasma jets with different configuration have been reported in the literature to generate CAP plasma. There exist various classifications schemes for CAP plasma jets. Some authors classify CAP plasma jets according to the power sources' excitation frequency to generate the plasma. This frequency range can vary from DC to GHz. Accordingly, they are named DC plasma jet, pulsed-dc plasma jet, KHz operated plasma jet, RF operated plasma jet and Microwave driven plasma jet [16, 23–25]. Some authors also use the names 'plasma flame', 'plasma plume', 'plasma gun', 'plasma stream', 'plasma pencil' etc., for plasma jets [24]. The plasma jets are operated with inert gases such as helium, argon etc. or a mixture of inert gas and a few percent of reactive gases of interest. The earliest known CAP plasma jet was developed by Koinuma et al. in 1992. It was powered by an RF source [26].

A comprehensive collection of various types of nonthermal plasma jet arrangements has been discussed in detail by Lu et al. [27]. They are classified into four different groups, namely dielectric-free electrode (DFE) jets, dielectric barrier discharge (DBD) jets, DBD-like jets and single electrode (SE) jets. The DFE jets consists of an inner powered electrode and an outer grounded electrode, as shown in **Figure 5(a)**. There is no dielectric material between the two electrodes. The gas temperature of this jet is relatively high, and cooling water is needed for continuous operation and to keep the temperature low. There is always a risk of arcing in this jet when standard operating conditions are not met. The DFE jet is not suitable for direct biomedical applications. However, it is effective for surface sterilization. It is operated by an RF power source.

In DBD jets, a dielectric layer is present between the two electrodes, and the plasma is not in contact with any electrode. The power consumed by this plasma jet is very less (of the order of few watts). Due to the presence of dielectric, these plasma jets are relatively safe as there is no risk of arcing and is ideal for biomedical applications. The DBD jets can be powered by a KHz ac source or by a pulsed-dc source. **Figure 5(b)** shows the typical electrode configurations of DBD jets.

In DBD-like plasma jets, the discharge is more or less DBD-like when plasma is not in contact with any object. There is no dielectric material between the live electrode and the object to be treated. In this type of plasma jets, easily more power can be delivered to the plasma, and the plasma can be very reactive. As long as arcing can be avoided, this type of jets has their own advantages. For biomedical applications, this kind of devices should be handled carefully because of the risk of arcing. These plasma jets can be powered by KHz ac, RF or pulsed dc sources. The typical configuration of a DBD-like plasma jet is shown in **Figure 5(c)**.

The SE jets are similar to DBD-like jets, except there is no electrode outside the dielectric tube. These jets can be operated by dc, KHz ac, RF or pulsed dc power sources. These kind of jets are not suitable for biomedical application due to the risk of arcing. **Figure 5(d)** shows the basic electrode configuration of a SE plasma jet.

Although the plasma produced by CAP plasma jets looks homogeneous to the naked eye, it is actually discrete in nature when observed by using fast imaging. The plasma volume consists of some "bullet "-like structure, with a propagation speed of more than ten kms^{-1}. This discrete nature of plasma jet was first reported by Teschke et al. using an RF-driven plasma jet [28] and by Lu et al. using a pulsed dc plasma jet [29].

3.3 Corona discharge

A corona discharge is a well-known non-equilibrium discharge that occurs around a pin or thin wire electrode where the electric field is higher near the
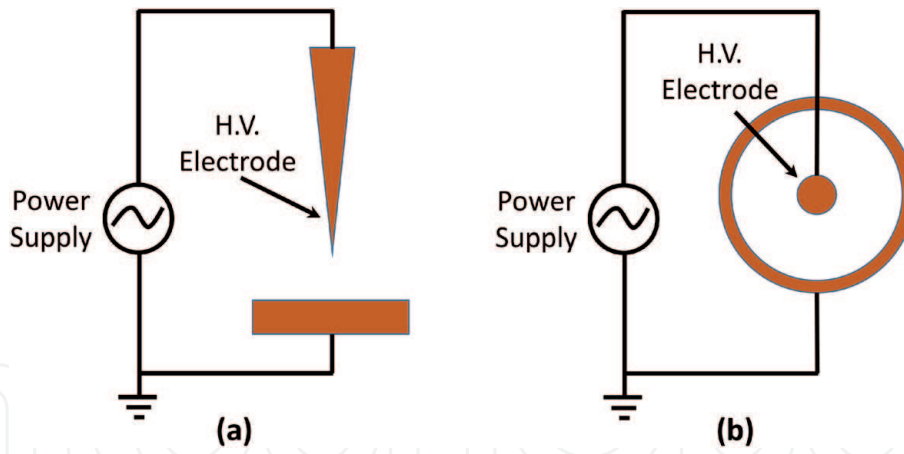
Figure 6.
Typical electrode configurations of corona discharge. (a) Point to plane and (b) wire to cylinder configuration.

electrode edge but decreases quickly with increase of distance [18, 30]. Due to this highly non-uniform electric field, the gas breakdown occurs near the pointed electrode. The electric field strength is high enough to form a conductive region but insufficient to cause electrical breakdown to nearby objects. This type of non-uniform electric field can be created using an asymmetric electrode pair arrangement such as point to plane or wire to cylindrical electrodes, as shown in **Figure 6**.

The corona discharge can be classified into two types depending on the polarity of the HV electrode. The physics of these positive and negative corona discharge is considerably different. This happens due to the vast difference in the mass of electrons and ions. In the positive corona, the electrons are attracted to the HV electrode, and the positive ions are repelled. The secondary electrons are created by photoionization in the gas near the electrode. The electrons are then attracted towards the electrode, which begins the process of further electron avalanche through inelastic collision with neutral gas molecules.

On the other hand, in the negative corona, the electrons move away from the HV electrode. In this case, the secondary electrons are primarily generated by the photo-electric effect from the electrode surface itself. The process is similar to the Townsend breakdown. The electron avalanche then multiplies through impact ionization. As we go away from the electrode, positive ion accumulation occurs, and the electric field becomes weak. As a result, ionization diminishes there.

A corona discharge can be driven by direct-current (DC), alternating-current (AC), or pulsed voltage. It has widespread applications in various fields, such as ozone synthesis, material processing, water purification, electrophotography, copier machine, bacterial inactivation, wound healing and medical surface preparation etc. This type of plasma provides substantial flexibility in treating various products and materials used in the medical industry, for example, syringe barrels, pill bottles, catheter tubing, IV tubes and surgical gowns etc.

4. Diagnostics of CAP plasma

Due to the small size and transient discharge behavior of CAP plasma, plasma diagnostics is very challenging. The use of invasive diagnostic techniques such as Langmuir probe is not suitable as they significantly disturb the plasma and, as a result, yield incorrect values. Therefore, various non-invasive optical diagnostic techniques are the choices of interest for determining the plasma characteristic of CAP plasma [31]. One such most widely used technique is Optical emission spectroscopy (OES).

It is a relatively simple and easy to implement method for determining various plasma properties. The light emitted by the plasma due to deexcitation contains various valuable information regarding the plasma. An optical emission spectrometer can capture this radiation, from which one can extract information on the different species present in the plasma. Also, using the emission spectrum, one can estimate various plasma parameters such as electron/excitation temperature, neutral or heavy particles gas temperature, electron density, concentration of different reactive excited species etc. **Figure 7** shows a typical emission spectrum obtained from an argon CAP plasma jet.

The emission spectrum of CAP plasma often contains emission from molecular species like N_2, N_2^+, OH etc. The highly energetic electrons of plasma can easily transfer their energy to the low lying molecular rotational and vibrational states. In atmospheric pressure condition, the rotational and translational degrees of freedom of gas molecules remain in equilibrium through collisions. Consequently, the rotational temperature gives the value of gas temperature. Generally, the OH rotational band around 306–309 nm, the second positive system of N_2 and the first negative system of N_2^+ are used to obtain the gas temperature. From the best fit between the experimental spectrum and a simulated theoretical spectrum of a particular molecular band, the rotational temperature or the gas temperature of the plasma can be determined. The simulated spectrum can be calculated using software like *Specair* [32] and *Lifbase* [33]. The Boltzmann distribution of the rotational levels is assumed to obtain the temperature.

Another important plasma parameter, the electron/excitation temperature, can be obtained using the Boltzmann plot technique [34]. In this technique, the spectral line intensity (I) and the excitation temperature (T_{exc}) is related by the formula: $\ln\left(I\lambda / g_k A\right) = -E_k / kT_{exc} + C$, where λ is the wavelength of the line, g_k is the upper state degeneracy, E_k is the upper level energy and A is the transition probability. If the Boltzmann law is satisfied, the plot of $\ln\left(I\lambda / g_k A\right)$ vs. E_k becomes a straight line, and the inverse of the slope gives the excitation temperature. Typically, the electron temperature is found to be near 1 eV in CAP plasmas. In low-temperature plasmas, the low energy electron number is much higher than that of high energy electrons. So the bound electrons on the higher excited levels can be in collisional equilibrium with the free electrons because the energy difference between higher excited levels and the ionization energy is small. So they can satisfy Boltzmann law.
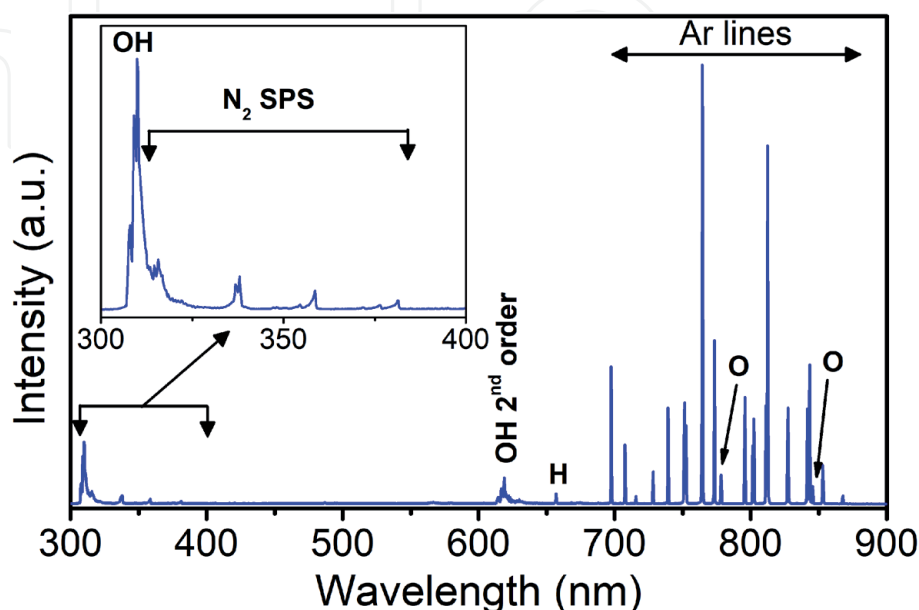

Figure 7.
Emission spectrum of an argon CAP plasma jet.

The population of electrons on the lower excited levels usually do not satisfy the Boltzmann law because they are not in collisional equilibrium with the free electrons. The excitation temperature can also be measured using another well-established method called the line intensity ratio method [35].

The electron density can be measured from the study of spectral line broadening. Spectral lines are always affected by various broadening mechanisms, such as Stark, Van-der-Waals, instrumental, Doppler broadening etc. By extracting the Stark part from the total broadening, the electron density can be determined. The popular lines used for this measurement is the hydrogen Balmer lines. These lines can appear as an impurity from the moisture, or hydrogen can be added in a small amount to the discharge for diagnostic purposes. The H_β 486.13 nm line is most widely used because of very strong Stark broadening and less self-absorption. It is also not much affected by broadening due to ion dynamics and temperature variations. Electron density as low as 5×10^{13} cm^{-3} can be measured using this method [31]. The H_α line at 656.3 nm can also be used for this purpose. However, the accuracy of the electron density value obtained from this line is relatively less. Other non-hydrogenic atomic lines can also be used to determine the electron density using this technique. For a detailed discussion on electron density measurement from the Stark broadening, an interested reader can go through the references [36, 37]. Typically, the electron density can vary between 10^{10} and 10^{14} cm^{-3} in CAP plasmas [31].

Apart from OES, there are many other techniques to study CAP plasmas. The active laser spectroscopy techniques such as laser induced fluorescence (LIF), two-photon absorption laser induced fluorescence (TALIF) can be used to obtain information on the ground state and long-lived, nonradiative excited atoms, molecules or radicals [2]. This technique has been used for many years for plasma diagnostics. Popular laser sources used in LIF are the Nd:YAG laser, dye lasers, excimer lasers, and ion lasers. Other well-known techniques such as Thompson scattering can give direct information on the electron density and temperature. Rayleigh and Raman scattering can provide information on the gas density and temperatures. The optical absorptions spectroscopy and cavity ring down spectroscopy (CRDS) can determine the absolute densities of certain plasma species. Other techniques known from chemical analysis such as UV and FTIR absorption spectroscopy, mass spectrometry, gas chromatography or electron paramagnetic resonance spectroscopy etc., are also used to identify and quantify ions and reactive species in the plasma and to track its transition from plasma phase to liquid phase [6, 18, 38].

5. Effects of CAP plasma on biomaterials

The bactericidal property of plasma was first demonstrated in the mid-1990s, and that started a new research field combining plasma physics and medicine, i.e. plasma medicine [39]. From the very beginning, it was realized that the plasma generated reactive species play a pivotal role in the observed biological effects of CAP plasma. With time, our knowledge of the mechanism of plasma action on cells and tissues has started growing significantly. The basic understanding of the mechanisms of CAP plasma effects on biomaterials is crucial to establish plasma technology application in the biomedical field.

Notwithstanding the use of different plasma sources, working gases, microorganism stains, cell types etc., some general biological plasma effects have been mentioned repeatedly in the basic research of plasma biomaterial interactions such as [9, 18]:

1. Lethal plasma effects

   a. Inactivation/killing of microorganisms

   b. Inactivation or destruction of cells by initialization of apoptosis in mammalian cells

2. Non-lethal plasma effects

   a. Stimulation of microorganism metabolism

   b. Detachment of cells from the cell cluster

   c. Influence on angiogenesis and cell proliferation and consequently promote wound healing and tissue regeneration

   d. Influence on cell migration, expression of cell surface proteins

The possibility to inactivate microorganisms on sensitive surfaces of living structures like intact or wounded skin has attracted the very early interest of physicians. The *in vitro* and *in vivo* results of plasma assisted wound healing showed that plasma acts in a two-stage process. The first one is the antiseptic effect to restore the physiological potential of the wound area by decreasing bacterial load, and the next one is additional stimulation of the healing processes by tissue regeneration independent from antiseptic effects.

Based on the current state of knowledge on the mechanism of plasma biomaterial interaction, it can be deduced that the biological effects of CAP plasma are based on two principles:

1. Biological plasma effects are primarily initiated by plasma induced changes of the liquid environment of tissue and cells.

2. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated in or transferred into liquid phases play a vital role in plasma-induced biological responses.

The biologically important ROSs include superoxide ($O_2^-\bullet$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), singlet oxygen ($^1O_2$), ozone (O_3) etc. The RNSs include nitric oxide ($\bullet NO$), nitrogen dioxide ($\bullet NO_2$), nitrogen trioxide (NO_3), peroxynitrite ($ONOO-$) etc. [7]. These reactive species are formed either by plasma–liquid interaction or by plasma–air interaction. These reactive species act on cells and tissues in the same way as that occur in the body's regular biochemical and physiological processes [40]. Based on this fundamental insight, the field of redox biology can now be used to explain the biological plasma effects. For example, hydroxyl radical causes peroxidation of unsaturated fatty acids present in lipids constituting the cell membrane. The strong oxidative properties of hydrogen peroxide affect proteins, lipids tend DNA. Nitric oxide is known to affect the regulation of collagen synthesis, cell proliferation, regulation of immune deficiencies, induction of phagocytosis and angiogenesis etc. In cancerous cells, it is suspected that the action of CAP plasma increases intracellular ROSs, which can lead to cell cycle arrest at the S-phase, DNA breaks, and induction of apoptosis (programmed cell death). Researchers have also shown that the plasma generated ROS and RNS can penetrate biological tissues up to more than 1 mm depth.

Therefore they can interact with the cells on the surface as well as with those beneath [41]. As these ROSs and RNSs regularly occur in cell biological processes, mammalian cells have mechanisms to protect themselves from over-concentration of these species, which might otherwise lead to oxidative stress with severe biological consequences such as genotoxic DNA changes. Detailed investigations till now indicate that the application of CAP plasma does not increase the risk of genotoxicity [6].

Other plasma components such as UV radiation, electric field/electric current also play an additional role. However, the role of UV in direct biological effects is estimated to be very low due to the low dose of UV in these plasma devices. But its supporting role in reactive species generation is essential. Electric fields or currents have varying direct biological effects on living tissue, and it strongly depends on the type of discharge. The magnitude of these electric fields can be as high as several kV/cm, and they are suspected of playing a part, such as in cellular electroporation, which may help larger molecules to enter the cells. Besides these, the plasma generated charged species such as electrons and ions are also assumed to play some roles in the observed biological effects. More research is needed to identify the role of these plasma components for their possible part in biological and medically relevant plasma action, above all reactive species.

6. Applications of cold atmospheric plasma in medicine and healthcare

CAP plasma can be employed in two different ways in biomedical applications. The first one is termed direct exposure or direct treatment, where plasma comes in direct contact with the biological sample to be treated. In this mode, all the plasma generated species, both long lived and short lived species, come in direct contact with the sample and work synergistically. The second mode is termed indirect exposure or indirect treatment. In this method, plasma is used to activate a liquid medium, and then the plasma activated liquid (PAL) is used for treatment. In this case, only the long lived chemical species such as nitrates, nitrites, and H_2O_2, which diffuse and solvate into the aqueous state, play a role. One advantage of PAL is that it can be stored and used at a later time and the composition may be tuned. Both types of CAP plasma treatment have shown significant success in biomedical applications. However, the amount of research reported using direct treatment is more than that using indirect treatment method.

Nowadays, CAP plasma has been successfully applied in various biomedical applications, including inactivation of microorganisms, sterilization of infected tissues, blood coagulation, skin regeneration, tooth bleaching, wound healing, cancer therapy etc. CAP plasma treatment for regenerative processes, such as wound healing, is one of the most advanced applications of plasma technology in the biomedical field. Three plasma devices have already been CE certified for medical use. The very first one is the medical device kINPen® MED (INP Greifswald/neoplas tools GmbH, Greifswald, Germany). The second one is PlasmaDerm® VU-2010 (CINOGY Technologies GmbH, Duderstadt, Germany), and the latest one is the SteriPlas medical device (Adtec Ltd., London, United Kingdom). Their specific purpose is wounds and skin diseases treatment. However, several more plasma devices are awaiting possible certification for clinical application [10, 42].

6.1 Use of CAP in disinfection, wound healing and dermatology

In the beginning, CAP plasma in medicine was applied to the treatment of chronic wounds [43]. Isbary et al. in 2012 first reported about the clinical trials

of CAP plasma treatment on chronic ulcer wounds [44]. The authors reported significant infection reduction without any side effect. After that, several clinical trials have proven that CAP plasma action sufficiently reduces the bacterial load on wounds and improve chronic ulcer healing [42]. CAP treatment is also found to accelerate the rate of wound closure at early stages after wounding. Various studies have shown that CAP plasma is an effective tool for disinfection of a variety of bacteria and fungi on the skin and wound pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa* etc. [45].

Besides wound healing, the CAP plasma has also been investigated in dermatological applications. These include treatment of infective and inflammatory skin diseases like atopic eczema herpes, zoster, athlete's foot, acne and others [42, 45]. Some studies have reported positive effects of CAP plasma, but still, more research is required to understand the biochemical processes involved in dermatological applications.

6.2 Use of CAP plasma in cancer therapy

Another most exciting and promising area of CAP plasma application in medicine is cancer treatment. Cancer cells appear to be more vulnerable to CAP plasma than healthy cells. Researchers have shown that CAP plasma can induce programmed cell death (apoptosis) in cancerous cells while leaving their nearby healthy cells essentially unaffected. Up until various *in vitro* and *in vivo* studies have been performed to study the CAP plasma effect on different cancer cells. These studies have repeatedly shown the anti-cancer capacity of CAP plasmas.

It has been reported that CAP plasma treatment increases intracellular ROS concentration. This, in turn, creates a severe redox imbalance in cancer cells as they are already under oxidative stress. Then, the redox imbalance leads to mitochondrial dysfunction, DNA damage, advanced state of oxidation of proteins, caspase activation, etc., and ultimately leads to death of cancer cells [11].

The early animal studies performed had shown the promising potential of CAP plasma in cancer therapy [46, 47]. Since then, many *in vivo* studies have been conducted, and similar positive results have been reported for various types of cancer cells injected under the skin of mice. To date, CAP plasma treatment has demonstrated a significant anti-cancer effect on approximately twenty cancer types *in vivo*. These cancer cell lines include skin, brain, head and neck, breast, leukemia, hepatoma, colorectal, bladder, cervical, lung etc. [48]. In 2019, Metelmann et al. reported the first clinical trials on patients with advanced head and neck cancer [49]. The trial demonstrated the clinical relevance of CAP plasma in cancer treatment and reported an overall positive effect. Some other researchers have shown that some radiation-resistant and chemo-resistant cancer cells are also sensitive to plasma treatment. Some studies have demonstrated CAP plasma as an intra-operative adjuvant treatment. It can be used to inactivate the remaining cancer cells after a surgery [42, 50]. In the USA, the Food & Drug Administration (FDA) approved the first clinical trials of CAP plasma to treat the cancer tumors remaining after surgery in 2019 [11].

6.3 Use of CAP plasma in dentistry

Another long time studied field of CAP plasma application is in dental medicine. The most predominant oral diseases are caries and periodontitis, which are initiated by dysbiotic biofilms. The application of plasma primarily aims to reduce these biofilms on tooth substances and surrounding tissues. Also, instead of using an antimicrobial solution to oral cavity sites for disinfection, CAP plasma treatment can eliminate the unpleasant side effects from anti-microbial solution use. There

is a broad spectrum of research going on the possible oral and dental application of CAP plasma ranging from disinfection of root canals, inactivation and removal of biofilm on teeth, treatment of infections and wounds of oral mucosa, dentures and on dental implants, tooth whitening, decontamination and coating of dental prosthesis, cleaning and optimization of tooth and implant surfaces to improve bonding of dental fillings [10, 42].

6.4 Use of CAP plasma in other biomedical areas

Another field explored from the beginning of plasma medicine research is CAP plasma use for hemostasis and blood coagulation [42]. Application of CAP plasma leads to blood coagulation in a much localized manner without damaging the tissue. Therefore it can be a valuable supporting technique in surgery. Aside from these large fields, the potential of CAP plasma in ophthalmology [51, 52] and neurology [53, 54] is also under investigation.

7. Summary and future outlook

The application of CAP plasma technology in the biomedical field has opened up new frontiers in science and technology. It has reached new heights of scientific progress in recent years and has been successfully applied in numerous applications, ranging from sterilization to wound healing to killing cancers. Three CAP devices have already been certified for clinical use for the treatment of chronic and infected wounds. Even if many details regarding the mechanism of interaction of plasma with biological matter are still not clear, some basic principles are known. The biological and medically beneficial plasma effects are primarily triggered by the plasma generated reactive oxygen species (ROS) and reactive nitrogen species (RNS). While CAP plasma has already reached standard medical care status in some areas, such as sterilization, disinfection, wound treatment etc., only primary and pre-clinical data for its effects are available in some other areas. A better understanding of the mechanism of its action will allow further improvement and extensions of the CAP plasma technology to achieve its full therapeutic potential. Also, the development of new plasma devices and modifications of the existing ones will open up new opportunities. In this case, international standardization of the methods to characterize the plasma devices is required to allow better comparability of results obtained from different findings.

It cannot be hypothetically omitted that CAP plasma application does not have some minimal adverse consequences at the molecular level. All these findings and the odds are subject to ongoing investigations, but the current results indicate that the various benefits of CAP plasma outweigh the unproven negative effects. What we all foresee is that the CAP plasma is on its way to clinical routine. Essentially a multidisciplinary research platform is growing with experts from many different fields like plasma physics, biochemistry, molecular biology, medicine etc. to address the issues with synergetic approach.

In the 20th century, laser technology caused a medical revolution with swift development. It successfully got integrated into medicine and created its own medical field called laser medicine. Now, CAP plasma has the chance to repeat this history and to be at the forefront of scientific and technological progress in medicine of the 21st century.

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