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REVIEW ARTICLE

Atmospheric Cold Plasma: A Brief Journey and Therapeutic Applications from Wound Healing to Cancer Biology

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Abstract

Cold Atmospheric Plasma (CAP) has now become a well-known new edge technology in the field of biomedical science to agriculture and food technology. Ionized gas known as cold atmospheric plasma has recently been the subject of intense inquiry by scientists for its potential application for treatment in oncology and dentistry. Air, Helium, Argon, Nitrogen, and other gases can all be used to create Cold Atmospheric Plasma. Cold plasma can effectively and safely inactivate spores, bacteria, fungi, viruses, and small molecules and thereby improving wound healing, combating microbial infections, and treating skin conditions with great efficiency. Interestingly the in vitro and in vivo demonstration of CAP has shown promising applications in cancer healing and treatment. The most widely employed technique for producing and sustaining a low-temperature plasma for use in technological and scientific applications involves applying an electric field to a neutral gas. The non-equilibrium atmospheric pressure plasma jet (NAPPJ) and the dielectric barrier discharge (DBD) have both been widely used in biomedical applications. This review aims to evaluate the emerging plasma technology - the basic science, technical aspects and provide insights of biomedical application in diverse area.

Key Words: *Atmospheric Cold Plasma (ACP); Cold Plasma Technology; Low Temperature Plasma (LTP); Biomedical science; Cancer therapeutic*

Introduction

On Earth, there are primarily three distinct states of matter including gas, liquid, and solid, but the cosmos possesses an abundant fourth state of matter named as plasma. In 1926, Irving Langmuir coined the term "plasma" to characterize this state of matter, stating that "...The ionized gas has about equal amounts of ions and electrons, leading to a very low space charge close to the electrodes, where there are sheaths with relatively few electrons." Therefore, the zone that has balanced charges of electrons and ions are referred to as plasma [1] which can be created artificially in the case of fluorescent and neon lights, plasma televisions, and other accessories or it can also be found in natural occurrences like "stars and lightning". Plasma technology is a rapidly expanding field of study as it has received particular attention for its applications in the field of biomedical science including materials and devices [2], textile surface modification [3], removing heat-

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sensitive chemicals from surfaces, sterilization of water, and more recently for healing of wound and decontamination of food. Use of cold plasma has enabled the medical sector in the areas of pathogen eradication by modulation of cellular activity and thereby implanting functionalization. Due to its advantageous combinations of reactive species and its ability to control inflammation in cells and tissues, previous researchers have demonstrated that plasma aids in the healing of wounds [4]. In our current review, the study aims to evaluate the emerging world of plasma technology, its application and provide valuable insight into the technical background associated with it.

Plasma Science and Technology

In the last several decades, low-temperature plasma (LTP) that can be produced at atmospheric pressure and temperatures below 40°C have paved the way for a brand-new field of plasma applications, especially in biological sciences. Notably, the density and temperature of the electrons vary according to the type of energy source and the amount of energy supplied to the plasma – thereby classifying it as high-temperature and ultra-low-temperature plasma. Since majority of the coupled electrical energy is moving towards the electron component instead of the entire gas stream (Figure 1) the temperature of the heavy particles stays close to that of the ambient air during the generation of cold plasma, making it suitable for use in processes where high temperature is undesirable [5].

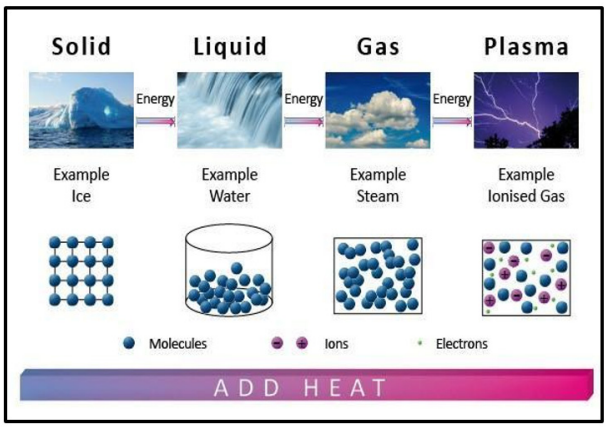

Figure 1) *Different states of matter.*

Interestingly, this adaptable sanitizing technique only needs an electrical charge and a carrier gas like air, O_2, N_2, or He. Numerous cold plasma systems are now being developed that can be operated in low-pressure treatment chambers or at atmospheric pressures [6].

Techniques for Generating Plasma

The most widely employed technique for producing and sustaining a low-temperature plasma for use in technological and scientific applications involves applying an electric field to a neutral gas [7]. Energy is added to a neutral gas to generate charge carriers (Figure 2) that create plasma [7]. When neutral molecules and atoms in the feed gas are struck by electrons or photons with enough energy, they form electrons and ions in the gas phase also known as electron-impact ionization or photoionization.

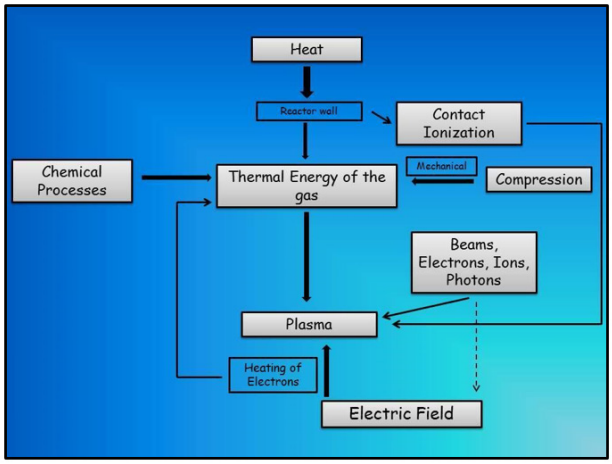

Figure 2) *Schematic diagram of plasma generation.*

Sources of Cold Atmospheric Pressure Plasma

The non-equilibrium atmospheric pressure plasma jet (NAPPJ) and the dielectric barrier discharge (DBD) have both been widely used in biomedical applications.

Dielectric Barrier Discharge (DBD)

Following its early and effective usage in the mid-1990s to inactivate microorganisms,

DBDs have gained potential applicability in the current field of biomedicine [8] including removal of tumors and cancer cells to healing of wounds [9]. Interestingly, the characteristic of plasma is determined and controlled by the electron energy distribution function (EEDF). Notably, the short repeated high-voltage pulses can increase ionization and excitation by preferentially heating the electron population [10]. Whereas the low-temperature plasma is maintained at a stable non-equilibrium state by pulses [11].

Non-equilibrium Atmospheric Pressure Plasma Jets (N-APPJ)

Although plasma jets have been used in the past for applications such as material processing [12], bio-tolerant plasma jets have also been used in plasma medicine recently as depicted in Koinuma et al. [13]. Low-temperature plasma plumes can be released by these jets into the atmosphere and can interact with soft materials, including biological tissues/material [14], without causing significant harm as it can be operated at temperatures below 40°C. Noteworthy to add, the plasma extends out from the high-voltage electrodes and into a low-voltage area, the target cells or tissues are not electrically shocked or harmed by the plasma. However, a very strong local and instantaneous electric field is visible near the tip of the plasma plume. This field may affect both the treated target and the plasma plume's propagation. Low-temperature plasma sources generate reactive oxygen and nitrogen species that are known to play important biological roles modulating redox regulation of cell signalling [15]. Additionally, other substances produced during generation of plasma sources including electric fields, UV and VUV light, and charged particles (electron and ion) are also actively contributing towards biological applications. For instance, the electric field can electroporate cell membranes, allowing chemicals to enter the cells and harm the mitochondria and other internal organelles as well as macromolecules like lipids, proteins, and DNA [16].

The capacity of CAP to inactivate germs has now been increasingly relevant as with the rise of significant healthcare issues in modern society. These include: (1) Antibiotic resistance, for example Methicillin Resistant Staphylococcus aureus (MRSA) and Clostridium difficile (C-diff) strains in hospital-acquired infections (HAI) as fatal to patients with weakened immune systems. (2) Chronic wounds, such as diabetic ulcers where healing is slow and are prone to high levels of infection advocated by a range of bacteria. The first clinical experiments on the use of cold atmospheric plasma to heal chronic wounds were conducted in 2010 where the outcomes were highly promising [17]. The enticing method has shown significant reduction of bioburden in infected chronic wounds and effectively inactivated germs like MRSA. Moreover, there are multiple plasma devices available today that are licensed for treating a variety of dermatological conditions.

Types of Exposure

Direct exposure

The biological targets are often exposed by plasma agents including charged particles, photons, an electric field and reactive species during direct exposure. While manifesting biological effects such as inactivation of germs by killing spores and lysis of gram-positive cells the above particles act alone or in combination [18]. Practical examples of LTP based application include inactivation of germs, sterilizing heat-sensitive medical equipment, destroying surface attached biofilms, cleaning wounds, decontaminating fluids, foodstuffs, farm commodities and so on. Through the modification of cell signalling pathways, direct exposure of LTP has also been employed to modify eukaryotic cell activities non-lethally [19] and on the other hand fatally on cancer cells and tumors [20]. Selective lethality of

LTP was reported previously in dose dependent manner on various cell lines including cancer cells at specific exposure rate with 100% killing efficiency. Interestingly, direct exposure enhances the level of reactive oxygen species inside the cells as reported earlier [21]. Due to the high levels of oxidative stress inside the cancer cells with the increase in ROS results in severe redox imbalance and thereby DNA damage, mitochondrial dysfunction, caspase activation, an advanced state of protein oxidation, or any combination of the above and ultimately killing the targeted cancer cells.

Indirect exposure

Indirect exposure of cancer cells with plasma-activated water (PAW) and plasma-activated media (PAM) had been optimized and the results were promising in both in vitro and in vivo studies for killing cancer cells and shrinking tumors to a large extent [22]. LTP exposure often triggered generation of long-lived species including peroxynitrite ($ONOO^-$), organic radicals, nitrite, NO_2, nitrate, NO_3, and hydrogen peroxide (H_2O_2) in the liquid phase during LTP exposure and have successfully been implicated in the anticancer therapy implicating PAM.

The liquid medium was exposed to an LTP source, typically the plasma plume of a plasma jet for a predetermined period to generate PAM. The media used for this application include Roswell Park Memorial Institute medium (RPMI), Ringer's Lactate solution (RL), Dulbecco's Modified Eagle Medium (DMEM), Eagle's Minimum Essential Medium (EMEM), and Dulbecco's Modified Eagle Medium (DMEM), with additives like a serum (for example, bovine serum), glutamine, and antibiotics (for example, mix of Penicillin/ Streptomycin). A 24-well plate was used where each well with media received LTP treatment for a specific amount of time, resulting in a variety of PAMs with different strengths.

Briefly, Cells were kept at 37°C in a humid incubator with 5% CO_2 after PAM administration. The control sample was also maintained using the same media that was not exposed to LTP. Trypan blue exclusion assay was utilized to quantify the MTS assay results and a representative Figure 3 illustrates how PAM produced by longer LTP treatment times results in a greater cell kill. More than 90% of the cells were reduced when PAM was produced with an exposure time of more than three minutes. Whereas in the case of exposure for two minutes duration an increase in viability was observed where multiplication of live cells outpaced the breakdown of cells. The results also showed the level of H_2O_2 in PAM rose with exposure time and had a strong correlation with the decline in cell viability. Generation of singlet oxygen is often associated with inactivation of catalase in cancer cells that typically produce it to shield themselves from intracellular ROS/ RNS signalling. When catalase is sufficiently inactivated, H_2O_2 enters the system via aquaporin and thereby inactivation of the antioxidant catalase and resulting ROS-mediated apoptosis. Strikingly, the healthy cells are also exposed to an inflow of ROS like H_2O_2 or peroxynitrite as they lack the expression of catalase on their surface. Therefore, the administered dose of ROS/RNS must be below a particular threshold in order to induce selective death of cancer cells.

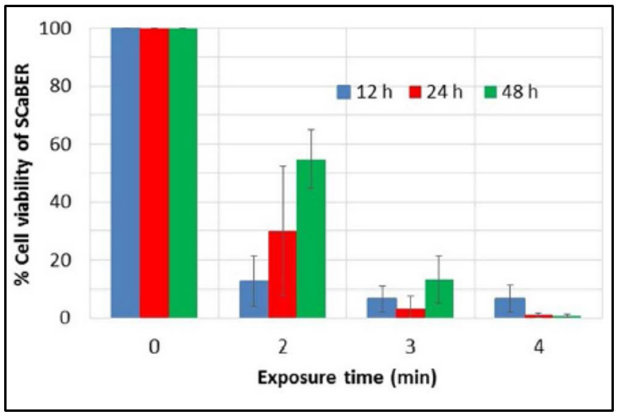

Figure 3) *Schematic representation of effect on cancer cell line (reconstructed).*

Advantages and Limitations of CAP

There are several advantages that Atmospheric Cold plasma technology (CAP) has to mankind. The technology has immense potential including, treatment of pancreatic cancer, acts as a powerful tool for modern medicine to handle chronic wounds, reducing microbial load without damaging the tissues and has adverse anti-tumor effects [23,24]. Additionally, CAP is also regularly used in dental treatment for example in the case of patients with dental plaque and related surgical as well as surface sterilization procedures effectively in dentistry [25]. Interestingly in the cases where mechanical/laser treatments can be of high risk, use of CAP for sterilizing infected dental tissues can be an effective alternative option especially during the time of dental cavity removal. CAP being a vibration and heat free method is more suitable for the patients and does not cause any healthy tissue damage.

The atmospheric pressure plasma jet (APPJ) has been used for material processing and surface cleaning since the late 90s. Very recently this technology has been used to sterilize surfaces during the SARS-CoV-2 outbreak. As per the study by Chen et al. [26], the APPJ treatment on different surfaces have shown significant inactivation of SARS-CoV-2 within 180s of exposure. Notably on metal surfaces, the exposure was able to decontaminate all viruses in the 30s. Similarly, Direct Barrier Discharge (DBD) was also being used in different fields such as controlling fungal infection in certain case studies [27].

The CAP has emerged as a technology to kill different infection-causing microorganisms however there is some limitation in this context which needs to be addressed. Notably CAP is admittedly weak in penetrating the plasma species into the deep layers of infectious films [28]. It also results in forming a viable but non-culturable state in the case of injured strains as confirmed by q-RT PCR of 16sRNA gene [29]. Similarly, another drawback reported previously was the use of Plasma treatment on small sample size in the case of cancer cells and small portions at once [30]. Finally, few infographic images of plasma-treated cell surfaces have shown the effect of elevated temperatures that can be harmful to cells and lead to tissue damage. Therefore, this technology needs further examination and numerous trial and error tests before it can serve its true purposes.

Conclusion

Low temperature plasma (LTP) has shown tremendous potential in modern biomedical and food technological applications. The plasma may be used either directly or indirectly depending on the site of action, dose response, exposure time, toxicity on healthy cells and finally side effects (if any). The sole advantage can be drawn in the case of antibiotic resistance conferred in hospital patients, post-operative care, wound healing and so on. Notably possible applications can also be extended in targeted therapy for oncogenic cases. Related studies have shown the potential application of CAP in cancer treatment, reducing infections, dentistry, and dermatological advances are worth mentioning. As of now, the technology withstands with a few limitations however, with the course of time and essential research and development, this technology will be improved to be more cost-effective, user friendly, improving penetrating power and finally aiming towards targeted therapeutic approach for better applicability and acceptability.

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