REVIEW ARTICLE

Cold Atmospheric Plasma as a Therapeutic Tool in Medicine and Dentistry

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Abstract

This comprehensive review explores decade-long research conducted on the applications of Cold Atmospheric Plasma (CAP) and Plasma Activated Liquids (PAL), including Plasma Activated Water (PAW), in the fields of Medicine and Dentistry. CAPs, operating at atmospheric pressure, offer unique advantages over conventional medical devices, generating Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) that interact with substances, enabling diverse applications. The review examined CAP and PAL efficacy against infectious diseases using in vitro, ex vivo, in vivo, and direct application methods. Significant strides were observed in wound healing, cancer treatment, and dental care. However, ensuring patient safety through rigorous plasma source standards remains crucial. The study underscores CAPs and PALs' potential to transform medical and dental therapies, urging further research and development in these groundbreaking technologies. These findings highlight the transformative impact of CAPs and PALs, offering promising avenues for innovative medical and dental treatments.

Keywords Cold atmospheric plasma · Plasma-activated liquids · Plasma-activated water · Plasma medicine · Plasma dentistry

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Introduction

In the fields of medicine and dentistry, a diverse range of therapeutic tools has been harnessed to address various health conditions and challenges. From traditional pharmaceutical interventions to cutting-edge surgical techniques, the quest for effective and innovative therapies continues to drive research and development in these crucial domains.

Among the recent advancements, Cold Atmospheric Plasmas (CAPs) have emerged as a promising technology with the potential to revolutionize various applications in academia and industry. What sets CAPs apart from conventional medical devices is their unique ability to operate at atmospheric pressure, distinguish them from other technologies requiring different pressure levels for therapeutic interventions.

The distinguishing feature of operating at atmospheric pressure empowers CAPs to generate a diverse array of active agents, each holding immense potential for applications across a multitude of medical and dental scenarios. Figure 1 showcases CAP generation with diverse sources, such as RONS, UV radiation, and electric fields, promising applications in direct and indirect treatments. Among the noteworthy active agents produced by CAPs are Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), collectively referred to as RONS. These reactive species have garnered significant attention due to their remarkable ability to interact with various substances and induce chemical reactions, thereby playing a pivotal role in numerous fields. In the biomedical sector, RONS have demonstrated valuable antimicrobial properties, making them particularly advantageous for sterilization and disinfection processes. Their potency against harmful microorganisms has shown promise in effectively combating infections and safeguarding patients in clinical settings [1, 2]. Moreover, CAPs have the capacity to generate ultraviolet (UV) photons, characterized by their high energy levels. These UV photons hold tremendous significance in a myriad of applications. For instance, they are instrumental in sterilization processes where their ability to initiate photochemical reactions proves invaluable. By targeting the DNA of microorganisms, UV photons can lead to their inactivation, ensuring a hygienic environment and enhancing patient safety [3]. Furthermore, CAPs possess the unique capability to generate charged particles, offering vast potential in numerous applications. In healthcare, these charged particles find utility in ionizing radiation treatments, while in technology, they contribute to the creation of microelectronic devices, among other uses. The cancer therapy is one area that stands to benefit significantly from CAPs, as the charged particles produced by these plasmas can cause DNA damage in cancer cells, ultimately leading to their death [4].

Over the past decade, CAPs have drawn considerable interest in the scientific community due to their distinctive properties and diverse potential applications in the fields of engineering, materials science, environment, medicine, etc. In the field of medicine, CAP have made a significant impact, providing innovative therapeutic applications such as wound healing, cancer treatment, and medical equipment sterilization [5]. Furthermore, the interaction of CAPs with living tissues has unveiled a multitude of simultaneous biological effects, encompassing antimicrobial, anti-inflammatory, tissue repair, and antineoplastic properties [2, 6]. In dentistry, CAPs have also demonstrated their transformative potential, contributing notably to dental implant sterilization, tooth whitening, and the treatment of various oral diseases [7], further underlining their promise in revolutionizing dental treatments, and enhancing patient outcomes.
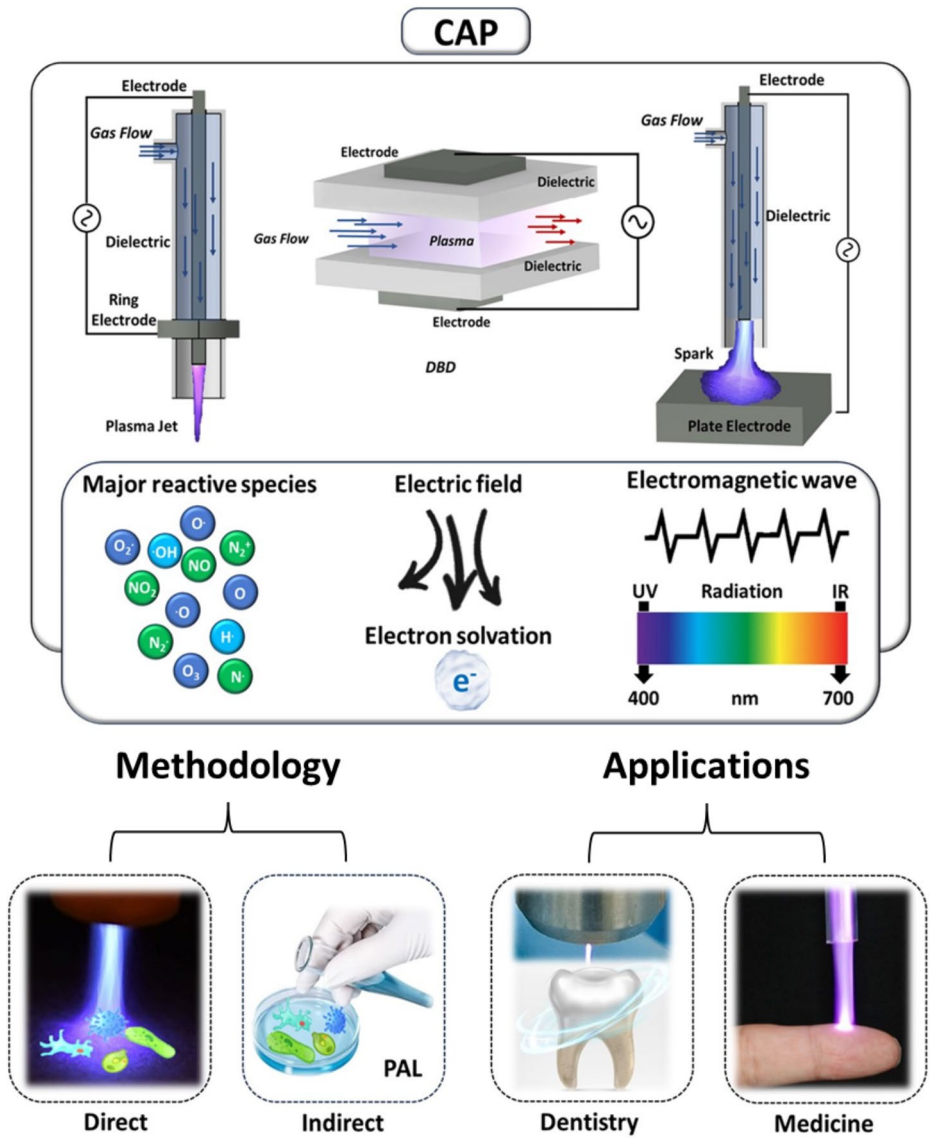
Fig. 1 A general scheme illustrating different sources of atmospheric cold plasma generation, capable of producing reactive agents such as reactive oxygen and nitrogen species (RONS), UV radiation, electric fields, among others, for direct and indirect treatment methodologies, with high potential for application in medical sciences. (adapted from [5])

Another exciting area of research within the field of CAPs is plasma-activated liquids (PAL), with plasma-activated water (PAW) being the most extensively studied. PAW refers to water that has been exposed to CAPs, resulting in the generation of reactive species and other active agents within the water [7]. This PAW has shown promising potential in various applications, such as disinfection, agriculture, food safety, and wastewater treatment [8–10]. Additionally, recent studies have introduced the concept of indirect plasma treatment,

wherein PAL is utilized as a treatment agent in various applications, further expanding the potential of CAP technology [9–11].

Understanding CAPs' biological effects, especially in treating infectious diseases, involves a comprehensive investigation employing diverse methodologies (in vitro, ex vivo, and in vivo). This approach provides valuable insights into the potential benefits and limitations of CAPs as therapeutic tools. Furthermore, different methods of CAP application, such as direct tissue contact and the use of PALs, including PAW, have been examined. The goal is to optimize the therapeutic potential of CAPs and PALs and develop tailored treatment strategies for specific medical and dental applications.

The development of plasma sources also comprises a significant aspect of the activities of several research groups in the CAP field. For a plasma source to be suitable for a given medical application, the resulting plasma must adhere to certain safety standards, particularly if the plasma plume is intended to come into contact with human tissues. The gas temperature of the plasma plume and the electrical current that leaks to the patient must not exceed 40 °C and 100 µA-AC, respectively, to avoid potential damage to living tissues [12, 13]. Evaluations of UV light emission and production of harmful gases by the plasma plume are also crucial, as these parameters not only affect the patient but also the operator, and thus determine the plasma exposure time and operating time of the plasma source [14]. Therefore, one of the main focuses in the development of plasma sources has been on creating devices that comply with these safety standards.

This comprehensive review investigates the diverse applications of CAPs in medicine and dentistry, spanning a decade-long research study. It offers an in-depth exploration of CAPs, including their generated chemistry and subsequent biological effects. A particular emphasis is placed on PALs and, more specifically, PAW. Furthermore, we delve into the evolution of CAP devices and their applications in medical and dental fields, revealing groundbreaking advancements in plasma technology that are revolutionizing medical and dental therapies. Safety standards for plasma sources are extensively discussed, along with insights from our work in developing compliant devices. The examination of CAPs and PALs applications provides a comprehensive understanding of their current status and future prospects in the medical and dental realms. As we explore potential applications and advancements, we also highlight the necessity for further research to fully unleash the transformative capabilities of PALs, potentially heralding a new era of medical and dental therapies.

Evolution of CAP Devices and Applications in Medical and Dental Fields

Currently, various sources of atmospheric plasma excitation are available, including high-frequency sources (in the order of kHz), radio-frequency sources (MHz), microwave sources (GHz), and the recent piezoelectric sources. A fascinating historical perspective reveals the initial application of high-frequency irradiation for various diseases, dating back to the early decades of the last century. Frederick Strong, an American physician and electrical engineer, pioneered the use of high-frequency currents for therapeutic purposes [15]. In his 1908 book "High Frequency Currents", Strong claimed to invent the vacuum electrode in 1897, later used widely in Europe around 1900. Apparatuses unintentionally produced spark effluvia via glass electrodes, representing CAP. Strong connected patients to a Tesla coil with a metal hand electrode, protected by a glass tube to prevent sparks. He replaced

it with a Geissler vacuum tube, inventing the Vacuum Electrode [15, 16]. Lowering gas pressure inside the tube generated X-rays and UV light. Strong found diverse physiological and medical effects, like promoting circulation and germicidal action [15]. Recent research shows the vacuum electrode (sometimes called the 'violet ray' or 'violet wand' [15]) generating antimicrobial plasma, suggesting CAP treatments as a rediscovery. Contemporary investigations echoed Strong's work, offering potential for further research and therapeutic efficacy rediscovery.

One recent paper compared antimicrobial effects of the historical vacuum electrode device with recent CAP devices [16]. Results align with observations in Crook's 1909 book on antimicrobial effects of brush discharges [15, 16]. In another study, Daschlein et al. also arrived at the conclusion that the vacuum electrode exhibited no significant difference in antimicrobial activity when compared to modern CAP sources [17]. They emphasized that this advantageous effect is not solely due to the device's psychosomatic or mystic light show aspects. The vacuum electrode was sold worldwide from the early 20th century until the 1950s and remains accessible today. While its medical value was likely overstated and misused, undeniable similarities to modern plasma devices exist.

In the medical field, surgical procedures have undergone a transformative revolution with the introduction of cutting-edge electrosurgical devices and endoscopic instruments, including plasma scalpels and argon plasma coagulators. Traditionally, these devices relied on high-temperature plasma, characterized by elevated gas temperatures. However, recent years have witnessed significant strides in the development of distinct CAP plasma sources, offering unique effects compared to their high-temperature counterparts [18]. This remarkable advancement has paved the way for specialized methods, particularly in dermatology and dentistry, focusing on treating solid surfaces.

Among the modern plasma devices, several stand out as parallel to historical devices used in local high-frequency current treatments. The dielectric barrier discharge (DBD) plasmas, gliding arc plasma jet, and surfatron microwave-driven plasma are noteworthy examples. These contemporary devices effectively utilize plasma in contact with cells, tissues, or organisms, showcasing similarities with historical devices like the vacuum electrode and other DBD-like configurations. This parallelism illustrates a continuous evolution of research and development in the field of plasma medicine.

Figure 2 provides an insightful timeline, depicting representative plasma devices that have been instrumental in medical and dental treatments from the beginning of the last century up to the present day. Moreover, it is worth noting that recent reviews have extensively explored a wide array of modern non-thermal plasma medical devices, offering comprehensive insights into their applications and potential advancements [18–21].

In the late 2000s, multiple Low-Temperature Plasma (LTP) sources received approval for both cosmetic and medical purposes. Notably, in 2008, the US FDA approved the Rhytec Portrait® (plasma jet) for use in dermatology. Additionally, several other plasma devices, including the Bovie J-Plasma®, the Canady Helios Cold Plasma and Hybrid Plasma™ Scalpel, are currently in use in the US for various medical applications [22].

In Germany, some CAP devices received "CE certification" for clinical testing in 2013. Notable examples include the kINPen MED (by Neoplas Tools GmbH in Greifswald, Germany), which utilizes an RF-powered (1 MHz) Ar jet [20], and the MicroPlasSter (by ADTEC in Hunslow, UK), operating as a microwave-powered (2.45 GHz) Ar plasma torch [21]. These devices are designed to treat tissues without significant thermal heating. Another
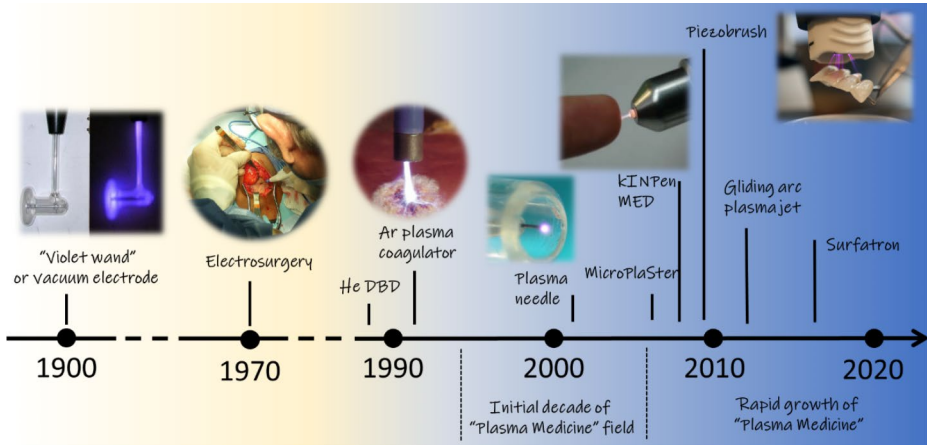
Fig. 2 Timeline showing the representative plasma devices for medical and dental applications. (adapted from [18–23])

device, the PlasmaDerm (by CINOGY GmbH in Duderstadt, Germany), operates with a DBD in open air, functioning in a non-thermal manner [21].

In 2009, the piezoelectric direct discharge (PDD), a new atmospheric pressure gaseous discharge producing cold plasma, was commercially released [23]. It utilizes a piezoelectric cold plasma generator (PCPG) with a resonant piezoelectric transformer (RPT) to generate > 10 kV output voltage at low input voltage (< 25 V). PDD ignites micro-discharges directly at the ceramic surface, distinguishing it from other discharges. Since 2009 the company Relyon Plasma GmbH commercialize PDD based piezo-brushes [23].

Over the last decade, Cold Atmospheric Plasmas (CAPs) have experienced a remarkable surge of interest within the scientific community, primarily due to their unique properties and diverse potential applications in medical sciences. This increasing trend is vividly illustrated by the substantial number of scientific papers published annually, as demonstrated in Fig. 3. The figure provides a breakdown of research areas in "Cold atmospheric plasma", including subdivisions related to medicine, as per the Scopus database.

Furthermore, Fig. 3**(b)** displays the number of publications over time for specific keywords like "Cold plasma + medicine" and "Cold plasma + dentistry". Notably, the data also encompasses information on "Plasma activated water + Medicine" and "Plasma activated water + Dentistry", particularly emphasizing the growth of research in these areas, especially within the medical field. However, it is important to note that in dentistry, the number of works is still relatively modest.

The information conveyed by Fig. 3 underscores the significant interest and investment in CAP research, especially its potential in medical applications. The emerging prominence of PAW research further demonstrates its relevance and promising role in medical science. Although dentistry shows comparatively fewer works, the overall trajectory indicates a compelling and dynamic landscape for the future of CAP and PAW applications in various clinical domains.

Table 1 provides a comprehensive overview of the diverse applications of CAPs in the field of medicine. The listed review papers highlight the significant research efforts into CAP's potential across critical areas such as wound healing, cancer treatment, dermatol-
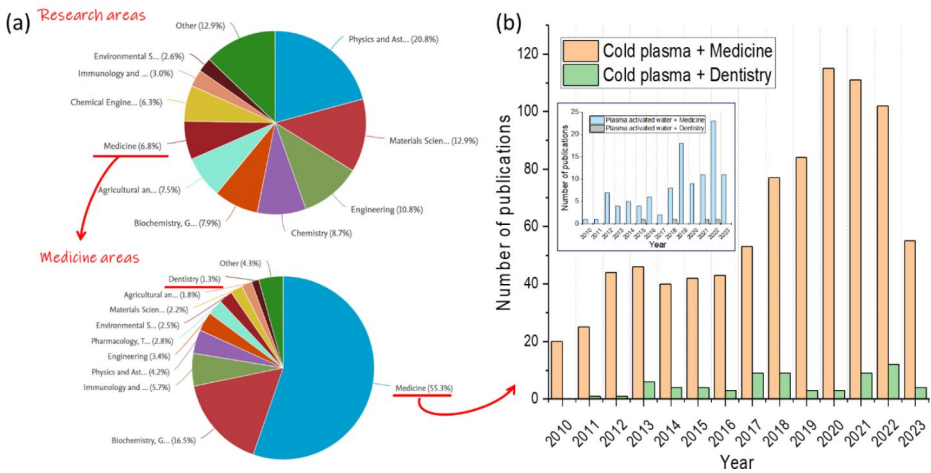
Fig. 3 **(a)** Division of research areas in "Cold atmospheric plasmas" with the subdivisions of areas comprising medicine according to the Scopus database. **(b)** Number of publications as a function of year for the keywords: "Cold plasma+medicine"; "Cold plasma+dentistry" (inside Figure **(b)**, data for "Plasma activated water+Medicine" and "Plasma activated water+Dentistry" are also presented). (Source: Scopus, accessed 07/17/2023)

ogy, virus inactivation, and pathogen inactivation. These reviews emphasize the efficacy of CAP-generated reactive species in addressing a wide range of medical challenges, ranging from cancer attenuation to the inactivation of viruses and pathogens. Particularly noteworthy is CAP's unique ability to generate Reactive Oxygen and Nitrogen Species (RONS), presenting a valuable resource in exploring new approaches for water treatment and other relevant fields.

In the context of virus inactivation, CAP has demonstrated a noteworthy contribution in addressing the SARS-CoV-2 pandemic, with several studies focusing on its potential for virus inactivation and destruction [32, 43, 48]. The generation of reactive species through physical and cold atmospheric plasma techniques has shown promising results in combating the virus and contributing to the efforts against COVID-19. CAP's ability to effectively inactivate viruses, including SARS-CoV-2, opens new possibilities in the field of medical science and presents a compelling area for further research and application.

Table 2 sheds light on the transformative potential of Cold Atmospheric Plasma (CAP) in the field of dentistry, encompassing a wide range of dental applications. The reviews emphasize CAP's role in dental implant sterilization, tooth whitening, and the treatment of oral diseases. Notably, CAP's antimicrobial properties emerge as a pivotal factor in effectively combating dental pathogens and supporting overall oral health. Its contributions to dental implantology and various dental procedures exemplify CAP's significance in advancing dental treatments and promoting oral well-being.

The review articles highlight the areas of dentistry where CAP has made significant strides, including endodontics [58, 62, 63], cariology [58, 59], oral oncology [58], oral candidiasis [57, 58], and periodontal disease [61]. CAP's therapeutic perspective in treating periodontal disease underscores its potential in addressing challenging dental conditions.

Table 1 List of the main review papers published on cold plasma in medicine / microbiology in the last 10 years

Review title (Year)	Main field of discussion	Ref.
Non–Thermal Atmospheric-Pressure Plasma Possible Application in Wound Healing (2014)	Would heating	[24]
Plasma Medicine: A Brief Introduction (2018)	Brief discussion	[22]
Safety implications of plasma-induced effects in living cells – a review of in vitro and in vivo findings (2019)	Safety of plasma in living cells	[25]
Plasma Medicine: Applications of Cold Atmospheric Pressure Plasma in Dermatology (2019)	Dermatology	[26]
Impact of ROS Generated by Chemical, Physical, and Plasma Techniques on Cancer Attenuation (2019)	Cancer treatment	[27]
Dosing: The key to precision plasma oncology (2019)	Cancer treatment; Plasma dosing	[28]
Cold atmospheric pressure plasmas in dermatology: Sources, reactive agents, and therapeutic effects (2020)	Dermatology	[29]
Cold Atmospheric Pressure Plasma in Wound Healing and Cancer Treatment (2020)	Would healing and Cancer treatment	[30]
Cold Atmospheric Plasma: A Powerful Tool for Modern Medicine (2020)	Would healing; Dermatology; Cancer treatment.	[31]
Molecular Mechanisms of the Efficacy of Cold Atmospheric Pressure Plasma (CAP) in Cancer Treatment (2020)	Cancer treatment	[32]
Cold Plasma, a New Hope in the Field of Virus Inactivation (2020)	Virus inactivation	[33]
The emerging potential of cold atmospheric plasma in skin biology (2020)	Dermatology	[34]
Cold atmospheric plasma cancer treatment, direct versus indirect approaches (2020)	Cancer treatment	[35]
Applications of Cold Atmospheric Pressure Plasma Technology in Medicine, Agriculture and Food Industry (2021)	Pathogen inactivation	[36]
A Review of Cold Atmospheric Pressure Plasmas for Trauma and Acute Care (2021)	Would healing	[37]
Non-thermal Plasma Treatment of ESKAPE Pathogens: A Review (2021)	Pathogen inactivation	[38]
Cold Atmospheric Plasma: A New Strategy Based Primarily on Oxidative Stress for Osteosarcoma Therapy (2021)	Osteosarcoma; Cancer treatment	[39]
Cold atmospheric plasma delivery for biomedical applications (2022)	Would healing; Blood coagulation; Cancer therapy;	[40]
Biomedical Applications of Cold Atmospheric Plasma (2022)	Pathogen inactivation; Would healing	[41]
Review of the cold atmospheric plasma technology application in food, disinfection, and textiles: A way forward for achieving circular economy (2022)	Pathogen inactivation	[42]
Recent advances in cold atmospheric plasma (CAP) for breast cancer therapy (2022)	Cancer treatment	[43]
Cold atmospheric plasma for addressing the COVID-19 pandemic (2022)	Virus inactivation	[44]
Cold Atmospheric Plasma: A Noteworthy Approach in Medical Science (2023)	Neurological Complications; Cancer; Would healing	[45]
Exploring the Use of Cold Atmospheric Plasma to Overcome Drug Resistance in Cancer (2023)	Cancer treatment	[46]

Table 1 (continued)

Review title (Year)	Main field of discussion	Ref.
Recent advances in cold atmospheric plasma for tumor therapy (2023)	Tumor therapy	[47]
The inactivation and destruction of viruses by reactive oxygen species generated through physical and cold atmospheric plasma techniques: Current status and perspectives (2023)	Virus inactivation	[48]
Cold atmospheric plasma: Novel opportunities for tumor microenvironment targeting (2023)	Cancer treatment	[49]

Table 2 List of the main review papers published on cold plasma in dentistry in the last 10 years

Review title (Year)	Main field of discussion	Ref.
The bright future of dentistry with cold plasma – Review (2014)	Brief discussion	[50]
Cold Atmospheric Plasma (CAP) in Dentistry (2013)	Brief discussion	[51]
Plasma Torch Toothbrush a New Insight in Fear Free Dentistry (2014)	Instrument	[52]
Translational plasma stomatology: Applications of cold atmospheric plasmas in dentistry and their extension (2017)	Wide discussion of plasma use in stomatology	[53]
The Emerging Role of Cold Atmospheric Plasma in Implantology: A Review of the Literature (2020)	Dental implants; Implantology	[54]
Effect of Non-Thermal Atmospheric Plasma on Micro-Tensile Bond Strength at Adhesive/Dentin Interface: A Systematic Review (2021)	Resin–dentin bonding	[55]
Effects of non-thermal atmospheric plasma on dentin wetting and adhesive bonding efficiency: Systematic review and meta-analysis (2021)	Dentin; Adhesive bonding	[56]
The Antimicrobial Effect of Cold Atmospheric Plasma against Dental Pathogens—A Systematic Review of In-Vitro Studies (2021)	Dental pathogens inactivation	[57]
Applications of Cold Atmospheric Pressure Plasma in Dentistry (2021)	Endodontics; Cariology; Oral Oncology; Oral Candidiasis.	[58]
Aurora Borealis in dentistry: The applications of cold plasma in biomedicine (2022)	Sterilization; Dental caries; Root canal disinfection; Tooth bleaching; Clinical removal of biofilms; etc.	[59]
Applications of Cold Atmospheric Pressure Plasma in Dentistry- A Review (2022)	Brief discussion	[60]
The therapeutic perspective of cold atmospheric plasma in periodontal disease (2023)	Periodontal disease	[61]

Additionally, CAP's applications extend to resin-dentin bonding, adhesive bonding efficiency, and dentin wetting, showing promise in enhancing dental materials and techniques [54–56].

Overall, the data of Table 2 emphasizes the wide-reaching impact of CAP in dentistry, reflecting its potential to revolutionize dental practices and enhance patient care. As CAP continues to show promising results and gain momentum in dental research, it opens up new horizons for innovative and advanced dental treatments across various fields within dentistry.

Overview of Cold Atmospheric Plasmas

As seen in the previous section, significant efforts have been devoted to the development and utilization of various distinct CAP systems. These systems, such as DBD-based plasmas, gliding arc discharges, and surface-wave discharges, have garnered significant attention and recognition within the scientific community. This is primarily attributed to their wide range of applications, including liquid activation, surface treatment, sterilization, and water treatment [64–70].

Each of these plasma generation techniques is characterized by unique plasma configurations and mechanisms that are tailored to facilitate the generation of highly reactive species. These reactive species, upon interaction with liquids, can initiate substantial chemical and biological transformations. Such transformations are pivotal in the fields where these plasma systems are implemented, leading to practical advancements and innovative solutions [71–73].

In the following sections, we explore the key features and characteristics of the three plasma generation techniques that have been extensively utilized and studied. To provide a comprehensive overview, we refer to Table 3, which presents the main characteristics of the cold atmospheric plasma devices most commonly used in the literature.

Dielectric Barrier Discharge (DBD)

DBD stands as a versatile and flexible plasma generation technique, characterized by its distinct construction. This technique typically consists of two parallel electrodes, with a dielectric barrier inserted between them, thus defining its name. The dielectric barrier plays a crucial role in preventing the transition from a glow discharge into an arc discharge, thereby facilitating a stable and uniform plasma field [84].

The inherent versatility of DBD is largely attributed to its ability to be tailored to a myriad of setups. These setups encompass but are not limited to, planar, cylindrical, and coaxial configurations (Fig. 4). Each configuration manifests its own unique set of properties, deriving from the geometry and arrangement of the electrodes and dielectric [84].

In a planar configuration, the electrodes are arranged flatly, in parallel to each other. This setup is particularly useful for surface treatment applications, where a large, flat surface needs to be uniformly treated. The cylindrical configuration, on the other hand, involves one electrode being wrapped around another, often with the dielectric material in between. This configuration can be especially effective for concentrated plasma applications, where a stronger, more focused plasma field is needed [85].

Table 3 Characteristics of Cold Atmospheric Plasma Devices: Power Range, Gas Temperature, Gases, RONS Generated, and Applications

Device	Power Range	Gas Temperature	Gases	RONS Generated*	Main Applications	Ref
Dielectric Barrier Discharge (DBD)-based plasmas	Operates at lower power levels up to hundreds of watts	Room temperature to a few hundred degrees Celsius	Air, oxygen, nitrogen, helium, argon, etc.	Ozone (O_3), atomic oxygen (O), singlet oxygen (O_2^*), hydroxyl radicals (OH*), nitrogen dioxide (NO_2), etc.	Surface treatment, Sterilization, Water treatment, Plasma activated Liquids (PAL).	[18–23, 74–77]
Gliding Arc discharge	Gliding Arc exhibits a wide power range from tens to hundreds of watts, making it suitable for various applications	Room temperature to a few hundred degrees Celsius	Air, oxygen, nitrogen, helium, argon, etc.	Hydroxyl radicals (OH*), nitrogen radicals (N*), atomic oxygen (O), ozone (O_3), etc.	Surface treatment, Sterilization, Water treatment, Plasma activated Liquids (PAL), Biomedical applications, Medicine, Dentistry.	[64, 78–81]
Surface-wave Discharge (Surfatron)	Operates at higher power levels, typically ranging from several hundred watts to kilowatts	Room temperature to a few hundred degrees Celsius	Helium, argon, etc.	Hydroxyl radicals (OH*), oxygen radicals (O*), ozone (O_3), nitrite (NO_2^-), nitrate (NO_3^-), hydrogen peroxide (H_2O_2), etc.	Water treatment, Plasma activated Liquids (PAL) Medicine, Dentistry	[70, 82, 83]

*Each technique generates different quantities of reactive oxygen and nitrogen species (RONS) based on the gas used

The coaxial configuration situates the electrodes on the same axis but with one inside the other. This design allows for a more focused and intense plasma field along the axis, making it ideal for applications such as plasma drilling or cutting. Lastly, the sliding configuration, a more complex and dynamic setup, can adjust the plasma field by moving one electrode relative to the other. This can allow for more precise control over the plasma field and is particularly suited to applications that require a varying plasma effect [86, 87].

A particularly important and frequently utilized application of DBD is the plasma jet. DBD plasma jets are typically used for surface treatment applications, biomedical applications, and even in environmental remediation. The plasma jet generates a directed stream of plasma that can be precisely controlled, allowing for localized treatment with high specificity [88].

The unique configuration of DBD equips it with the capability to generate a multitude of reactive species, both in gas and liquid phases. When we delve into the gas phase, DBD facilitates the production of several species, each of which serves specific roles in a variety of applications [89]. For instance, DBD assists in the formation of ozone (O_3), a strong oxidant known for its disinfecting and deodorizing properties, making it particularly useful in air and water purification processes. Atomic oxygen (O), another species generated by DBD, is an integral part of many chemical reactions due to its high reactivity, and it is especially significant in combustion processes. Singlet oxygen (O_2^*), another reactive species produced in the gas phase by DBD, holds notable potential in applications involving disinfection and sterilization [77–79, 89]. This is due to its higher reactivity compared to ground-state oxygen, making it more efficient in destroying bacteria and other harmful organisms [79]. Hydroxyl radicals (OH*), also generated by DBD, are powerful oxidants that contribute to numerous reactions and play a crucial role in many environmental and atmospheric processes. Lastly, nitrogen dioxide (NO_2), another product of DBD's gas phase reactions, is an important reactive gas in various industrial and environmental contexts, including air pollution monitoring and control.

Switching to the liquid phase, DBD primarily contributes to the formation of hydrogen peroxide (H_2O_2). This is achieved through reactions involving hydroxyl radicals (OH*) and atomic oxygen (O) [77, 89]. Hydrogen peroxide is a valuable compound in many applications, such as disinfection, sterilization, and wastewater treatment, to name a few. However, it is crucial to note that the production rate of H_2O_2 can be influenced by specific experimental conditions and the presence of additional species within the system [76, 77, 90]. This highlights the importance of careful control and understanding of the system to achieve the desired results.

Furthermore, DBD exhibits a unique capability of generating nitrite (NO_2^-) and nitrate (NO_3^-), through the activation of reactive species such as hydroxyl radicals (OH*) and atomic oxygen (O) [91]. These are important compounds in a range of biological and environmental contexts, including nitrogen cycling in ecosystems, water quality monitoring, and food preservation [77, 78, 85, 91–93].

Gliding Arc

In the medical and dental fields, gliding arc plasma has emerged as a promising and cutting-edge technology with diverse applications. Y. D. Korolev conducted a comprehensive review exploring low-current discharges in a gas flow at atmospheric pressure, highlighting
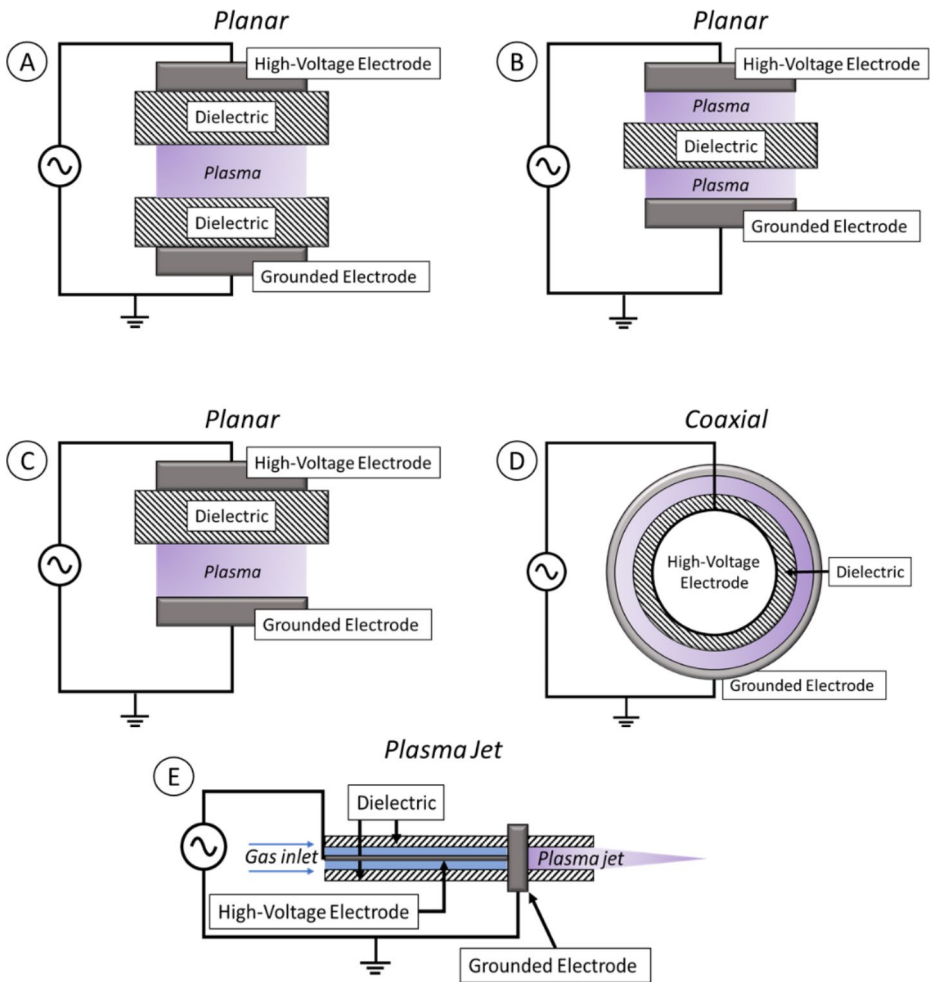
Fig. 4 Some types of DBD reactors: **(A)** Planar with two dielectrics in contact with both electrodes; **(B)** Planar with one dielectric without contact with both electrodes; **(C)** Planar with one dielectric in contact with the high-voltage electrode; **(D)** Coaxial with one dielectric in contact with the high-voltage inner electrode; and **(E)** Tubular DBD jet reactor with ring grounded electrode

the study of glow discharges in coaxial plasmatrons and gliding arcs [94]. These gliding arcs are known for generating plasma jets containing active chemical species with extensive applications in surface modification, biology, and medicine. Notably, plasma discharges in nitrogen-oxygen mixtures have been utilized to produce nitric oxide (NO), which serves as a valuable vessel dilator in inhalation therapy.

Figure 5 illustrates the structure of a rotating gliding arc reactor. The Gliding Arc (GA) is a plasma system that lies between non-thermal and thermal plasmas, offering unique characteristics and potential applications. It has the capability to simultaneously produce high plasma densities, a high degree of non-equilibrium, higher electron temperatures, lower gas temperatures, and the ability to stimulate specific chemical processes without quenching [94]. This dynamic feature enhances the interaction of the GA plasma with liquids and sur-
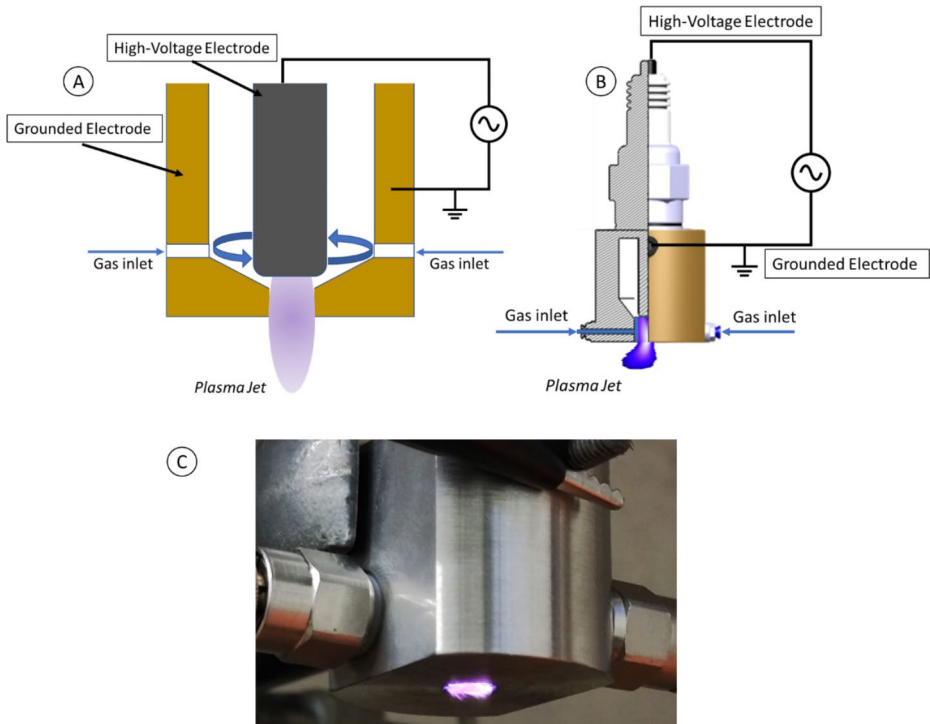
Fig. 5 **(A)** Concept of rotating GA reactor, **(B)** The scheme and **(C)** Photograph of the GA plasma used in our research group [97, 98]

faces, making it a versatile tool with broad applications [72]. In the healthcare realm, especially in medicine and dentistry, the GA technique's capacity to generate highly energetic and chemically reactive plasma is particularly promising. This leads to the production of various reactive species, including hydroxyl radicals (OH*), nitrogen radicals (N*), atomic oxygen (O), ozone (O_3), and nitrogen oxides (NO_x) [79, 80]. The high electron density and energy of the GA plasma stimulate intense chemical reactions, enabling the production of these species in significant quantities, expanding its applicability in medicine and dentistry [95, 96].

In dentistry, plasma-liquid technology, such as the generation of plasma-activated water (PAW) through the GA, is gaining significant attention [80]. PAW, rich with active constituents, offers potential clinical advantages in treating various oral diseases [80]. The versatility and potential of plasma liquid technology, found in applications ranging from agriculture and the food industry to healthcare, make it an exciting approach in dentistry. The use of PAW in dentistry is supported by the potential of direct and indirect application of non-thermal atmospheric pressure plasmas, including the GA technique [99–101]. The GA technique capabilities open new possibilities for effective and innovative dental treatments, promising advancements in oral healthcare and disease treatment.

Surface-Wave Discharge

The Surface-wave discharge technique, often referred to as the surfatron, is a plasma methodology featuring a plasma source encased within a microwave cavity (Fig. 6). This cavity hosts a plasma-quartz tube, the interior of which is equipped with a slit. The slit is a crucial component of the surfatron, as it allows for the effective coupling of electromagnetic energy to the plasma via surface waves. These waves subsequently propagate along the quartz tube, thereby enabling the creation of plasma in regions distal from the surfatron while maintaining the self-consistent propagation of the surface wave. The production of the plasma column persists until the power supply is insufficient for its sustenance.

The surfatron has been expertly adapted for usage at elevated frequencies, specifically 2.45 GHz, catering to situations that require high microwave power levels over 500 W, or necessitate large tube diameters, measuring 15 mm or more.

As a versatile tool, the surfatron can generate a broad spectrum of reactive species. These include, but are not limited to, hydroxyl radicals (OH*), oxygen radicals (O*), ozone (O_3), nitrite (NO_2^-), nitrate (NO_3^-), and hydrogen peroxide (H_2O_2) [20]. Notwithstanding, the precise production levels of these reactive species can fluctuate, subject to a variety of factors. Influencing variables include the choice of gas, the composition of the liquid, the power applied, and the specific experimental conditions. Thus, meticulous optimization of these parameters is paramount to maximize the production of specific reactive species and to ensure the overall efficacy of the process [102].

In the context of medical and dental applications, the surfatron is making strides as a potential tool for advanced treatment protocols. Previous research indicates the promise of plasma technologies in fields such as dentistry, where PAW is being explored for various oral diseases [14, 99–101, 103–108].
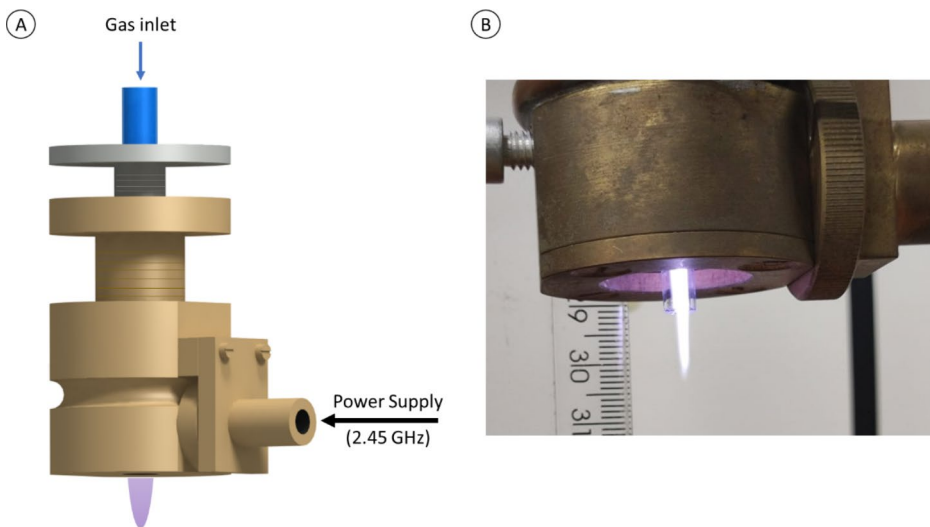
Fig. 6 **(a)** Schematic drawing and **(b)** Photograph of a surface-wave plasma jet reactor operating with argon gas

Critical Safety Standards of Plasma Sources

Plasma sources, a critical component in a variety of applications, particularly in the medical and industrial sectors, are required to adhere to a comprehensive set of safety standards [14]. These standards, which encompass a wide array of physical and chemical properties, play a pivotal role in ensuring the safe and effective operation of plasma sources. The adherence to these standards not only ensures the safety of the end-users but also enhances the overall performance and reliability of these plasma sources.

One of the primary standards that plasma sources must comply with is the DIN SPEC 91315:2014-06 (General requirements for plasma sources in medicine) [109]. This standard, recognized globally, provides a detailed framework for the general requirements that plasma sources in the medical field must meet. It mandates a series of rigorous experiments designed to assess the potential health hazards associated with atmospheric pressure plasmas [14]. Key properties, including gas temperature, thermal output, heat flow to the treated surface, patient leakage current, UV radiation, and ozone emission, are carefully evaluated to ensure the plasma source operates within safe parameters for a given "plasma dosage." Depending on the exposure or treatment intensity, influenced by plasma device, treatment, and target parameters, plasma can have cell stimulatory or inhibitory effects or induce sub-lethal cellular damage (Fig. 7). This principle aligns with the concepts of "oxidative eustress," promoting physiological responses, and "oxidative distress," causing pathophysiological signaling, proposed in redox biology [14, 24].

The safety of plasma exposure is of utmost importance, given the potential risks associated with cell toxicity, immunogenicity, sensitization, genotoxicity, and potential carcinogenicity. This concern carries significant weight in various sectors, including medicine, healthcare, food, agriculture, and other fields utilizing plasma technology. Prioritizing safety and comprehending the potential side effects of plasma exposure are critical in unlocking its full potential and expanding its applications in the biomedical domain [24].

Another crucial standard that plasma sources must adhere to is the IEC DIN EN 60,601 [110]. This standard, developed by the International Electrotechnical Commission (IEC), guarantees the safe operation of the plasma jet as a piece of medical-electrical equipment. It outlines four types of leakage currents: ground leakage current, housing leakage current, patient leakage current, and patient auxiliary current. Each of these leakage currents is carefully measured and controlled to ensure the safety of the patients and the operators. Of these, the patient leakage current under standard conditions is particularly significant in the

Fig. 7 The balance between cell stimulatory and cell inhibitory effects is dependent on the plasma exposure/treatment intensity, which is determined by device and treatment parameters, as well as characteristics of the target cells and their environment. Genotoxic effects can occur when DNA damage exceeds or escapes the capacity of cellular repair mechanisms at sub-lethal plasma treatments. (adapted from [24])
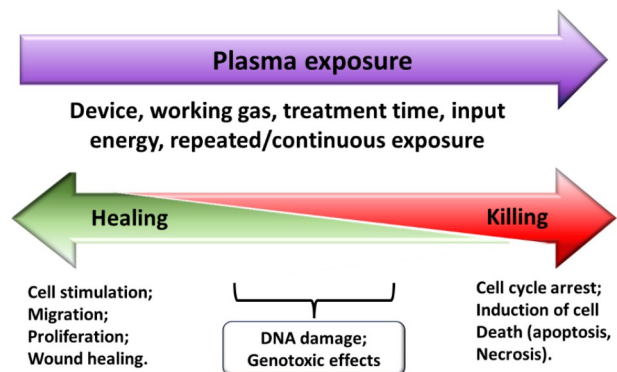
context of plasma sources [14]. This is because any excessive leakage current can pose a risk to the patient, making its control paramount.

The gas temperature and heat flow of the plasma source are also vital safety considerations that must not be overlooked [14]. Cold plasmas, known for their lower gas temperatures, are commonly employed in medical applications due to their safety and effectiveness. The heat flow of the atmospheric pressure plasma jet (APJ) is ascertained by a detailed examination of the transient temperature curve during the heating-up phase. This analysis helps ensure that the heat flow remains within safe operating limits, thereby preventing any potential thermal damage to the treated tissues or surfaces.

UV emission is another safety factor that requires meticulous monitoring and control [14]. Plasma sources, especially those incorporating nitrogen as a shielding gas, can emit dominant nitrogen bands in the UVB and UVA regions. Exposure limits and threshold values for UV radiation are contingent on various factors, including the velocity and movement of the plasma source over the skin and the skin's condition. Therefore, careful assessment and control of UV emission are necessary to prevent any potential harm to the patients or operators.

Despite the implementation of nitrogen shielding, a notable concentration of ozone can be detected in the vicinity of the plasma source. This ozone emission, while a natural byproduct of the operation of plasma sources, is a critical safety consideration that must be factored into the final design of a medical device. Excessive ozone concentration can be harmful, and therefore, appropriate measures must be taken to control its emission.

In sum, the safety standards for plasma sources are comprehensive and multifaceted, encompassing a wide range of physical and chemical properties. These standards ensure that the plasma sources operate safely and effectively, thereby enhancing their reliability and performance. Nevertheless, compliance with these standards is not the end of the road. Additional investigations and certifications, such as ISO 13,485 and risk assessment in accordance with DIN EN ISO 14,971, are required to fulfill the stipulations of the Medical Device Directive (MDD) for CE marking [14]. These additional certifications provide an extra layer of assurance, further reinforcing the commitment to safety and quality in the operation of plasma sources. By adhering to these rigorous safety standards and certifications, plasma sources can be confidently employed in biomedical applications, from disinfection and wound healing to blood coagulation and cancer treatment, with the assurance of safety and efficacy.

Evolution of the Plasma Sources Used to Produce He-APPJs

A plasma source can be separated in two basic parts: the power supply and the plasma device. The first provides the energy required to ignite a discharge with the second one. The studies in plasma medicine from our research group started using a parallel plate dielectric barrier discharge (DBD) device in which the discharge was driven by a low-frequency (60 Hz) high-voltage (HV) power supply [111]. In the later years, the research started to be developed using He-APPJs. Then, the plasma source was based on a commercial power supply composed of an AC generator from GBS Elektronik GmbH (model Minipuls4), an arbitrary function generator from RIGOL (model DG1012) and a DC voltage generator. With that power supply it was possible to compare the use of sinusoidal voltage in continu-

ous wave mode or in burst mode. The last can be seen as a pulse-like waveform. An example of that is shown in Fig. 8, where T_r is the repetition period of the burst and Δt is the time interval in which the plasma discharge occurs [112]. The oscillation frequency inside each burst is of the order of 30 kHz.

One of the requirements for the application of APPJs in dentistry is that the plasma plume must be produced by a handable device in a way that it fits in the oral cavity of a patient. This problem was solved by our research group after the development of a device that is able to produce a remote plasma plume at the end tip of long, thin and flexible plastic tubes [113]. The complete plasma device consists of a dielectric barrier discharge (DBD) reactor, composed by a metal pin electrode encapsulated into a closed-end quartz tube, which, in turn, is placed inside a dielectric chamber. A 1-meter long flexible plastic tube is connected to the reactor exit. Inside this tube is placed a thin copper wire, which ends 2 mm before the outlet tip of the plastic tube.

The basic functioning of this plasma source is as follows: a continuous gas flow is injected inside the reactor and flows out through the long tube. When the high-voltage is applied to the pin electrode, a primary plasma discharge is formed inside the reactor chamber. Once this primary DBD discharge is ignited, it polarizes the wire inside the long tube. Eventually, the electric field induced on the wire tip is sufficient to generate a small (remote) discharge, the plasma jet, at the distal end of the plastic tube. The tip of the conducting wire does not touch the glass tube or any electrified part of the DBD device, so the wire acts as a floating electrode. This plasma production method is also referred to as jet transfer technique. The generation of remote plasma jets was also studied by other research groups. More recently, this technique has been employed in a device aimed for endoscopic applications [114, 115].
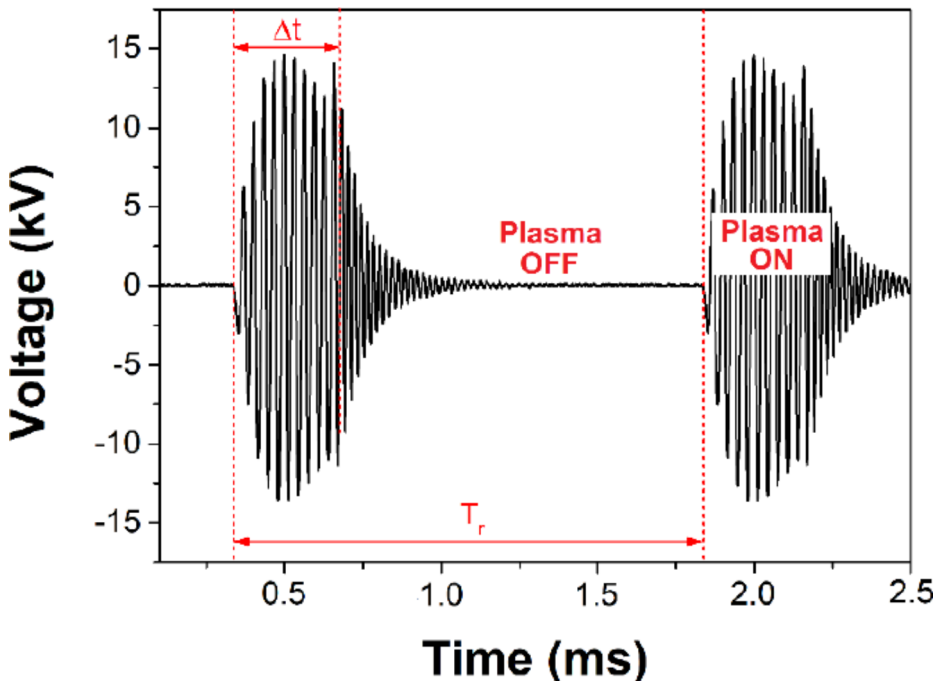

Fig. 8 Burst waveform [112]

By using the plasma device with a long tube and the HV in burst mode, it was possible to generate He-APPJ with a good safety degree. In addition, the physical properties of the plasma jet, mainly production of reactive species and discharge power, proved to be suitable for medical applications [116]. Thus, the studies focused on plasma medicine and dentistry that followed were based on this configuration [117–124]. Most importantly, with such configuration working as expected and proving antimicrobial efficacy it was possible to establish an effective and safe protocol for in vivo treatment of oral candidiasis using He-APPJs [119].

In recent years, the research group started new studies aimed to reduce the dimensions of the plasma source. For this purpose, the first step was to replace the above-mentioned commercial power supply by a home-made one, which is portable and has considerably smaller dimensions. The main physical features of the initial plasma source were maintained (use of a long tube and the jet transfer technique). The waveforms produced by each power supply are quite different, but both produce pulse-like high-voltage signals. The homemade power supply generates a damped-sine waveform, as can be seen in Fig. 9 [125, 126].

All those differences between the electrical characteristics of the power supplies result in significant differences in some properties of the plasma jets produced by each plasma source, being that the production of reactive species and the discharge power are the main ones. However, an important parameter, the gas temperature (T_{gas}) in the plasma plume, remained at the same order and below 40 °C when operating in the free jet mode [112, 126]. When using the portable device with the plasma plume impinging on a conducting surface, T_{gas} is also lower than 40 °C [126]. An interesting phenomenon pointed out in the work by Nascimento et al. [126] was that one of the main heating sources of a He-APPJ can be the helium gas itself and the T_{gas} values tend to increase as the distance from the plasma outlet is incremented.

Besides the evolution in the development of the plasma source using the jet transfer technique with a thin wire inside the long tube, our research group developed an alternative

Fig. 9 Damped-sine waveform (reproduced from [126])
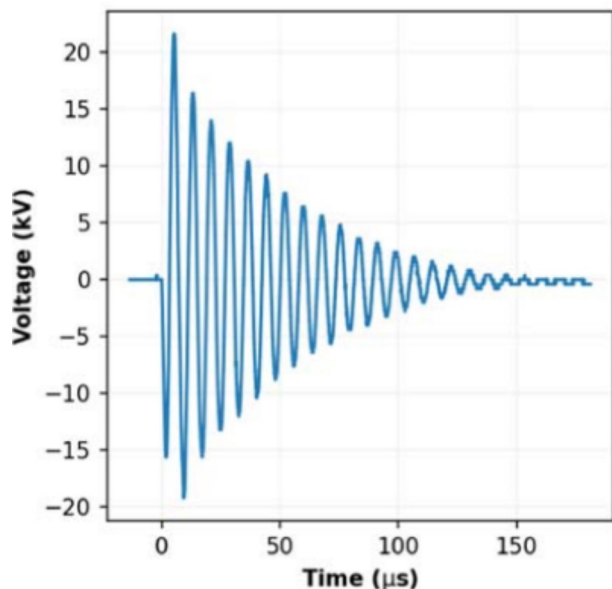
configuration for the long tube part of the plasma device. Such configuration consists in a concentric arrangement of a long tube composed by a metallic mesh, with 90% closed area, over a polytetrafluoroethylene (PTFE) tube with both placed inside a Nylon 6 tube [126, 127]. Initial tests showed that with this setup the effective electric current passing through the plasma can be up to three times smaller than in the configuration that uses the thin wire [128]. This is basically due to the additional dielectric barrier, the PTFE tube, between the conductor and the plasma jet. A reduction of such electrical current is one of the necessary steps to increase device safety in plasma medicine applications.

Most of the studies with the mesh assembly were carried out using argon as the working gas. However, basic tests with such configuration producing He-APPJs were also performed to assess its antimicrobial efficacy. Satisfactory results were achieved with the formation of inhibition zones in *Escherichia coli* colonies in in vitro assays. A plasma source employing the mesh configuration is still under development and will eventually replace the one assembled with the wire.

Exploring the Potentialities of Non-Thermal Atmospheric Pressure Plasma Jets

Cold atmospheric plasmas (CAPs) have emerged as a promising technology with remarkable potential in the field of medicine and dentistry due to its antimicrobial, anti-inflammatory, wound healing, and anti-cancer potentialities which encompass a wide range of clinical applications.

CAPs have demonstrated efficacy against a range of microbial species, including bacteria, fungi and virus making them valuable tools for combating various infections. In particular, CAPs have shown inhibitory effects on both planktonic cells and biofilms, which are highly organized microbial communities known for their increased resistance to antimicrobial agents. This resistance poses a significant challenge in medical and dental settings. Therefore, the ability of CAPs to target and reduce the viability of biofilms offers new possibilities for overcoming this obstacle.

Extensive studies have been conducted on the effectiveness of Helium CAP and Helium non-thermal atmospheric pressure plasma jets (He-APPJs) in combating microbial infections. These investigations have revealed the inhibitory effects of CAPs on a variety of microbial species, such as *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans*, and methicillin-resistant *Staphylococcus aureus* (MRSA) [129].

Kostov et al. reported the inhibitory effect of Helium CAP generated inside Petri dishes on bacterial suspensions, observing a significant reduction in viable cells of both *S. aureus* and *E. coli*, with evidence of cell wall damage detected by scanning electron micrograph [130]. Subsequently, our focus shifted to Helium non-thermal atmospheric pressure plasma jets (He-APPJs), which have the advantage of generating cold plasma plumes not confined by electrodes, making them suitable for biomedical applications. Nishime et al. findings indicated that He-APPJ exhibited antimicrobial effects on the Gram-positive bacterium *E. faecalis* (ATCC 29,212), the Gram-negative bacterium *P. aeruginosa* (ATCC 15,442), and the fungus *C. albicans* (SC 5314) [131]. Notably, *E. faecalis* was found to be the most susceptible species, while *C. albicans* demonstrated higher resistance.

CAP's antifungal effect against medically relevant species has been observed, positioning it as a potential therapeutic alternative for recalcitrant fungal infections. Fungal infections present a significant challenge in medicine due to increasing antifungal resistance and limited therapeutic options. CAP has shown the ability to reduce Trichophyton rubrum growth, germination, and adherence to the nail. Fungal suspensions exposed to the plasma jet for 10 and 15 min were unable to infect nail specimens in an ex vivo infection model [132].

Wang et al. [133]. studied the production of N_2O_5 using dielectric barrier discharge (DBD) and gliding arc reactor. They found that the presence of N_2O_5 in the effluent gas led to efficient sterilization effects against bacteria. Moreau et al. [134] provided a comprehensive overview of plasma biodecontamination, emphasizing GA discharge's ability to generate an abundance of reactive species. Gliding arc techniques showed high efficiency against various bacterial species, making it a promising option for decontamination processes.

In addition to antifungal and antibacterial studies on plasma, plasma-mediated virus inactivation is a recent tool of study on plasma medicine that has shown interesting results. Different kind of viruses such as respiratory and sexually transmitted viruses have been evaluated [32]. CAP treatment inactivated respiratory syncytial virus, the main responsible for lower respiratory tract infections in infants, on a glass surface after 5 min of exposure [135]. Chen et al. investigated CAP effect on SARS-CoV-2 distributed on the surface of metal, plastic, cardboard, and composite leather used for various kind of balls. CAP showed good potential inactivating SARS-CoV-2 on all the evaluated surfaces [136]. These findings point out that CAP could be an interesting source of respiratory virus decontamination, mainly in hospital environments where virus can spread easily. In other study focused HIV virus, macrophages were previously exposed to CAP for three times in a total of 45 s of exposure. After, these cells were infected with HIV and the authors observed that CAP treatment reduced the viral reverse transcriptase activity and impaired the steps necessary for successful of virus infection, with no cytotoxic effects to the cells [137]. These findings hold great promise for the development of future innovative therapeutic approaches for viruses' treatment.

Considering the broad antimicrobial spectrum, CAPs prove to be particularly interesting for the treatment of infectious diseases. Importantly, our studies have revealed that CAPs exhibit inhibitory effects not only on planktonic cells but also on biofilms, which are highly organized microbial communities. Biofilm formation is one of the most relevant virulence factors of pathogenic microorganisms. Moreover, biofilms have been shown to be more resistant to external factors, such as antimicrobials, when compared to planktonic cells [138].

The utilization of CAPs in various applications, including wound healing has been a key focus of several research groups. The migration and proliferation of keratinocytes and fibroblasts as well as the formation of the new blood vessels (angiogenesis) is necessary to complete wound healing. In this scenario is important the decontamination of the wound as the bacterial colonization with biofilm formation can impair the proper tissue repair [139]. In the context of wound healing, CAPs have demonstrated their potential to reduce the overall viability of biofilms associated with chronic wounds, including those caused by MRSA, *P. aeruginosa*, and *E. faecalis.* The protocol was effective against these microorganisms after 5 min of exposure, with low cytotoxicity and genotoxicity to a mouse fibroblastic cell

line (3T3) [140]. Additionally, our ongoing in vivo studies on mice and rabbits have shown promising results in terms of tissue repair after exposure to CAPs for 5 min.

Similarly, other studies focused on CAP applications for wound healing have demonstrated satisfactory outcomes in acute and chronic medical conditions that require wound healing. The exposition of acute ulcers to CAP lead to reduced traumatic wound and its associated inflammation, progressive wound repair in genital wart and decreased urticarial and pain of burn wound. For chronic ulcers, the exposition to CAP has also brought positive outcomes such as reduced microbial burden, faster collagen synthesis, angiogenesis, re-epithelialization and finally progressive repair [41].

In vivo studies focused on skin inflammatory diseases have demonstrated an anti-inflammatory potential of CAP, which is crucial for the tissue repair. Positive findings were observed after CAP treatment of allergic contact dermatitis resulting in reduction of inflammation and accelerated healing and for atopic dermatitis, where CAP promoted a decrease of eosinophil and mast cell infiltration, in addition to an inhibition of T helper 2 (Th2) cell differentiation [141, 142].

Vasilets reviewed the potential therapeutic applications of nitric oxide-containing plasma gas. The antimicrobial properties of NO and other species, along with the promotion of tissue regeneration, make it valuable for medical purposes across diverse fields [143]. Nguyen et al. demonstrated the application of GA plasma for the treatment of a failed finger perforator flap [144]. The treatment stimulated tissue repair, exhibited antimicrobial effects, and improved blood circulation, promising advancements in regenerative medicine.

Concern the investigations with surface-wave plasmas, E. Benova et al. have discovered operational regimes that allow the application of a microwave plasma torch for treating biological systems without causing thermal damage. Their investigations have shown significant bactericidal [145] and virucidal [146] effects within remarkably short treatment times (5–30 s), much faster than the typical 2–5 min needed with MicroPlaSter [147]. Furthermore, preliminary experiments on wound treatment in mice [148] have inspired further research in this area. Notably, the production of crucial RONS species for bio-medical plasma effects occurs even when pure argon is used as the working gas, due to the plasma torch's interaction with ambient air [149]. These findings underscore the potential of microwave plasma torches for diverse bio-medical applications, offering rapid and effective treatments without significant thermal damage.

In the field of dentistry, CAPs offer simultaneous antimicrobial, anti-inflammatory, and tissue-repairing effects, making them highly valuable for the treatment of multiple oral diseases (Fig. 10).

CAP properties on decontamination of caries lesions in minimally invasive techniques have been reported. Dental caries is a multifactorial disease influenced by interactions between the tooth structure and the microbial biofilm formed on the tooth surface. These interactions are modulated by factors such as sugars, salivary properties, and genetic factors [150]. A systematic review that evaluated the effect of CAP on dental pathogens observed that CAP is a promising tool against the bacteria associated with dental caries, such *as S. mutans, L. casei and L. fermentum*. However, the authors pointed out that most of the studies have evaluated mono-species biofilms [151]. Considering that multi-species biofilms are more representative of a real clinical situation and more difficult to be completely eliminated, future studies should focus on CAP treatment applied to different kind of multi-specie biofilms.
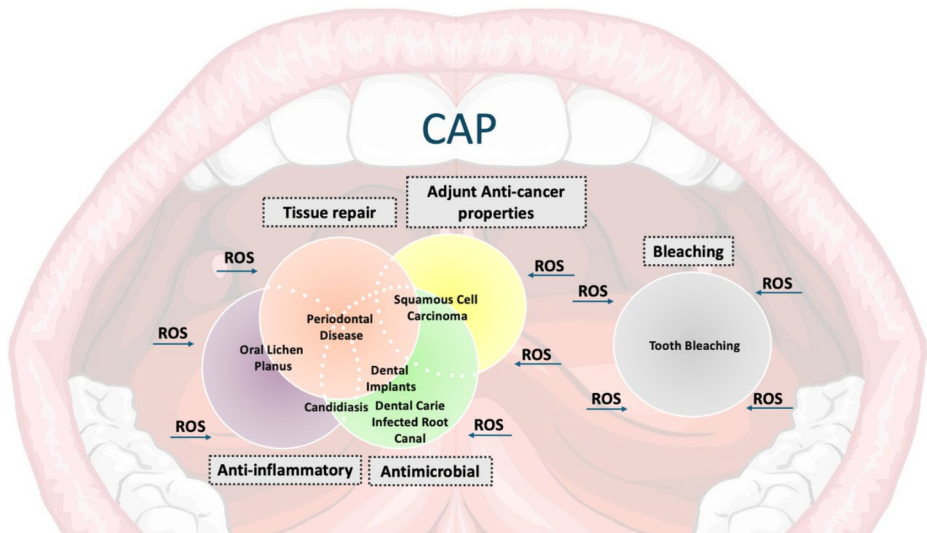
Fig. 10 Different biological subsets related to CAP exposure and ROS generation and their clinical applicability in dentistry. Note that some conditions encompass different biological subsets generated by CAP

Previously, our group showed that Argon CAP produced by kINPen09™ demonstrated inhibitory effects on multispecies cariogenic biofilms composed of *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus gordonii* on hydroxyapatite discs [152]. Ongoing studies on the effect of Helium CAP on these biofilms are also showing promising results.

Microorganisms and their products can reach the root canal, promoting the evolution of dental caries to endodontic/periapical disease. *E faecalis* is a bacterium that can penetrate the radial dentin tubules up to 1000 micrometers. Besides that, *E faecalis* is highly associated with endodontic reinfection. Effectiveness of CAP in eliminating this pathogen has been observed [151]. Its antimicrobial activity against ex vivo root canals biofilms from extracted teeth has also been reported, although in this study the CAP treatment was not so effective as the use of 6% NaOCl [153].

Candidiasis is a fungal infection commonly caused by the opportunistic pathogen *C. abicans*, which can proliferate and cause disease in the presence of local and/or systemic predisposing factors. In immunocompromised patients the superficial lesions may progress to life-threatening disseminated infections. The inhibitory effect of Helium-CAP has been reported against *C. albicans* (SC 5314 and ATCC 18,804) as well as against five clinical isolates from denture stomatitis lesions [154]. Helium-CAP has demonstrated the ability to modulate *C. albicans* virulence traits, significantly reducing the filamentation rate by nearly 40 times, as well as decreasing adherence to epithelial cells and biofilm formation [155]. Importantly, the CAP protocol showed low cytotoxicity to Vero cells [156]. In an in vivo study on the treatment of experimentally induced oral candidiasis with Helium-CAP, simultaneous anti-inflammatory and inhibitory effects on fungal morphogenesis were observed, with a marked reduction in tissue invasion [156].

CAP effectiveness against *C.albicans* was also reported by Ebrahimi-Shaghaghi et al., who also analyzed some mechanisms of action related to this potential. CAP showed inhibi-

tory effects on planktonic and biofilm forms of *C. albicans* [157]. It was observed a reduction of a critical sterol of fungal membrane (ergosterol) in a time dependent manner from 0 to 210 s. The expression of the heat shock protein 90 kDa (HSP90) which is important in the pathogenesis of *C. albicans* was inhibited in the strains ATCC 10,231 and PFCC 9362 after 210 s of exposure. Additionally, CAP exposition induced intracellular ROS, which may induce membrane damage and fungus cell death [157].

Periodontal disease is an inflammatory oral condition that affects the supporting structures of the teeth composed by the gingiva, periodontal ligament and bone. It is a common cause of tooth loss and may contribute to systemic inflammation, leading to worsening of other pathological conditions. The dysbiosis of oral microbiota and dental plaque formation are crucial for the development of the disease. The bacteria from the dental plaque interacts with the immune defenses of the host, leading to inflammation which is responsible for tissue loss and progression of the disease [158].

In a previous study, inhibitory effects of Helium CAP on *Porphyromonas gingivalis* / *Streptococcus gordonii* and *P. gingivalis* HW24D-1 mature biofilms were reported, along with low cytotoxicity to human gingival fibroblasts [159]. Moreover, it exibited minimal genotoxicity to keratinocytes (OBA-9) [152]. In vivo testing using a model of experimental periodontitis induced in c57bl/6 mice demonstrated that a 5-minute CAP exposure tended to improve periodontal tissue recovery, as evaluated by mineral tissue improvement and Type I collagen percentage [159]. These findings suggest the potential of CAP in promoting periodontal tissue healing.

CAP potentialities on periodontal disease were previously explored in other studies [160]. The authors concluded that the efficacy is firstly related to the breakdown of bacterial DNA, RNA and proteins via ROS/RNS-mediated oxidative stress. Additionally, CAP can attenuate the inflammatory response and help in the soft and hard tissue remodeling. The anti-inflammatory property is related to the downregulation of pro-inflammatory cytokines such as TNFα, IL-6 and IL1-β and upregulation of the anti-inflammatory cytokine IL-10 in human periodontal ligament cells (1hPDLCs). Less number of inflammatory cells has been observed in in vivo CAP-treated periodontal disease, which demonstrate this anti-inflammatory potential. The soft tissue remodeling probably occurs via upregulation of cell proliferation and extracellular matrix factors in human gingival fibroblasts (hHGFs), human gingival keratinocytes (hHGKs) and hPDLCs. Moreover, CAP can also be useful to hard tissue regeneration by osteoblastic differentiation of hPDLCs [160].

The anti-inflammatory potential of CAP has also been explored in the context of oral lichen planus disease, which is an inflammatory disease with an associated autoimmune component that can reach the oral mucosa. The treatment of ex-vivo tissues promoted downregulation of many pro-inflammatory cytokines and reduced number of T-helper (CD4+) and cytotoxic T cells (CD8+) in CAP treated tissues [161]. These promising results still need further in vivo validation, but open perspectives for application of CAP in the field of stomatology focused on inflammatory and immune-mediated diseases.

Another CAP ability that has been explored in medical and dentistry fields is the anti-cancer property. Cancer cells have higher amount of aquaporins (water channels) while they have lower amount of cholesterol compared to normal cells, which make them more susceptible to reactive oxygen and nitrogen species [162]. Some mechanisms of action for plasma efficacy on cancer cells are the impairment of cell division and migration ability, gene expression modulation and apoptosis, induction of immune system, enhance of drug

effectiveness and direct tumor cell death by ROS. Many in vitro studies have investigated cancer cell lines from different lineages treated with CAP in combination with the most used medications for cancer treatment. Interesting findings as synergistic anti-cancer effect with increase in the cytotoxic potential of the drug have been observed after CAP treatment [163].

Head and neck squamous cell carcinoma (SCC) represent more than 90% of oral cancers [164]. A previous clinical study with advanced cases of SCC showed that CAP may reduce the pain, the typical fetid odor related to the lesions and may decrease the microbial load with some partial remission of the tumor or wound healing of infected ulcerations [165]. In addition, in vitro studies have demonstrated that CAP may induce EGFR dysfunction in EGFR-overexpressing oral SCC cells through reactive species and may increase the number of dead cancer cells [166]. In this scenario, CAP seems to be a promising tool for (1) ameliorating the side effects of advanced oral cancers and (2) playing an interesting role as an adjunct treatment.

CAP has also been investigated in context of dental implants. A previous study indicated that CAP treatment has not interfered in the elemental composition of different dental implant materials. Additionally, higher proliferation of human gingival fibroblast (HGF-1) and osteoblast-like cells (MG-63) were observed after CAP treatment of the cells for 120 s [167]. CAP treatment of contaminated implant surfaces has demonstrated inactivation or reduction on viability and quantity of biofilms in vitro studies. Moreover, a highly uniform presence of osteogenic tissue and a closer interaction with CAP-treated implant surfaces with improvement of the osseointegration has also been reported in vivo studies [54]. Considering that infection is one of the causes of dental implant failure and the desirable improvement of osseointegration [168], these positive outcomes indicate that CAP could be used in dental implant practice. Its application could be in the biomaterial surface or directly in the treated area at the time of the implantation.

Tooth bleaching is another tool of CAP in dentistry field, which differently from the other conditions previously discussed is this section, is not related to CAP antimicrobial, anti-inflammatory, tissue-repairing or anti-cancer effects. Despite, the generation of ROS is also responsible for tooth bleaching. High-efficiency tooth bleaching has been reached using low concentration of hydrogen peroxide associated with CAP for 10 min [169] or only with CAP treatment after 3 exposures of 60 s [54]. In the last case, CAP was applied in different days simultaneously with root canal disinfection, which is not possible with other bleaching procedures [54]. These findings open perspectives for CAP acting as bleaching agent. The possibility of replacing conventional light sources is desirable as they may present controversy bleaching efficacy and induce high temperatures that can be harmful to surround tissues [54, 169].

Taken together, the potentialities of non-thermal atmospheric pressure plasma in medical and dentistry fields are multiple and dependent on the applied substrate i.e., mammalian cells, cancer cells, different kind of microorganisms, infected ulcers, tooth surface or biomaterial surfaces, such as dental implants. Optimized conditions for each clinical setting should be used to achieve the desired goal for each condition. The adaptation of the plasma device for intra-oral application was a crucial experimental step of our group. A small plasma jet was launched from the end of a long flexible plastic tube, allowing for precise targeting of the plasma jet within the oral cavity [170].

Exploring the Potential of Plasma-Activated Liquids (PAL)

In a recent review by Noala et al. extensive research on Plasma-Activated Liquids (PAL), with a focus on Plasma-Activated Water (PAW), within dentistry is presented [7]. PAL has proven highly advantageous in clinical settings, revolutionizing the treatment of challenging conditions that conventional plasmas struggle to address. Notably, these innovative liquids show promising results in dental applications, such as surgical wound washing, root canal irrigation, and treatment in difficult-to-reach areas within the body.

Various plasma-based devices, including GA plasma jets, surface or coaxial Dielectric Barrier Discharges (DBDs), and surfatron plasma jets, have been utilized to generate PAL from different sources like deionized water, distilled water, tap water, and saline water. For example, the GA plasma jet is used to produce PAL, investigating its antimicrobial properties through direct liquid application [171] and nebulized PAW against bacteria like *S. aureus* and *E. coli* [172]. Nebulized PAW has consistent physicochemical properties even during transportation through tubes, making it effective for controlling microbiota in respiratory tract appliances [172].

In another study, the GA device was used to activate different liquids with varying properties, such as deionized water, distilled water, and tap water. The resulting PAL showed significant inhibitory effects on *E. coli*, and their safety for medical and dental applications was confirmed through minimal cytotoxicity to normal oral keratinocytes (NOK) [173, 174].

The effectiveness of PAW in disinfecting various dental materials, including endodontic devices, silicone, diamond dental drills, metals, titanium alloy surfaces, and titanium disc surfaces, has been demonstrated [7]. The use of PAW as an alternative disinfection method presents advantages over traditional techniques, and its antimicrobial effects are attributed to the main reactive species and low pH acquired during plasma activation [175]. PAW's long-lasting antimicrobial effect when stored at low temperatures further supports its potential for dental sterilization [176]. Studies also indicate that PAW is effective in decontaminating dental unit waterline system tubes and shows promise in the decontamination of other dental materials [177, 178]. These findings highlight PAW's potential as a new sterilization method in dentistry, with opportunities for further exploration of other materials and polymicrobial biofilms relevant to clinical settings [179].

Treatment of oral infectious diseases using PAW is a promising field of research, with potential applications in various oral conditions, such as caries, periodontitis, and candidiasis [7]. In the case of dental caries, PAW has shown efficacy against cariogenic bacteria like *Streptococcus mutans* and *Lactobacillus spp.*, which are responsible for tooth demineralization [180]. PAW activated with a non-thermal plasma of argon and oxygen reduced the viability of *S. mutans* in both planktonic and biofilm forms [181]. PAW also demonstrated a reduction in *S. mutans*, *Porphyromonas gingivalis*, and *Actinomyces viscosus* within minutes of treatment [180]. Moreover, studies suggest PAW's potential role in enhancing bond strength in adhesive restorations, which could prevent microleakage and secondary caries [59].

Regarding oral candidiasis, PAW has shown antimicrobial effects against *C. albicans* in planktonic and biofilm forms [119]. Investigations into the effects of PAW on *C. albicans* revealed a reduction in viability, and the main components responsible for its antifungal activity were suggested to be hydroxyl radicals and NO_3^- [182]. However, one study reported no effect of plasma-activated tap water on *C. albicans* [171]. In endodontic infec-

tions, PAW has been investigated for its potential as an antimicrobial irrigator in root canals [182]. Studies demonstrated a reduction in *Enterococcus faecalis* viability in biofilms after short exposures to PAW [183]. PAW also exhibited antimicrobial effects against *E. coli* and *S. aureus*, which are occasionally found in root canals [184]. In periodontal disease treatment, PAW showed a progressive inhibition of *Porphyromonas gingivalis* in both planktonic and biofilm forms [122]. Considering the restricted access of subgingival biofilm, PAW's ability to reach such areas makes it an interesting treatment option [180].

PAW has been extensively studied and found to contain various reactive oxygen and nitrogen species (RONS) that offer promising applications in the antimicrobial field [173]. Research has demonstrated its efficacy in treating surfaces contaminated by bacterial biofilms, including those associated with dental devices [174]. Furthermore, PAW has shown potential in reducing bacterial colonies associated with oral infectious diseases [89]. Further in vitro and in vivo investigations are needed to standardize the best parameters for each solution and microorganism to harness the full potential of PAW in treating oral infectious diseases [7].

The non-thermal plasma treatment using PAW has shown promising anti-inflammatory properties and wound healing capabilities, making it a potential alternative for the treatment of infectious diseases in dentistry [7].

PAW has demonstrated selectivity in its antimicrobial action, causing no damage to mammalian cells [185]. Long-term exposure of PAW on vital mouse teeth did not result in significant changes in tooth structure or toxicity to surrounding tissues [186]. Furthermore, PAW has been shown to decrease inflammation in various studies, including oral candidiasis treatment in mice and as an adjuvant therapy for periodontitis in rats [119]. It was observed that PAW could suppress pro-inflammatory genes and cytokines, thus potentially acting against both acute and chronic inflammation [187].

PAW has also demonstrated favorable effects on wound healing, promoting cell proliferation and migration in human keratinocytes [188]. Although direct studies on the effect of PAW or PAL on oral wound healing are lacking, observations in rats and mice suggest a tendency to improve periodontal tissue loss after CAP treatment [189].

The potential applications of PAW in oral surgery and implantology are promising. CAP treatment has shown positive effects on osteoblast-like cells, suggesting potential use in oral surgery for wound closure and healing [190]. Moreover, PAW could enhance dental implant surfaces, promoting better osseointegration [59].

The simplicity of PAW's use as a mouthwash or irrigation agent, along with its antimicrobial, anti-inflammatory, and wound healing properties, makes it a potential adjuvant tool in dentistry for conditions requiring tissue repair [7].

Indirect CAP treatment using PAW has demonstrated promising anti-cancer properties, with cancer cells being more sensitive to the harmful effects of reactive oxygen and nitrogen species than healthy cells [32]. The higher expression of water channels (aquaporins) in cancer cells allows for easier penetration of reactive species, leading to various types of cell death, such as apoptosis, necrosis, or senescence [32].

Using PAW in cancer therapy is promising due to its ease of use and ability to treat large or deep tumors [32, 191, 192]. The treatment is faster, more comfortable for patients, and can inhibit tumor growth and metastasis [191–193]. In oral squamous cell lines, PAL has shown anti-cancer properties, with reductions in cell viability [194].

The potential applications of PAW in oral cancer therapy are significant. PAW could be used before surgery by washing or injecting into deep neoplasms and after surgery to reduce microbial load and promote wound healing, potentially preventing recurrences [7]. PAW could also be used in oral premalignant lesions, offering a non-invasive approach alongside other therapies or as a standalone treatment, depending on individual patient factors [7].

Conclusion

In conclusion, the exploration of CAP and its various applications, including PAL, has opened exciting possibilities in the fields of medicine and dentistry. CAPs have proven to be highly effective in combating a wide range of microbial species, including bacteria, fungi, and viruses. Notably, CAPs have shown inhibitory effects on biofilms, which are notoriously resistant to conventional antimicrobial agents, presenting a significant challenge in clinical settings. Moreover, CAPs demonstrate remarkable potential in wound healing, tissue repair, and inflammatory conditions. The generation of RONS in PAL, such as PAW, further enhances their antimicrobial properties. PAW has shown promise in treating contaminated surfaces, decontaminating dental devices, and addressing oral infectious diseases. The versatility of CAPs lies in their ability to be applied through various plasma sources, such as gliding arc, DBDs, and surfatron plasma jets, each offering specific advantages in the activation process of PAL. Importantly, safety studies have demonstrated minimal cytotoxicity to healthy cells, making these innovations potentially safe for use in diverse medical and dental applications. The ongoing research and development in this field hold great potential for revolutionizing clinical approaches, providing effective and safe treatments for infectious diseases, wound healing, and oral conditions. The antimicrobial, anti-inflammatory, and tissue-regenerating properties of CAPs make them valuable tools for improving patient care and enhancing treatment outcomes. As the understanding of plasma science and its potential applications continues to expand, we can look forward to witnessing even more innovative and transformative uses of CAPs in the medical and dental fields.

Data Availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

Ethical Approval Not applicable.

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