Applications of Cold Atmospheric Pressure Plasma in Dentistry

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Abstract: Plasma is an electrically conducting medium that responds to electric and magnetic fields. It consists of large quantities of highly reactive species, such as ions, energetic electrons, exited atoms and molecules, ultraviolet photons, and metastable and active radicals. Non-thermal or cold plasmas are partially ionized gases whose electron temperatures usually exceed several tens of thousand degrees K, while the ions and neutrals have much lower temperatures. Due to the presence of reactive species at low temperature, the biological effects of non-thermal plasmas have been studied for application in the medical area with promising results. This review outlines the application of cold atmospheric pressure plasma (CAPP) in dentistry for the control of several pathogenic microorganisms, induction of anti-inflammatory, tissue repair effects and apoptosis of cancer cells, with low toxicity to healthy cells. Therefore, CAPP has potential to be applied in many areas of dentistry such as cariology, periodontology, endodontics and oral oncology.

Keywords: cold atmospheric pressure plasma; antimicrobial agent; plasma medicine; dentistry

1. Introduction

Plasma is frequently referred to as the fourth state of the matter and can be described as a gaseous mixture of neutral particles, electrons and ions at different densities and temperatures. Most of the visible matter in the universe (about 99%), such as stars, nebula and interstellar medium, is in the state of plasma. Plasma can be generated by heating a gas or by subjecting it to strong electromagnetic fields to the point that the gas particles become ionized. Thus, plasma is an electrically conducting medium that responds to electric and magnetic fields, which is also a source of large quantities of highly reactive species, such as ions, energetic electrons, excited atoms and molecules, ultraviolet photons, metastable, and active radicals [1]. In laboratory conditions, plasma is generally produced by an electric discharge in noble or molecular gases, such as argon (Ar), helium (He), oxygen (O2) and nitrogen (N2), using different excitation schemes, such as microwaves, radiofrequency and DC or AC electric fields [2].

Normally, the electron and ion densities in plasmas are approximately equal (a condition called quasi-neutrality), but the respective electron and ion temperatures can be quite different. Plasmas are usually classified as thermal and non-thermal plasmas. Thermal plasmas are in thermal equilibrium, which means that their temperatures are relatively homogenous throughout the heavy particles (i.e., atoms, molecules, and ions) and...
electrons that usually span in the range of thousands of K [1]. The so-called non-thermal or cold plasmas are partially ionized gases and their electron temperatures exceed several tens of thousands K, while the heavy particles (ions and neutrals) have a much lower temperature [2]. Plasma can be also generated under different pressure conditions, including atmospheric pressure [1]. In the last decade, atmospheric pressure plasmas have become a very attractive tool for material processing applications because they are generated in an open environment and can be easily implemented in online processing. However, working at atmospheric pressure has some disadvantages. For instance, gas breakdown at atmospheric pressure occurs at much higher electric field, typically in the order of ten of kV cm⁻¹ [1]. Additionally, if special precautions are not taken, the atmospheric plasmas have the tendency to become thermal i.e., hot plasmas that can damage heat sensitive materials or burn living tissues [2].

A gas discharge in which a dielectric layer covers one or both electrodes is called Dielectric Barrier Discharge (DBD) and it was first introduced by Siemens—in 1857—for the production of ozone. Large number of DBD systems has been reported [3] with the planar and cylindrical geometries as the most common employed configurations. Figure 1a depicts a typical planar DBD reactor, while Figure 1b shows the so-called floating electrode DBD (FE-DBD) [4,5]. The plasma, in this case, is formed between an insulated high voltage electrode and a target (human skin or living tissue), which acts as a floating counter electrode.

![DBD reactors schematic](image)

**Figure 1.** Schematic representation of DBD reactors in planar geometry: (a) conventional two electrodes DBD and (b) floating electrode DBD (FE-DBD). Adapted from [5].

Due to the current limitation caused by the charge accumulation on the dielectric surface, the gas temperature in DBD can be quite low (about the room temperature), which makes it adequate for biological applications [4]. However, the plasma in DBD devices is confined into small gaps between two electrodes (usually in the order of few mm), which is a disadvantage for some applications. For instance, FE-DBD was successfully used in a number of clinical trials for the treatment of skin diseases, to control melanoma development, blood coagulation and antisepsis of open wounds [5]. However, it is not suitable for plasma application inside the human body’s cavities, such as tooth root canals or internal organs.

On the other hand, in the so-called atmospheric pressure plasma jets (APPJs) the plasma generated into a dielectric enclosure (tube or syringe) is expelled through a small orifice into the ambient atmosphere by gas flow (usually a noble gas). The ejected plasma forms a plasma plume that extended several cm into the air and can be easily directed to a target. Figure 2 shows a drawing of the APPJ concept. In the last decade many APPJ configurations, different electrodes arrangements and excitation schemes have been reported in the literature. More details about APPJs and their characteristics can be found in some recent review papers [6–8].
Depending on the jet’s operating conditions, the plasma plume tip can be maintained below 40 °C, enabling the contact with living tissues without any risk of burns and electric shock. Thus, cold atmospheric pressure plasma (CAPP), such as FE-DBD and APPJ, have been appointed as the most promising tools for biomedical and hospital applications [9–12].

Since CAPPs are generated in ambient air, large quantities of reactive oxygen and nitrogen species (RONS) are produced. Therefore, when CAPP enters in contact with living tissues, the synergistic action of several plasma components, such RONS, energetic (UV) photons, and charge particles should be considered. The biochemical mechanisms involved in the interaction of plasma species with microorganisms and cells, as well as the plasma application for tissue healing and disinfection are extensively studied in a novel interdisciplinary field called Plasma Medicine [9,10]. Recent studies have demonstrated that RONS are the main factor responsible for plasma antimicrobial and tissue healing effects, while the UV photons have only minor effect [13].

CAPP can be applied directly on living tissues and, in this case, RONS reach directly the target. Alternatively, CAPP can be applied indirectly, by previous exposure of liquids (i.e., water, liquid culture media) to the plasma, creating solutions containing RONS, known as plasma-activated media (PAM) or plasma-activated water (PAW) [14,15]. In general, the plasma-liquid interaction generates hydrogen peroxide, nitrites, nitrates and others RONS [16]. However, the composition of PAM depends not only on the plasma-liquid interactions but also on the subsequent chemical reactions in the liquid phase that can cause further changes into PAM composition [15].

2. CAPP Biological Activities

The biological effects of CAPP enable several applications in the medical area [9]. Laroussi [17] was the first author to report on the antibacterial effect of CAPP. After, an expressive number of manuscripts, review articles, contributions to conference and books on the antimicrobial potential of CAPP and on the physicochemical mechanisms for antimicrobial inactivation have been published. The growth control of several pathogenic microorganisms, such as Gram-positive and Gram-negative bacteria, fungal species and bacterial spores have been reported [18–23]. Additionally, an antibiofilm effect has also been observed for bacteria and fungi [24–28].

Interesting data point out to the anti-inflammatory and tissue repair effect induced by CAPP [29,30]. CAPP improved wound healing in mice with induction of type I collagen and MCP-1 protein production in keratinocytes and fibroblasts [31,32]. Brun et al. [33] observed increased migration and proliferation of fibroblasts in response to the production of RONS during CAPP exposure. Similar effects were observed by Bourdens et al.
[34] and Haralambiev et al. [35]. Stimulation of keratinocytes by antioxidant pathways was also reported [30,36]. CAPP showed positive effect in the cutaneous microcirculation, increasing the tissue oxygen saturation and radial blood flow [37,38] that can contribute to improved tissue repair.

A remarkable feature of CAPP is its highly selective toxicity that highlights the potential for clinical treatment of infectious diseases [9,39,40]. This differential activity is based on differences in the cellular metabolism in presence of RONS. Eukaryotic cells exhibit protection to RONS, while prokaryotic ones do not demonstrate such protective mechanism [39,41–43]. The disparity in the cell sizes influences response to CAPP. For instance, bacterial cells (typically between 0.2 and 10 μm) have higher surface-volume ratio, which favours the plasma action, while eukaryotic cells are much bigger, from 10 to 100 μm [10,39,42]. Moreover, the organization of eukaryotic cells into tissues additionally increases their resistance to CAPP effects. Therefore, by adjusting the treatment parameters, plasma can be used to eliminate bacteria in planktonic or biofilm forms without damage to the surrounding host tissues [9,44–46].

In this context, treating fungal diseases has an additional challenge, as both fungal and host cells are eukaryotic. Hence, in this particular situation, the simultaneous study of fungal inhibition and toxicity to host cells is extremely important. Interestingly, there have already been some encouraging results in the literature. Borges et al. [24] reported that CAPP treatment for 5 min has antibiofilm effects on Candida albicans with low cytotoxicity to Vero cells [47]. In the same study, CAPP was also applied in vivo to treat oral candidiasis in mice without damaging the surrounding tissues.

The ability of CAPP to induce cell death by inducing apoptosis [48] can be also very useful for therapeutic purposes, and it has been applied in the control of cancer cells. In this case, metabolic differences between healthy and malignant cells favour the selectivity of CAPP. Constant cell replications, observed in malignant cells, can expose their DNA to CAPP more frequently, favouring to the cell structural damage [39,42,49–51].

RONS generated by CAPP, including hydroxyl radicals (OH), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), superoxide anion (O²⁻), atomic oxygen (O), atomic nitrogen (N), nitric oxide (NO), nitrogen trioxide (NO₃), influence redox-regulated cell processes [9,11,18,42,52–56]. In particular, reactive oxygen species (ROS) can react with many biological macromolecules, causing oxidative structural modification and the loss of their biological function [57]. At the cellular level, ROS regulate growth, apoptosis and other signalling processes, while at the system level, they contribute to complex functions, including regulation of immune response [58]. In addition, ROS are emerging as the most important agents in the bacterial response to lethal stress. Currently, the effects of superoxide, hydrogen peroxide and hydroxyl radical have been studied. Superoxide and hydrogen peroxide arise when molecular oxygen oxidizes redox enzymes that transfer electrons to other substrates. Hydrogen peroxide that can be produced from the dismutation of superoxide serves as a substrate for the formation of hydroxyl radicals. If this oxidative process is not controlled, an accumulation of hydroxyl radicals can occur. The hydroxyl radical breaks down nucleic acids, carbonylated proteins and peroxidised lipids which can lead to cell death [59].

Reactive nitrogen species (RNS) can be both harmful or beneficial to living systems. At low concentrations, RNS can play an important role as a regulatory mediator in signalling. On the other hand, at moderate or high concentrations, RNS are harmful to living organisms and can inactivate important cellular molecules [60]. Nitric oxide is an important regulator of physiological processes [61] and can mediate the harmful cellular toxicity of metabolic enzymes, generating nitrite peroxide as a final product of reaction with superoxide [62].

The exact mechanism of CAPP and microbial cell interaction is not fully understood yet, but presently it is widely accepted that its antimicrobial activity is associated with synergistic action of two major CAPP components, UV radiation and RONS. They can break covalent bonds of stable compounds, such as the peptidoglycan from the bacterial
cell walls and peroxidation of lipids in the cell membrane [17,44,56,63–65]. Also, CAPP interaction with prokaryotic cells can cause cellular rupture by electro erosion with formation of ionic pores and subsequent loss of cellular content [11,63]. CAPP can also break covalent bonds in the polymeric matrix of microbial biofilms, favouring their disruption [28,45,46,56,66].

3. CAPP Application in Periodontology

Periodontal disease (PD) affects the dental support tissues and it is a major cause of tooth loss impacting on individual’s function and social behaviour. Nowadays, it is well established that the presence of biofilm formation itself can lead to gingivitis but not to periodontitis. Among the bacteria involved in periodontitis development, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, known as red complex, are the most studied and associated to tissue destruction. In particular, *P. gingivalis* can modulate the host response leading to bacterial unbalance into the gingival sulcus [67]. Due to the limited gain of the traditional periodontal treatment, it is necessary to find new adjuvant therapies.

Mahasneh et al. [68] treated blood agar plates previously inoculated with *P. gingivalis* (ATCC 33277) with He-CAPP jet from 5 to 11 min, and found significant difference in the diameter of inhibition zone in a time dependent manner for all periods of application when compared to the control group. Authors attributed the results to cell damage induced by RONS, though no specific analysis was performed to confirm the hypothesis. Liu et al. [69] studied *P. gingivalis* mono species biofilms using confocal microscopy and also evaluated the effect of CAPP on rabbit mucosa. Differently of Mahasneh et al. [68] that used He-CAPP, Liu et al. [69] tested He/O₂ mixture as working gas and observed that 5 min of treatment inhibited the most bacterial cells contrary to negative control. Moreover, after one or five days of CAPP exposure for 10 min on healthy rabbit mucosa, they found no signs of irritation.

Kuçuk et al. [70], in the first clinical trial using CAPP as an adjuvant therapy for nonsurgical treatment in one-time application protocol, found significant gain of clinical attachment length after three months. They also observed the elimination of microorganisms in the red complex and recolonization reduction.

In terms of tissue repair, Kwon et al. [71] evidenced that CAPP treatment for 1 and 2 min could improve cell morphology of human gingival fibroblasts and enhance the mRNA expression of TGF-β and VEGF. Though, when authors tested CAPP for 4 min, both morphology and growth factors expression were better in control group. Interestingly, Eggers et al. [72] observed that one day after a 60-s CAPP application on osteoblast-like cells, the mRNA of proinflammatory cytokines such as IL-6, IL-8 and IL-1 were up-regulated as well as TNF-α, COX₂, CCL₂ mRNA and COL 1, important genes for wound healing. Plus, proliferation genes such as PCNA and Ki-67 mRNA expression was significantly upregulated.

More recently, most of the studies have been focused on peri-implantitis as the study of Shi et al. [73]. Similar to periodontitis, peri-implantitis is an inflammatory disease dependent on biofilm that causes loss of hard and soft tissue, but it occurs around the dental implant. The authors evaluated the effects of CAPP (3 min) as an adjuvant to clinical treatment in ligature induced peri-implantitis in beagle dogs. The study compared this treatment with the traditional clinical treatment using 0.2% chlorhexidine digluconate (3 min) as decontaminant. Clinical and bone analyses (micro-CT and histology) showed better recovery in CAPP-treated group three months after treatment. Microbial recovery by PCR showed that *P. gingivalis* and *T. forsythia* quantity was significantly reduced when compared to control and baseline. *A. actinomycetemcomitans* had a significant decrease in the first month that was not maintained for the next two months of follow up.

Carreiro et al. [74] showed that CAPP can reduce viability and, supposedly, quantity of *P. gingival* biofilm in titanium discs after 1 and 3 min with no epithelial harm for gingival tissues, in vitro. The same results were obtained by Lee et al. [75] using He-CAPP for
3 and 5 min. Authors noted that not only the exposed, but also the peripheral area of the titanium discs were decontaminated by CAPP. The authors also observed an increase in anti-VEGF expression after CAPP treatment using an in vitro gingival epithelium model.

Not directly focused on periodontitis, some studies on CAPP therapy let us intrigued on its potential on periodontal therapy. Arndt et al. [31,76] observed an increase in the β-defensins levels in fibroblasts and keratinocytes, and type I collagen induction after 2 min of cell activation in vitro using a microwave plasma torch (MicroPlaSter β plasma torch system) with argon. These findings are extremely important since β-defensins is a component of the innate immune response that avoid the increase in the count of pathogenic bacteria in the gingival sulcus during the first stages of periodontal disease, and expression of human β-defensins mRNA is decreased in patients with chronic periodontitis when compared to healthy individuals [77]. Additionally, induction of type I collagen indicates wound healing stimulation.

Meanwhile, Brun et al. [33] demonstrated increase on migration and proliferation of fibroblasts in response to ROS produced by He-CAPP. In a recent study, the same group [78] studied P. aeruginosa and S. aureus biofilms and demonstrated that CAPP damaged the cell membrane in a way that prevents antimicrobial resistance, and has a synergistic effect with other antimicrobial drugs.

These antimicrobials, antibiotic, and tissue stimulating evidences of CAPP therapy taken together show the importance of studying this technology as an adjuvant therapy for periodontitis that remains as a chronic disease where current gold standard therapy (scaling and root planning) is based only on damage control, in a sense that pathogenic microbial colonization can be reduced but colonization commonly occurs in the short-term, and tissue reattachment can be achieved, but still there is no gain of lost tissue.

4. CAPP Application in Endodontics

Endodontic infection occurs in the tooth root canal system that gets exposed to the oral environment. The microorganisms can reach the intra-radicular region from a carious lesion or after a traumatic injury to the coronal tooth structure [79]. Primary endodontic infections are caused by polymicrobial biofilms dominated by aerobes and facultative anaerobes microorganisms [80]. The persistence of Enterococcus faecalis in the root canal system is the main cause of post-treatment infection followed by Fusobacterium and Propionibacterium [80,81].

Endodontic infection treatment involves removing of remnants vital or necrotic tissues, the elimination of microorganisms within the root canal system, and removal of hard tissue debris that is formed during root canal instrumentation. The root canal disinfection is considered the pivot of this therapy [81]. NaOCl solution is commonly used to clean and disinfect the root canal due to its antimicrobial activity, however, it can also affect vital pulp and decreases the mechanical resistance of dentin [82].

Some studies have shown that CAPP is able to inhibit the persistent root canal microorganism Enterococcus faecalis. Chang et al. [83] evaluated the effect of CAPP against E. faecalis suspensions spread on the surface of sterile glass slides. They observed that CAPP was able to reduce the number of colonies forming unities after 2 min of exposure. They also observed that the antimicrobial effects are time-dependent and the exposure for 3 min showed the best results. In situ studies using dental tissue as the surface for biofilm formation could better represent the root canal system. Armand et al. [84] reported that the viability of E. faecalis biofilms formed on the surface of tooth fragments were significantly reduced after exposure to He-CAPP or He/O₂-CAPP for 4, 6 and 8 min.

The association of He-CAPP with other substances can also be a strategy for clinical application. Li et al. [85] observed that Ar/O₂-CAPP treatment for 12 min eliminated 3-week E. faecalis biofilms without changes of root canal dentin. Zhou et al. [86] observed that He-CAPP jet flowing through 3% hydrogen peroxide (H₂O₂) showed better effects against E. faecalis biofilms formed inside extracted teeth root canals when compared to He-CAPP alone. Optical emission spectroscopy analysis showed that stronger emission
lines of atomic oxygen and hydroxyl radical in the He/H₂O₂-CAPP when compared with He-CAPP.

More recently, the indirect CAPP treatment also appeared as an alternative for the disinfection of root canals system, although few information is available so far. Yamamoto et al. [87] showed that plasma-treated water (PTW) can be used as an endodontic irrigant. The in-situ experiment showed that PTW was able to disinfect the root canal experimentally infected with E. faecalis without adverse effects to oral mucosa.

The study on the CAPP effects against Propionibacterium is scarce in the literature. According to Ali et al. [88] CAPP exposure inactivated P. acnes cells in suspensions and biofilm formed on glass slides. They also observed that the antimicrobial effect increases with time.

No study reporting the antimicrobial effects of CAPP against Fusobacterium could be found in the literature. However, CAPP has already showed antimicrobial activity against other Gram-negative and anaerobes oral pathogens as P. gingivalis [68].

The aim of endodontic treatment is to eliminate the pathogenic microorganisms and prevent reinfection, avoiding clinical failures. The studies shown that CAPP can be an alternative to the conventional irrigant solutions which can damage healthy tissues. The antimicrobial effect of plasma jet seems to be correlated with the time of exposure and the working gas. Then, an efficient protocol should be developed to the use of CAPP in the endodontic clinical routine.

5. CAPP Application in Cariology

Carious dental tissue infected by cariogenic microorganisms is usually removed by using rotating instruments that can cause pain and discomfort to the patient. However, the treatment of carious lesions has been changing in the last years. Minimal intervention dentistry (MID) is one of the proposed alternatives that aims to prevent or paralyze disease’s activity [89]. In this context, atraumatic restorative treatment (ART) is one example of MID and it has been used with success [90]. This method shows positive results in the treatment of caries in childhood and injuries in children and elderly [91–94]. In ART, carious tissue is removed by hand instruments and the restoration is carried out [94]. It has been described that the performance of ART can be improved by the elimination of the cariogenic microorganisms during the cavity’s cleaning process carried out before the restorative procedure [95].

CAPP has been suggested as a promising therapeutic tool in cariology due to its antimicrobial efficiency [96]. Promising results have been obtained against cariogenic bacteria [97]. Sladek et al. [98] were the first researchers to suggest the use of CAPP for the disinfection of caries cavities. The researchers concluded that it represents an efficient technique that promote the disinfection of irregular structures and canaliculi inside the affected tooth. Plasma needles generated by specific devices allow the penetration of reactive RONS inside the dental canaliculi inhibiting the cariogenic microorganisms [98]. In addition, plasma has the advantage to inhibit microbial biofilms, without damaging the normal tissue [28,97] and without causing hyperaemia, swelling, ulcer or anabrosis, resulted from absence of thermal damage [28,69].

Subsequent studies also reported the effect of CAPP against cariogenic microorganisms. Hirano et al. [99] analysed the effect of CAPP on free-floating planktonic cells. The treatment with CAPP reduced the counts of Streptococcus mutans in 4-logs after 3 min of exposure. Park et al. [100] described the inhibitory effect of atmospheric pressure plasma associated with gold nanoparticles on S. mutans. The group observed that gold nanoparticles conjugated to the bacterial surface and stimulated by the CAPP affected the bacterial cell wall, suggesting that this association may be a future alternative for caries treatment.

The inhibitory effect on S. mutans and Lactobacillus acidophilus biofilms grown on hydroxyapatite discs has been observed a few seconds after the CAPP treatment [101]. The study suggested that CAPP deactivation of microorganisms was caused by bombardment of charged or neutral species or an accumulation of electric charge a few seconds after
treatment. Recently, the inhibition of a cariogenic multispecies biofilm formed by *S. mutans*, *S. sanguinis* and *S. gordonii* by Ar-CAPP was reported [102]. Despite of these positive evidences, studies about the effectiveness of CAPP treatment on polymicrobial cariogenic biofilms is still needed [96].

CAPP can also improve the conventional restorative methods. It can optimize the adhesion between the tooth and the restorative material [103]. Another study demonstrated that the CAPP induces the polymerization of a dental adhesive by direct and indirect energy transfer [104]. In this sense, CAPP can control the moisture of demineralized dentin surfaces, improve adhesive penetration and the mechanical properties of the adhesive/dentin interface [105].

Considering all the characteristics and effects of CAPP, the application of this innovative technique can be an alternative for the treatment of caries diseases in the near future, allowing more conservative procedures and improving restorative methods.

6. CAPP Application in Oral Oncology

The incidence and prevalence of oral cancer have been continuously increasing in numbers, as well as the mortality rates, especially among younger patients [106]. Oral cancer stands as an international public health problem and one of most common cancer with more than 177,000 deaths and 354,500 new cases year worldwide [107].

Recent advances and future directions have been proposed on oral oncology [108,109]. In addition to surgical approaches, the stereotactic body radiotherapy associated with smart drug delivery systems (SDDSs) have been proposed to oral cancer therapy [109–112]. The immunotherapy also advanced considerably in the last years [111,113–115]. However, those new discoveries involve expensive treatments and ultra-expensive drugs [116–118]. For this reason, the search for alternative is needed.

The potential of CAPP in oral oncotherapy is based on its selectivity towards malignant cells, capacity to induce cell death, immune response, and controlled discharge of RONS that can interfere on the molecular mechanisms of the disease [51,119,120]. The role of oxidation/reduction potential is already understood as key factor for the progression and establishment of the disease based on the HOCl or the ·NO/ONO− signalling pathway [119,121]. In this way, NO and nitrite therapies have already been applied as anti-cancer based on their effects on cancer cells and on catalase-dependent apoptotic pathways, which are implied in development and regression of the disease [51,121,122].

CAPP treatment as a tool to control oral cancer cells has been studied in the last years. Han et al. [123] reported that N2- CAPP induced DNA damage in SCC-25 oral cancer cells. The effect on head and neck squamous cell carcinoma (HNSCC) was also detected [124]. CAPP can also be an alternative for the treatment of oral lichen planus, a precancerous lesion [125]. Interestingly, cancer cells such as SCC-15 and HNSCC were more sensitive to CAPP when compared to non-cancer cells lines [124,126].

Clinical researches showed that CAPP can reduce the microbial load in the lesions and the pain, as well as partial remission on head and neck cancer patients [127,128]. The proposal to use CAPP for oral cancer treatment is recent and the understanding of in situ effects requires more studies as a promising pro-oxidant therapy [120,121].

7. CAPP for the Treatment of Oral Candidiasis

Oral candidiasis is an opportunistic disease with high prevalence among immunocompromised patients [129]. Lately, reports on refractory cases of oropharyngeal candidiasis are increasing [130,131] and the treatment of these cases has faced considerable challenges due to the increasing occurrence of antifungal resistance and low number of new antifungal molecules [132].

Proton ATPases, efflux pumps, adherence, morphogenesis, and resistance to oxidative stress have posed as new targets to the development of novel antifungal agents [133,134]. In this context, CAPP antifungal effect has been studied.
Anti-*Candida albicans* effect was reported by some studies [21,135–137]. Additionally, CAPP showed modulatory effects on *C. albicans* virulence factors, such as adhesion and filamentation [24,136]. Suppression of ergosterol biosynthesis has been observed [138].

Antibiofilm effect is considered a key factor to superficial candidiasis treatment. Exposure to He-CAPP for 150 s reduced significantly the viability of *C. albicans* biofilms, with low cytotoxicity to Vero cells [24]. The same effect was detected when Ar-CAPP [28,139,140] and a microwave-Induced Plasma Torch were used [141]. Recently, Singh et al. [142] reported that RONS from CAPP inhibited *C. albicans* biofilms by affecting the fungal cell wall.

Murine experimental model of oral candidiasis treated with CAPP displayed marked reduction in inflammation and fungal tissue invasion [47].

8. Conclusions

Cold atmospheric pressure plasma (CAPP) has antimicrobial and anti-inflammatory effects that are useful in several areas of Dentistry, such as in Cariology, Periodontology and Endodontics. Additionally, CAPP has been showing potential to be used in the treatment of oral fungal diseases and to control oral cancer. However, additional in vivo studies to standardize clinical protocols are still needed.

9. Future Perspectives

CAPP has a great potential to be used in dentistry in the near future, with applications in several dental specialities. CAPP might contribute to the treatment of refractory infectious diseases and control of oral cancers. Currently, the major challenge to be overcome is the determination of standardized therapeutic protocols to each disease that can be validated by controlled clinical trials.

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