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Beyond Conventional Disinfectants: A Comparative Review of Cold Atmospheric Plasma and Photodynamic Therapy against Oral Pathogens

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Abstract

Introduction: Microbial resistance is a global challenge for conventional antimicrobial methods such as chlorhexidine (CHX) and sodium hypochlorite (NaOCl). Among the recently developed approaches, photodynamic therapy (PDT) and cold atmospheric plasma (CAP) have garnered attention. This study reviews the effectiveness of CAP and PDT relative to each other and to other antimicrobial methods.

Methods: The primary keywords, including "antimicrobial photodynamic therapy," "cold plasma," "disinfection," and "oral pathogens," were used in a literature search across databases such as Google Scholar and PubMed, covering the period from January 2016 to September 2025.

Results: CAP and PDT showed effective antimicrobial effects against oral pathogens. For CAP, main parameters include gas composition (He/O_2), power (55 W), and exposure duration (60-180 seconds). A He/O_2 plasma jet reduced the bacterial load in *Enterococcus faecalis* biofilms. The efficacy of PDT was different based on the types of photosensitizers and light parameters. Indocyanine green (0.2%) with an 810-nm laser (12 J/cm², 60 s) was effective against *Streptococcus mutans*, while Methylene blue (0.02%) with a 660-nm laser (20 J/cm², 100 s) showed higher biofilm reduction. Both methods surpassed conventional antimicrobial agents such as CHX and NaOCl, especially in biofilm penetration.

Conclusion: CAP and PDT are promising alternatives to conventional antimicrobial methods, offering effective control of oral pathogens with lower side effects than CHX and NaOCl. However, further research is needed to optimize treatment parameters and determine the most suitable clinical applications for each method.

Keywords: Antimicrobial photodynamic therapy, Cold plasma, Disinfection, Oral pathogen

Introduction

Oral health is inextricably linked to the balance of the oral microbiome. The emergence of microbial biofilms on hard and soft tissues is a primary etiological factor in prevalent oral diseases such as dental caries, periodontitis, and endodontic infections.[1] The management of these infections has long relied on conventional antimicrobial agents such as chlorhexidine (CHX) and sodium hypochlorite (NaOCl), which are considered gold standards in dental practice due to their broad-spectrum efficacy.[2, 3]

However, the escalating challenge of microbial resistance, coupled with the inherent limitations of these biocides, underscores an urgent need for alternative therapeutic strategies.[4] A significant drawback of conventional agents is their poor penetration into complex biofilm structures and dentinal tubules, often leading to treatment failure and persistent infections.[5] Furthermore, CHX is associated with undesirable side effects, including tooth staining and altered taste perception, while NaOCl poses a risk of severe cytotoxic damage to periapical tissues if extruded beyond the root canal system.[2, 3] These limitations have catalyzed the exploration of novel, physical antimicrobial approaches that are less prone to inducing resistance and offer improved safety profiles.

Among the most promising alternatives are cold atmospheric plasma (CAP) and photodynamic therapy (PDT). CAP is a non-thermal, ionized gas that generates reactive oxygen and nitrogen species (ROS and RNS) such as ozone, hydroxyl radicals, and singlet oxygen.[6-8] These species damage microbial membranes, proteins, and nucleic acids, giving CAP strong antimicrobial activity and

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suitability for biomedical use.[8] In contrast, PDT uses light to activate a photosensitizer dye, producing ROS—mainly singlet oxygen—that irreversibly destroy microbial cells. PDT is highly selective and causes minimal harm to host tissues when applied under controlled conditions.[9, 10]

Although both CAP and PDT have demonstrated significant antimicrobial efficacy as standalone treatments, a direct comparative analysis of their mechanisms, penetration depth, and clinical applicability against oral pathogens remains an area of active investigation.[11] Some evidence suggests that their combination could yield synergistic effects, potentially overcoming the individual limitations of each method.[11-16]

While independent studies on CAP and PDT exist, there is a scarcity of review articles that directly compare these two novel technologies against each other and conventional methods. Therefore, this review aims to comprehensively analyze and compare the antimicrobial mechanisms and efficacy of CAP and PDT against key oral pathogens. It will critically evaluate existing in vitro studies, discuss their potential as alternatives or adjuncts to conventional disinfectants, and identify future research directions for optimizing their clinical translation in dentistry.

The antimicrobial mechanism of photodynamic therapy

PDT is one of the most effective antimicrobial treatments, as it uses light of a specific wavelength to activate a photosensitizing agent.[17] The therapeutic efficacy of PDT depends on the interaction between light, interstitial oxygen, and the photosensitizer, resulting in the production of ROS that inactivates adjacent cellular structures such as microbial cells.[18] Photosensitizers are known to distribute within or near the bacterial cytoplasmic membrane. When illuminated with a suitable wavelength, the photosensitizer is excited by photons and transitioned from its ground singlet state to an excited singlet state. From there, it can either fluoresce if it returns to the ground state or undergo intersystem crossing to a more stable triplet state. In the triplet state, the photosensitizer transfers energy to adjacent (interstitial / tissue) molecular oxygen, generating two major photochemical pathways (Figure 1), referred to as type I and type II reactions.[19]

In the Type I mechanism, the excited photosensitizer undergoes electron or hydrogen transfer reactions with surrounding molecules in the cell, forming free radicals. These radicals then react with oxygen to generate various ROS, including superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2), which damage the membrane integrity and result in irreversible cell damage. The type II process, in contrast, is characterized by a direct transfer of energy from the triplet state photosensitizer to molecular oxygen, resulting in the production of singlet oxygen ($^1O_2$), a highly reactive but short-lived form of oxygen. Singlet oxygen induces oxidative damage to cellular structures, such as membranes and cell walls, ultimately leading to the destruction of bacteria, fungi, and viruses. Due to its very short lifetime and short diffusion range (~0.02 μm), singlet oxygen acts locally and is thus less likely to inflict damage on nearby tissues. Therefore, the type II mechanism is believed to be the dominant pathway of microbial inactivation in PDT.[20-22]

The antimicrobial mechanism of cold atmospheric plasma

Plasma, which is sometimes referred to as the fourth state

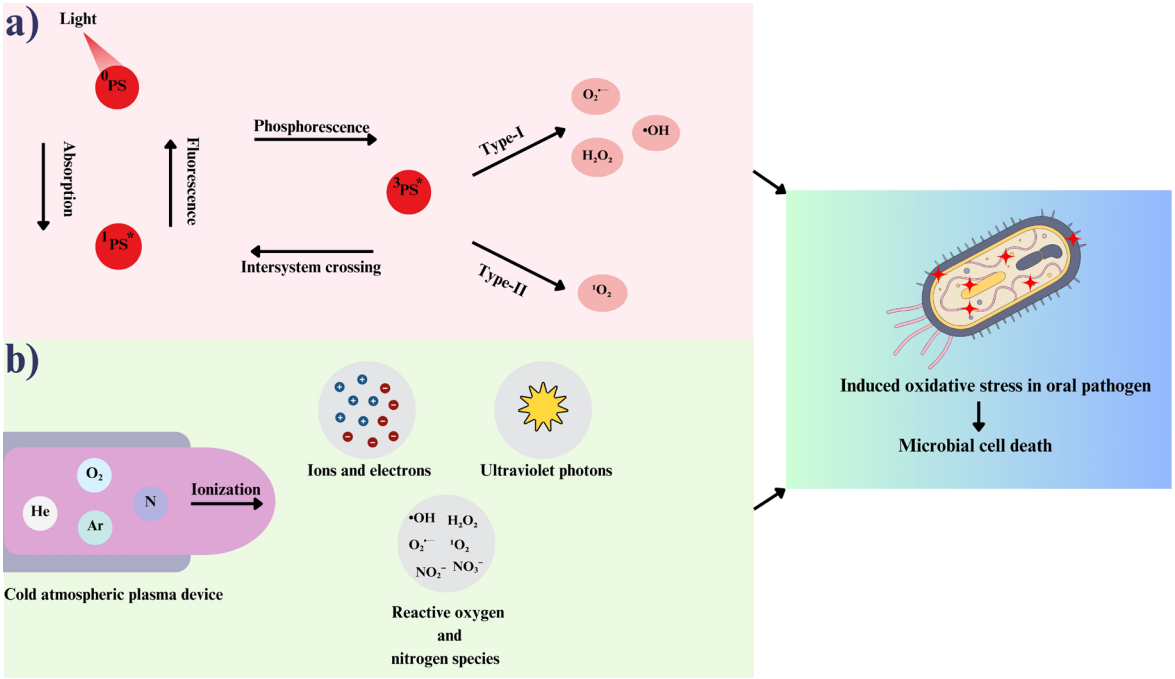

Figure 1. The antimicrobial mechanisms of a) photodynamic therapy and b) cold atmospheric plasma (Ar, Argon; PS, photosensitizer; ^{0}PS, Ground singlet state; ^{1}PS*, Excited singlet state; ^{3}PS*, Excited triplet state; •OH, Hydroxyl radicals; He, Helium; H_2O_2, Hydrogen peroxide; $O_2^{\bullet-}$, Superoxide anions; $^1O_2$, Singlet oxygen; NO_2^-, Nitrite; NO_3^-, Nitrate; N, Nitrogen; O_2, Oxygen)

of matter, is an ionized gas that is electrically neutral overall.[23] It can be roughly divided into high-temperature plasma and low-temperature plasma:[24]

1. *High-temperature plasma* (as found in stars and nuclear fusion reactions) is in thermal equilibrium with electrons and heavy particles having the same temperature, sometimes reaching up to 10^8 K. Reproducing such a plasma under laboratory conditions is extremely challenging.[25]
2. *Low-temperature plasma* is a non-thermal equilibrium system and can be further classified into thermal and non-thermal types. With recent technological advancements, it is now possible to produce non-thermal plasma at room temperature and atmospheric pressure, known as CAP or low-temperature atmospheric pressure plasma. As CAP does not generate deleterious heat, it has been especially appealing for biomedical applications, where it can interact with tissue in a safe manner.[26]

Typically, plasma machines are categorized into indirect and direct types. In indirect (jet-type) devices, plasma is generated by ionizing a working gas, usually between two electrodes, and it is propelled outwards by the flow of the gas. In direct devices, including plasma brushes, corona discharges, and dielectric barrier discharge (DBD) systems, plasma is generated between the electrode and the target surface, which acts as the grounded electrode. These systems can even operate using ambient air.[27]

At the microscopic level (Figure 1), plasma is a mixture of charged and neutral species formed through the ionization of gases such as argon, helium, oxygen, and nitrogen. It comprises ions, electrons, atoms, excited molecules, and ultraviolet photons. Despite containing these charged species, plasma remains electrically neutral. When in contact with an oxygen or nitrogen-containing atmosphere, plasma generates ROS and RNS, including hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), nitrites (NO_2^-), and nitrates (NO_3^-). These reactive species are the primary drivers of plasma's antimicrobial activity, damaging microbial targets through various mechanisms, including membrane damage, oxidative degradation of nucleic acids, and protein modification.[27-29]

Comparative Efficacy Analysis: CAP and PDT versus Conventional Methods and versus Each Other

In recent years, CAP and PDT have emerged as potential alternatives to conventional antimicrobial agents, such as CHX and NaOCl, for treating biofilm-associated infections. Some studies have compared the efficacy of CAP and PDT with that of conventional disinfectants. Table 1 summarizes some of the key findings from these studies, as they relate to the common oral pathogens.

Conventional agents, such as CHX and NaOCl, in therapeutic dose applications, have several drawbacks, including ineffective penetration of biofilms, shallow disinfection depth, and reliance on patient adherence. Moreover, CHX has undesirable side effects, such as tooth staining and taste perception modification, while NaOCl poses a cytotoxic risk if it leaks out of the root canal, which can damage the soft tissue, nerves, or airway.[2,30] Thus, researchers have been seeking safe yet effective alternatives to antimicrobials. As reported by Yeganehfard et al., CHX remains the most effective agent against *Streptococcus mutans*, whereas CAP (Plasma art [He gas], 1.85 cm^3/s, 8 W, 100 kHz) and PDT (especially PDT with indocyanine green (0.2% ICG [5 mL], 810 nm, 12 J/cm^2, 60 seconds)) exhibit strong antimicrobial activity with no toxicity.[11] All treatments tested showed a significant reduction in bacterial colony-forming units (CFUs) compared to untreated controls. The largest reduction was obtained with CHX, followed by ICG-mediated photodynamic therapy (810 nm), CAP exposure for 180 seconds, and methylene blue-mediated photodynamic therapy (660 nm). Interestingly, the bactericidal activity of CAP increased with increasing exposure times, and ICG-mediated PDT was demonstrated to be as effective as CHX, a commonly acknowledged gold standard.[11] While CHX is still potent, its poor penetration through biofilms and deeper tissue layers enhances the potential of CAP to be a more versatile and tissue-safe disinfection technique.

In terms of low tissue toxicity and biofilm resistance, which are problems with NaOCl, the standard disinfectant in root canal treatment, Haghighi et al. found that CAP and 2.5% NaOCl had no significant difference in antibacterial activity (both achieved 99% bactericidal activity).[14] Also, 10.2% curcumin-mediated PDT (445 nm) and 002% methylene blue-mediated PDT (660 nm) resulted in substantial bacterial reductions, with slightly greater efficacy observed in the case of curcumin. In contrast, the bacterial reduction obtained with the 940-nm diode laser was the lowest (about 80.5%). The results showed that CAP and NaOCl had the greatest effect against the *Enterococcus faecalis* biofilm (one of the main causes of endodontic treatment failure), suggesting that CAP could be used as a safe and effective alternative to NaOCl, especially in the disinfection of primary teeth.[14]

With the increasing problem of microbial resistance, CAP and PDT have emerged as promising alternative antimicrobial strategies. However, a comparative analysis of these two methods is crucial for determining the most effective way to treat oral infections. Their main differences lie in their mechanism and depth of penetration. PDT uses light to activate a photosensitizer that produces ROS, which can damage bacterial cells. CAP, in contrast, generates a mixture of ROS and RNS that act on bacterial membranes and on protein and nucleic acids. Although both methods are effective in reducing bacterial populations, their different modes of action make them appropriate for use in different clinical situations. The effectiveness of PDT depends on the type of photosensitizer used and the wavelength of light. For example, PDT using ICG at a wavelength of 810 nm can penetrate deeper into tissues. In contrast, lower wavelengths result in reduced light penetration, making PDT more suitable for treating superficial infections.[31]

Table 1. Main characteristics of studies comparing the antimicrobial effect of cold atmospheric plasma and photodynamic therapy against oral pathogens.

Cold atmospheric plasma					Photodynamic therapy					Other antimicrobial methods	Antimicrobial assay			Refs
Device	Gas flow rate	Power output	Frequency	Duration	Laser source	Wavelength	Energy density	Wave mode	Photosensitizer (concentration)	Time of irradiation		Microorganism	Phase	Test	
Plasma Jet (He gas)	3.8 L/min	8 W	100 kHz	180 s 210 s	Diode	450 nm 660 nm	12 J/cm² 20 J/cm²	Continuous	Curcumin (10.2 mg/cc) 0.02% Methylene blue	60 s 100 s	Nystatin (0.1 mL)	*Candida albicans*	Planktonic	Colony-forming unit count	12
Plasma art (He gas)	1.85 cm³/s	8 W	100 kHz	90 s 20 s 150 s 180 s	N/A	810 nm 660 nm	12 J/cm² 20 J/cm²	Continuous	0.2% Indocyanine green (0.5 mL) 0.02% Methylene blue (0.5 mL)	60 s 100 s	2% Chlorhexidine	*Streptococcus mutans*	Biofilm	Colony-forming unit count (to assess bactericidal effects)	11
Plasma art	3 L/min	55 W	50 kHz	60 s	N/A	660 nm	15.6 J/cm²	Continuous	0.01% Methylene blue (125 µL)	60 s	Aforementioned diluted penicillin (125 µL)	*Streptococcus sanguinis*	Biofilm	Colony-forming unit count	13
Plasma art (He gas)	3 L/min	55 W	50 kHz	60 s	Diode laser	445 m, 660 nm	N/A	Continuous	10.2% Curcumin (100 λ) 0.02% Methylene blue (100 λ)	60 s (Photosensitizers were applied 120 seconds prior to laser irradiation)	2.5% Sodium hypochlorite (2mL) 940 nm diode laser (twice/each time was 5 s with 10 s interval)	*Enterococcus faecalis*	Biofilm	Colony-forming unit count	14
Dielectric barrier discharge plasma jet (He and He/ 0.5% O_2 gases)	He: 4 slm, O_2: 20 sccm	N/A	N/A	4 min 6 min 8 min	Diode laser	810 nm	N/A	Continuous	Toluidine blue (0.1 mg/mL)	30 s (repeated 3 times with 5-second intervals between each application/ Photosensitizer was applied 5 min prior to laser irradiation)	No treatment	*Enterococcus faecalis*	Biofilm	Colony-forming unit count	15
Plasma ONE	N/A	5 W	420–1220 Hz	120 s	HELBO® Blue	660 nm	N/A	Continuous	0.01% Methylene blue	30 s	0.04% Polihexanide	*Acinetobacter baumannii* *Staphylococcus aureus*	Planktonic	Colony-forming unit count (to assess bactericidal effects)	16

Abbreviations: cm³/s, cubic centimeter per second; He, Helium; Hz, Hertz; O_2, Oxygen; J/cm², Joules per square centimeter; kHz, Kilohertz; S, Second; sccm, Standard cubic centimeters per minute; slm, standard liter per minute; L/min, liters per minute; Min, Minute; mL, Milliliter; Nm, Nanometer; N/A, Not applicable; Refs, References; W, Watt.

Additionally, the gas-phase chemistry of CAP provides deeper tissue penetration, making it beneficial for treating slightly complex or deeper infections.

Armand et al. made a direct comparison between CAP (cold plasma jet using He and He/O_2 gases) and 0.1% toluidine blue-mediated PDT (810 nm) for root canal disinfection of *E. faecalis*-infected root canals.[15] All treatment methods showed a significant decrease in bacterial load, with He/O_2 plasma being the most effective, followed by PDT and He plasma. Further increasing plasma exposure from four to eight minutes increased antibacterial performance to nearly complete bacterial killing. Scanning electron microscope images validated the significant disruption of biofilm and the opening of dentinal tubules after He/O_2 plasma treatment, highlighting its excellent biofilm-degrading activity. The study ultimately found that He/O_2 plasma is a potentially effective technique for disinfecting root canals and is particularly effective for treating infections associated with biofilms.[15]

On the other hand, Ghaiyoomi et al. studied the synergistic use of 0.01% methylene blue-mediated PDT (660 nm, 15.6 J/cm², 60 seconds) and CAP against *Streptococcus sanguinis*, a major pathogen associated with oral and cardiac infections.[13] The combined PDT and CAP treatment resulted in the most significant bacterial reduction compared to using either method individually. While CAP or laser irradiation without a photosensitizer seemed to be of limited efficacy, PDT alone was more effective; however, the synergistic approach produced the most significant bacterial inhibition. The authors concluded that the combination of PDT and CAP can be a potent non-invasive treatment for managing infections caused by biofilms in the oral cavity. However, CAP efficacy can be reduced in a moist environment, such as the oral cavity.[13] Hafner et al. found that CAP's bactericidal efficacy was reduced in humid conditions and was effective only for biofilms up to a thickness of

0.3 mm, with no significant bactericidal effect observed in thicker biofilms (>0.6 mm).[16] In contrast, the results of methylene blue-mediated PDT (660 nm) showed greater than 5-log bacterial reductions for *Staphylococcus aureus* and *Acinetobacter baumannii*, which were higher than those achieved with CAP. Polihexanide (0.04%) was also effective, with a more than five orders of magnitude reduction in bacteria load. Although CAP performed marginally better against *A. baumannii* than against *S. aureus*, likely due to its greater penetration with gas, overall, its performance was inferior to that of PDT and Polihexanide in moist conditions.[16]

A noteworthy finding from the reviewed studies was the research conducted by Azizi et al.[12] on *Candida albicans*. They demonstrated that CAP using helium gas for 180 seconds and low-power 660-nm laser treatment had inhibitory effects on *C. albicans* colonies that were more effective than PDT. However, the application of nystatin (100,000 units) significantly reduced the number of *C. albicans* colonies and proved to be more effective than both CAP and laser irradiation, as well as PDT. The differences in these results, along with the increasing challenge of microbial resistance, emphasize the need for future research. Such studies should involve a more detailed examination and consider various factors, including the type of microorganism and the parameters used in PDT and CAP.

Conclusion

This review demonstrates that both CAP and PDT are promising alternatives to conventional disinfectants, each with distinct mechanisms and potential clinical niches; however, studies directly comparing these two compounds are limited. The emerging evidence suggests that both modalities have distinct benefits and can play a significant role in controlling oral pathogens. Although classical disinfectants CHX and NaOCl are still commonly used against *S. mutans* and *E. faecalis*, their disadvantages, such as limited penetration into tissues and the resistance of microorganisms, highlight the increasing need for a new generation of disinfectants. Future studies are recommended to standardize and optimize treatment parameters for CAP (e.g., gas composition, power level, and exposure duration) as well as PDT (e.g., light wavelength, energy density, photosensitizer type and concentration, and irradiation time). Furthermore, the development of combined CAP-PDT protocols could enhance their efficiency in controlling deep-seated infections; it should be considered that the optimal choice (or combination) of CAP and PDT may depend on the specific pathogen, anatomical site, and clinical setting, so personalized or context-specific application is another reason highlighting the importance of future studies in this field. Filling these knowledge gaps will lead to more efficacious, safe, and clinically relevant antimicrobial solutions, ultimately resulting in a revolution in disinfection methods in dentistry and biomedicine in general.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All data generated or analyzed during the current study are included in this article.

Ethical Approval

Not required.

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