

## REVIEW

# TRANSLATIONAL APPLICATION OF COLD ATMOSPHERIC PLASMA IN PERIODONTOLOGY AND IMPLANTOLOGY: WHERE ARE WE? A SYSTEMATIC REVIEW OF IN VIVO STUDIES IN HUMAN AND ANIMAL MODELS



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## ABSTRACT

### Aim

The utilization of cold atmospheric plasma (CAP) in dentistry presents a promising avenue for novel therapeutic interventions. This systematic review of in vivo studies aimed at summarizing the existing evidence regarding the efficacy of CAP as a treatment for biofilms associated with periodontitis and peri-implantitis. The objective was to advance the definition and standardization of protocols and facilitate the integration of CAP treatment as a chair-side practice.

### Materials and Methods

This review was registered in PROSPERO database (CRD42023404757), and a comprehensive search was conducted following PRISMA guidelines and using PubMed, Scopus, Web of Science, and Embase databases.

### Results

In total, 9 in vivo studies were included, 1 on humans and 8 on animal models. A notable reduction in residual bacteria when CAP was combined with conventional therapies in both periodontitis and peri-implantitis models was observed. Biochemical and histological assays demonstrated a decrease in inflammatory cytokines within crevicular fluid and oral tissues.

### Conclusions

Current evidence suggests that CAP shows promise for periodontitis and peri-implantitis treatment, but further clinical trials with larger cohorts and standardized protocols are needed to confirm its efficacy and safety.

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### KEYWORDS

Alveolar bone loss, Antimicrobial, Biofilms, Cold atmospheric plasma, Peri-implantitis, Periodontitis

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## INTRODUCTION

Plasma is a partially ionized gas composed of free electrons, ions, radicals, excited atoms and molecules and UV-Vis radiation and it is often referred to as the fourth state of matter.<sup>1,2</sup> The goal of generating low-temperature, non-equilibrium plasmas at atmospheric pressure has been pursued since the early 1900s.<sup>3</sup> However, stable non-equilibrium atmospheric pressure plasmas, mostly using dielectric barrier discharge (DBD) arrangements with helium as the operating gas, were not reported until the late 1980s and early 1990s.<sup>4,5</sup> The use of fast-rise time voltage pulses, which improved plasma chemistry control, marked the next step in the progression in the late 1990s and early 2000s. These advancements in the mid-1990s signaled a turning point toward applications in biology and medicine.<sup>6</sup> Cold atmospheric plasma (CAP) was developed in response to the need for devices that could deliver reactive species in quantities that exceeded volume limitations.<sup>7</sup> Oxygen, nitrogen, and their admixtures with argon or helium used as carrier gases have been employed in modern configurations to achieve medically acceptable gas temperatures below 40°C.<sup>8</sup> Earlier plasma jets had high gas temperatures that were unsuitable for clinical purposes. These developments make it possible to precisely control the formation of low-temperature plasma plumes, which makes them useful for highly precise biomedical therapies.<sup>9</sup> Indeed, CAP has been tested in a wide range of applications. It was shown to be effective in the decontamination and sterilization of surfaces,<sup>10,11</sup> in the improvement of biomaterial properties,<sup>12</sup> and in interacting with living tissues, to promote healing,<sup>13</sup> and impacting on cancer cell proliferation<sup>14-16</sup> due to its tolerability and safety for human tissues.<sup>17,18</sup>

In recent years, the expanding Anti-Microbial Resistance (AMR) phenomenon has become a major issue, and thus, CAP application in dentistry has gained interest as a novel therapeutically alternative or adjunctive strategy<sup>14,17,19</sup> for the treatment of oral pathologies, especially those caused by disequilibrium in the oral microbiota and characterized by an overgrowth of potential pathogens,<sup>20</sup> such as periodontal and peri-implant diseases. Indeed, the antimicrobial properties of CAP technology hold interesting translational potential for these conditions. However, only a few studies have investigated the use of CAP for the management of periodontal and peri-implant diseases, and most of them are *in vitro*<sup>21,22</sup> or *ex vivo* studies.<sup>23,24</sup> Specifically, CAP seems promising as a potential tool for controlling of biofilms – communities of microorganisms – which are formed when bacteria attach to a surface and secrete a protective matrix of Extracellular Polymeric Substances (EPS), making them resistant to antimicrobials, other treatments, and the host immune system. Biofilms are responsible for almost all human infections and show a causal connection with periodontal and/or peri-implant diseases. Consequently, the removal of

biofilms may represent a field of plasma application in dentistry.<sup>25</sup>

Regarding CAP mechanisms of action, very little is known. Generally, the CAP effect may derive from a mixture of reactive oxygen and nitrogen species (RONS) produced by plasma both in direct (e.g. plasma touching the target) or indirect (e.g. plasma remotely applied, or application of plasma treated liquids or hydrogels) application to cells or tissues.<sup>26,27</sup> Such studies<sup>28-30</sup> showed that CAP can penetrate the protective matrix of biofilms and damage bacterial cells, leading to their death. Charged particles and RONS produced by plasma can greatly compromise the integrity of bacterial walls, coats, and membranes. Additionally, the reactive species including RONS, energized electrons, and electric field, generated by CAP during direct approaches can disrupt quorum sensing, the signaling pathways that bacteria use to communicate with each other, making it more difficult for them to form biofilms. One advantage of direct CAP treatment is that it can be applied on a variety of surfaces, including those that are difficult to clean using traditional methods, such as dental implants. Matthes et al.<sup>31</sup> stated that microbial biofilms of the oral cavity are highly diverse and can also attach on artificial surfaces, such as implants. Thus, CAP devices could be very suitable for the treatment of peri-implant infection. Application of CAP has proven beneficial not only in the decontamination and cleaning of dental implants<sup>32</sup> but also in the therapeutically care of periodontal diseases<sup>28,33-35</sup> as well as in oral candidiasis,<sup>36,37</sup> by exerting both anti-bacterial and anti-fungal properties. However, studies applied up to now<sup>38-40</sup> employed heterogeneous protocols, such as varying distances and treatment times, gas sources, and power levels, which might contribute to different outcomes with under-investigated effects, make it difficult the definition of clinical protocols for CAP application in humans.

This systematic review of *in vivo* studies aims to summarize the current evidence on the effects of CAP treatment on bacterial biofilms associated with periodontal and peri-implant diseases, focusing on its antimicrobial, anti-inflammatory and regenerative potential. This review will provide insights to optimize the technology, define standardized protocols and ultimately advance toward its translational application as chair-side approach.

## MATERIALS AND METHODS

This systematic review was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement.<sup>41</sup> The review record was approved by the international prospective register of systematic reviews PROSPERO on March 14th, 2023 under the identification number CRD42023404757 and can be accessed via the following reg-

istration link: [https://www.crd.york.ac.uk/prospero/display\\_record.php?RecordID=404757](https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=404757).

### Search Strategy

An electronic search of scientific databases (PubMed, Scopus, Web of Science, Embase, Cochrane Library, ISRCTN Registry and clinicaltrials.gov) as well as grey literature (The New York Academy of Medicine, DANS Data Life Sciences), was performed to identify suitable studies. The following terms and keywords, alone or in combination, were used: ("nonthermodynamics equilibrium plasma" OR "nonequilibrium plasma" OR "dielectric-barrier discharges" OR "barrier discharges" OR "non-thermal plasma" OR "glow discharge" OR "cold atmospheric plasma" OR "cold plasma" OR "low-temperature plasma" OR "kinpen med" OR "non-thermal atmospheric pressure plasma" OR "nonthermal atmospheric pressure plasma" OR "cold physical plasma" OR "plasma medicine" OR pam OR cap OR "plasma activated medium" OR "cold atmospheric-pressure plasma" OR "plasma gases" OR "plasma activated liquid" OR "cold argon plasma" OR "plasma jet" OR "air plasma") AND (biofilm OR biofilms OR "oral biofilm" OR "oral microorganism\*" OR "periodontal pathogen\*" OR "biofilm colonization\*" OR "dental plaque" OR "dental deposit\*" OR "materia alba" OR "periodontal disease\*" OR periodont\* OR parodont\* OR "pyorrhea alveolaris" OR "peri-implant disease\*" OR peri-implant\* OR perimplant\* OR peri implant\*). The adapted search strategy for PubMed, Web of Science, Scopus, Embase, Cochrane Library, ISRCTN Registry and clinicaltrials.gov is shown in Supplemental Material Table S1.

The initial search was conducted on March 21st, 2024. The last electronic search was performed on May 5th, 2024, and updated on December 9th, 2024. In addition to the electronic search, a hand search was undertaken by checking the references of the included studies to identify further eligible papers. A reference management software program (End-Note) was used, and duplicates were discarded first electronically and then manually by checking the resulting list.

### Eligibility Criteria

#### Inclusion Criteria

The search was limited to studies published in English without any year restrictions. To be eligible for inclusion, the following criteria had to be met:

1. Case report, case series, cohort, case-control, and randomized design where humans or animal models were treated by CAP.
2. Patients had to be over 18 years old with a diagnosis of periodontitis or peri-implantitis or the study had to involve animal models of periodontal or peri-implantitis diseases.
3. Biological, physic-chemical and/or clinical outcomes had to be reported, such as the impact of CAP treatment on oral

microorganisms, periodontal or peri-implant tissues, and disease outcomes.

#### Exclusion Criteria

The listed exclusion criteria were applied:

1. Proceedings, systematic reviews, and meta-analyses.
2. Ecological studies.
3. In vitro or ex vivo models.
4. Clinical studies including patients with systemic and uncontrolled diseases, which could affect periodontal or peri-implant tissues.
5. Clinical studies including patients who underwent antibiotic or corticosteroid treatment, which could impact on periodontal or peri-implant disease or the oral microbiome.

#### Focused PICO Question

**Participants:** patients affected by periodontitis or peri-implantitis, or animal models of periodontitis and peri-implantitis;

**Intervention:** documented protocol of direct and indirect CAP irradiation;

**Comparison:** when available, the studied model was exposed to other treatments or different CAP treatment protocols;

**Outcomes:** antimicrobial effects and periodontal or peri-implant hard-soft tissue response to CAP treatment.

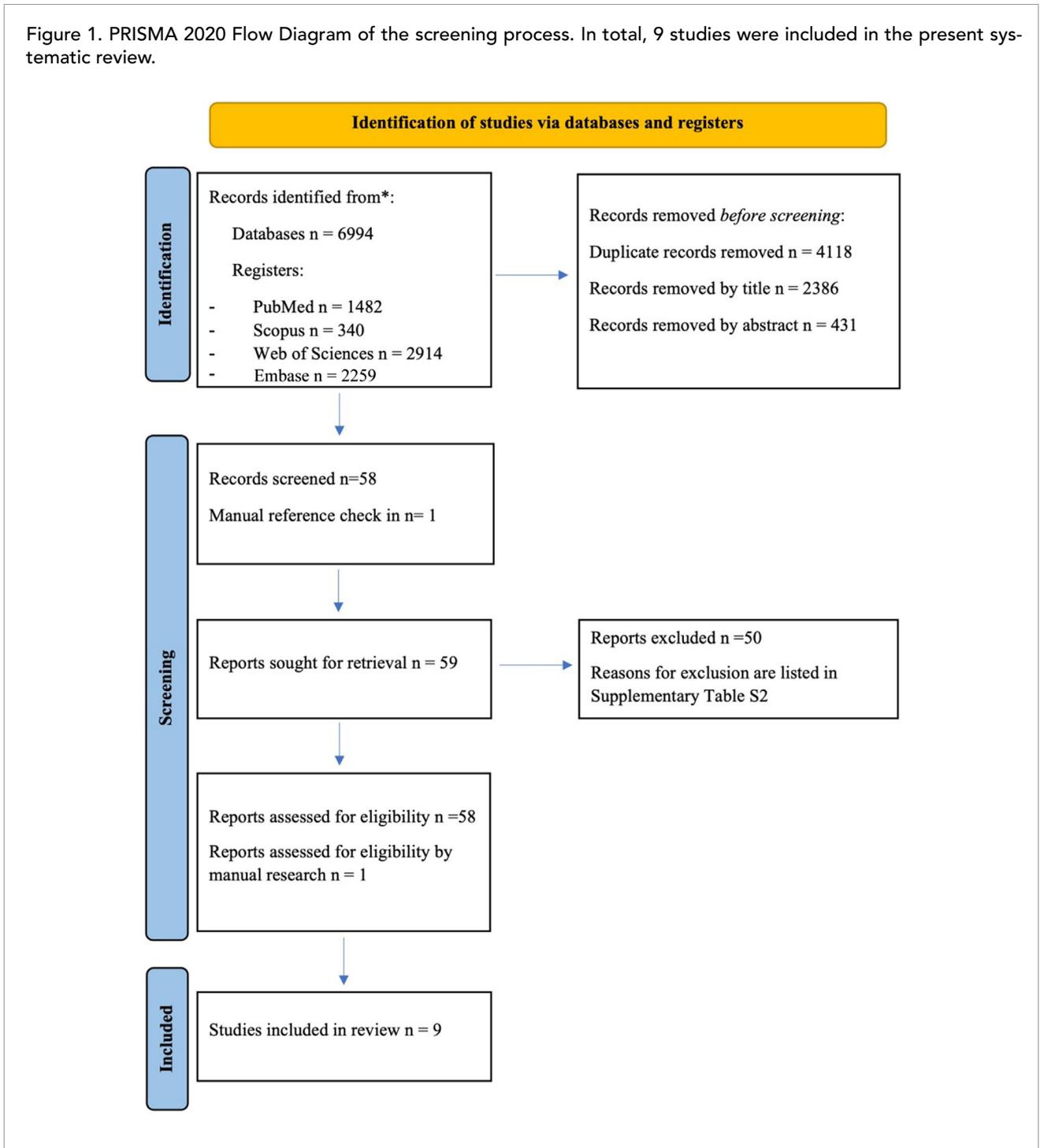
#### Selection of Studies

The retrieved citations were independently reviewed by 3 authors (GS, GP and GB). Relevant studies were identified based on the title and abstract. If these did not provide sufficient information about the inclusion criteria, the full text was evaluated to assess eligibility. Disagreements were solved in a joint meeting, and a fourth reviewer was consulted to make the final decision (VCAC). This author also calculated a k-statistic value to ascertain the level of agreement among reviewers.

#### Data Extraction and Method of Analysis

Three reviewers (GS, GP and GB) independently extracted data from all the included studies using a predesigned extraction form. Microsoft Excel 2020 (Microsoft Office, Microsoft Corporation, Redmond, WA, USA) was used for data collection. Different extraction sheets were created based on the outcomes (antimicrobial effects or periodontal, peri-implant tissue response). Concerning the hard-soft tissues response, the following data were extracted: study design; human/animal model; control and test groups, evaluated tissue; implant type, surface, brand; CAP treatment protocol;

Figure 1. PRISMA 2020 Flow Diagram of the screening process. In total, 9 studies were included in the present systematic review.



primary outcomes; other outcomes; assays, and biological and chemical results. For anti-microbial effects the following data were extracted: study design; human/animal model; control and test groups, the method of inoculation microorganisms in animal models; CAP treatment protocol; primary

outcome; other outcomes; performed assays; and summarized results.

The features and settings of the CAP devices were also summarized in a different extraction sheet (e.g. trade name, flow

rate, feeding gas, pulse frequency, voltage, pulse, application distance, application time, total energy, and power).

### Risk of Bias Assessment

Two reviewers (GB and VCAC) assessed the quality of studies by evaluating the risk of bias. For human studies, the risk of bias was evaluated according to the Cochrane Risk of Bias in Randomized Interventional Studies tool (Rob 2) in its current version, dated 22 August 2019.<sup>42</sup> For in vivo animal studies, the Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) risk of bias tool was used.<sup>43</sup>

## RESULTS

### Study Selection

A total of 6994 potentially relevant records were identified from the databases and further processed following the PRISMA statement (Figure 1). After the removal of duplicates, 2876 records were further examined based on their title and abstracts, and 2817 studies were excluded as they did not meet the specific eligibility criteria for the present study. The reference lists of eligible studies were also reviewed to identify any further studies that had been missed in the electronic searches and an additional article was found through a manual search. A total of 59 full-text articles were finally evaluated, and 50 were subsequently excluded (Table S2). The value of the K-statistic was 0.86, which indicates an optime level of agreement between the reviewers.

### General Characteristics of Included Studies

Nine studies were included, 1 on humans and 8 performed on animal models (Table 1), this is schematized in Figure 2. The studies were published between 2015 and 2024, and they were conducted in China (n = 4),<sup>35,40,44,45</sup> in Turkey (n = 2),<sup>33,38</sup> Brazil (n = 2)<sup>39,46</sup> and Korea (n = 1).<sup>47</sup>

Based on the effect of CAP treatment in periodontitis and peri-implantitis models, the included studies were categorized into 3 main groups:

- three<sup>33,35,40</sup> investigated the anti-microbial properties
- six<sup>33,35,38,45,46</sup> assessed the anti-inflammatory effect on crevicular fluid as well as periodontal tissues<sup>47</sup>
- eight<sup>33,35,38-40,44,45,47</sup> evaluated the protective effect improvement of the clinical index of periodontal health along with the ability of CAP to reduce alveolar bone loss.

### Results of Risk of Bias Assessment

The Cochrane Risk of Bias in Randomized Interventional Studies tool (RoB 2) indicated an overall moderate, risk of bias for the article by Küçük et al.<sup>33</sup> (Table S3). For the studies involving animal models, the SYRCLE tool was employed.

It revealed that 4 studies<sup>39,40,44,46</sup> reported sequence generation for allocation based on the use of randomization software, whereas the remaining 4 articles<sup>35,38,44,47</sup> did not mention it and were therefore considered to be at high risk. Baseline characteristics were reported in all the included studies, except for Park et al.<sup>47</sup> Three items of the SYRCLE assessment tool – “allocation concealment,” “random housing,” and “blinding” – contributed to an unclear risk of bias because they were not described in detail in 7 studies.<sup>35,38-40,45-47</sup> Only Tang et al.<sup>44</sup> specified that the animals were housed individually in kennels or cages during the experiments. The risk was again unclear for the items “random outcome assessment” and “blinding of outcome assessment” in all articles. Additionally, all articles were at low risk for “incomplete data,” “other sources of bias, and regarding “selective outcome reporting,” only in Lima et al.<sup>39</sup> and Tang et al.<sup>44</sup> was the risk unclear, whilst in Park et al. it was high.<sup>47</sup> Information about the condition of living animal models was described in all studies, except for Park et al.<sup>47</sup> (Supplementary Table S4).

### Effects on Microbiological Community

Three articles<sup>33,35,40</sup> evaluated the antimicrobial effects of CAP on periodontal pathogens associated with periodontal and peri-implant diseases: 1 in a human model<sup>33</sup> and 2 in animal models.<sup>35,40</sup> The human study compared CAP in combination with SRP in patients with periodontitis. Conversely, the 2 animal model studies,<sup>35,40</sup> conducted on rats and beagle dogs respectively, investigated the effect of CAP, as stand-alone treatment, on experimental induced periodontitis and peri-implantitis. The short-term antimicrobial effect was assessed by quantifying residual bacteria through PCR analysis.

#### Human Study

Küçük et al.<sup>33</sup> analyzed microbiological data from subgingival plaque samples. All bacterial species showed reductions in both groups – CAP+SRP-treated group and SRP alone group - with significant decreases in *T. denticola* and *P. gingivalis* at 1 month. However, the decrease of *P. gingivalis* and *T. forsythia* at 3 months was significantly greater in the combination group when compared to SRP alone. Additionally, the CAP-treated group showed reduced recolonization of red complex bacteria.

#### Animal Model Studies

In a beagle dog study, Shi et al.<sup>40</sup> examined the detection rates of *P. gingivalis*, *A. actinomycetemcomitans*, and *T. forsythia* over time in plasma-treated and control groups. At baseline, there were no significant differences in the detection rates between the 2 groups. After 1 month, both groups showed significant reductions in *P. gingivalis* and *T. forsythia* ( $P < .05$ ). However, *A. actinomycetemcomitans*

Table 1. Overview of the included studies.

Authors	Study design	Study model	Study groups	Patients' characteristics	Outcomes and experimental times	Results
Küçük et al., <sup>33</sup>	Prospective, split-mouth, double-masked, placebo controlled randomized clinical trial	Periodontitis	Addition of NAPP to SRP for treatment of chronic periodontitis in humans: CG: SRP; TG: SRP + NAPP	25 (M:13; F:12) 26-48 years – mean age 38.2	Measurement before periodontal therapy (baseline), at 30 days and 3 months after treatment of clinical parameters: PD, CAL, PI, GI, BoP; measurement of biochemical parameters at baseline, 30 days and 3 months after treatment on GCF: (IL)-1 $\beta$ , IL-10, VEGF; microbiologic analysis: analyze the differential expressions of stain specific genes for Pg, Tf, and Td at 30 days and 3 months	<b>Clinical examination (full mouth): CAL (mm)</b> CG: = 3.45 $\pm$ 0.71 $\downarrow$ TG: = 3.47 $\pm$ 0.53 $\downarrow$ <b>PD (mm)</b> CG: 3.26 $\pm$ 0.63 mm $\downarrow$ TG: 3.32 $\pm$ 0.46 mm $\downarrow$ <b>PI</b> <i>Baseline:</i> CG: 1.64 $\pm$ 0.33 TG: 1.71 $\pm$ 0.29 <i>1st month:</i> CG: 0.61 $\pm$ 0.34 $\downarrow\downarrow$ TG: 0.65 $\pm$ 0.35 $\downarrow\downarrow$ <i>3rd month:</i> CG: 0.53 $\pm$ 0.32 $\downarrow\downarrow$ TG: 0.54 $\pm$ 0.31 $\downarrow\downarrow$ <b>GI</b> <i>Baseline:</i> CG: 1.77 $\pm$ 0.33 TG: 1.67 $\pm$ 0.44 <i>1st month</i> CG: 0.34 $\pm$ 0.18 $\downarrow\downarrow$ TG: 0.54 $\pm$ 0.27 $\downarrow\downarrow$ <i>3rd month:</i> CG: 0.39 $\pm$ 0.18 $\downarrow\downarrow$ TG: 0.25 $\pm$ 0.14 $\downarrow\downarrow$ <b>BoP (% of site)</b> <i>Baseline:</i> CG: 60.88 $\pm$ 23.59 TG: 63.45 $\pm$ 23.76 <i>1st month:</i> CG: 10.89 $\pm$ 8.06 $\downarrow\downarrow$ TG: 10.91 $\pm$ 7.48 $\downarrow\downarrow$ <i>3rd month:</i> CG: 9.86 $\pm$ 6.83 $\downarrow\downarrow$ TG: 8.82 $\pm$ 7.15 $\downarrow\downarrow$ <b>Biochemical analysis on GFC by ELISA:</b> <b>IL-1<math>\beta</math> (pg)/(60 s):</b> <i>Baseline:</i> CG: 12.19 $\pm$ 1.17 TG: 12.08 $\pm$ 1.14 <i>1st month:</i> CG: 10.77 $\pm$ 1.13 TG: 11.24 $\pm$ 0.94 <i>3rd month:</i> CG: 10.9 $\pm$ 0.76 TG: 10.52 $\pm$ 1.00 <b>IL-10 (pg)/(60 s):</b> <i>Baseline:</i> CG: 0.83 (0.33-2.17) TG: 0.75 (0.31-2.00) <i>1st month:</i> CG: 0.68 (0.32-2.07) TG: 0.67 (0.30-4.14) <i>3rd month:</i> CG: 1.06 (0.30-1.85) TG: 0.87 (0.36-1.86) <b>VEGF (pg)/(60 s):</b>

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Authors	Study design	Study model	Study groups	Patients' characteristics	Outcomes and experimental times	Results
						<p><i>Baseline:</i>            CG: 99.03 ± 28.02            TG: 101.95 ± 31.24  <i>1st month:</i>            CG: 84.19 ± 23.42            TG: 86.58 ± 26.33  <i>3rd month:</i>            CG: 92.65 ± 27.19            TG: 94.01 ± 27.60            Not significant differences  <b>Microbiologic analysis</b> on the subgingival plaque samples by RT-qPCR:  <b>Pg</b> (log<sub>10</sub> relative quantity)  <i>1st month</i>            CG: 0 ↓            TG: -2.2 ↓↓  <i>3rd month</i>            CG: 0.4 ↓            TG: -0.9 ↓↓  <b>Tf</b> (log<sub>10</sub> relative quantity)  <i>1st month</i>            CG: -0.3 ↓            TG: -1.1 ↓↓  <i>3rd month</i>            CG: 0.4 ↓            TG: -0.7 ↓↓  <b>Td</b> (log<sub>10</sub> relative quantity)  <i>1st month</i>            CG: -1.2 ↓            TG: -2.1 ↓↓  <i>3rd month</i>            CG: -1.1 ↓            TG: -1.7 ↓↓</p>
Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
de Oliveira et al. <sup>46</sup>	Case – control	Periodontitis	Randomly divided into 2 groups <b>CG:</b> periodontitis without treatment group (P group) (n=28); <b>TG:</b> periodontitis with atmospheric plasma treatment group (P + AP group) (n = 28)	Fifty-six rats male Wistar rats (aged 90 days, weighing 250-300 g)	Tissue samples were collected at 2 and 4 weeks after periodontitis induction. (n = 14/group) <b>Biochemical analysis:</b> MPO: quantified in the supernatant of the macerated gingival tissue; NAG: quantified in the supernatant of the macerated gingival tissue <b>Histomorphometry:</b> gingival tissue and periodontal ligament were	<p><b>Biochemical analysis</b> (optical density of MPO-NAG/mg tissue):  <b>MPO:</b> After 2 and 4 weeks            CG:            TG: ↑↑  <b>NAG:</b> After 4 weeks            CG:            TG: ↑↑  <b>Histomorphometric analysis</b> (quantified by Plugin "Cell Counter" of the ImageJ software):  <b>Inflammatory infiltrate:</b> After 2 and 4 weeks            CG:            TG: ↓↓</p>
						(continued on next page)

Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
					subjected to hematoxylin and eosin (HE) staining: to measure inflammatory infiltration, blood vessels, and fibroblasts <b>Immunohistochemistry:</b> antibodies against IL-1 $\beta$ , TNF- $\alpha$ , and IL-10: gingival sections; antibodies against RANK, RANKL, OPG: alveolar bone sections	<b>Amount of blood vessels:</b> After 2 and 4 weeks CG: TG: $\uparrow\uparrow$ <b>Fibroblasts:</b> After 2 and 4 weeks CG: TG: $\downarrow\downarrow$ <b>Immunohistochemical (% areas of positively stained):</b> <b>TNF-<math>\alpha</math>:</b> After 4 weeks CG: TG: $\downarrow\downarrow$ <b>IL-1<math>\beta</math>:</b> After 2 weeks CG: TG: $\uparrow\uparrow$ After 4 weeks CG: TG: $\downarrow\downarrow$ <b>IL-10:</b> After 2 and 4 weeks CG: TG: $\uparrow\uparrow$ <b>RANK:</b> After 2 and 4 weeks CG: TG: $\downarrow\downarrow$ <b>RANKL:</b> After 4 weeks CG: TG: $\downarrow\downarrow$ <b>OPG:</b> After 2 and 4 weeks No differences
Kusakci-Seker et al. <sup>38</sup>	Case - control	Periodontitis	Rats were randomly divided into 3 groups: CG: n = 8; PG: n = 10 NTAP group (NTAPG): n = 10	Thirty 12-week-old male Wistar albino rats - weighing between 200 and 220 g - 2 rats were excluded because they didn't meet the weight standards	<b>Micro-CT:</b> alveolar bone measurement <b>Histological analysis:</b> inflammatory cells and the presence of severe alveolar bone resorption <b>Immunohistochemical analysis:</b> antibodies OCN and ALP values	<b>Micro-CT:</b> <b>Alveolar bone loss (mm)</b> CG: $0.1 \pm 0.1$ PG: $1.3 \pm 0.1 \uparrow$ NTAPG: $0.6 \pm 0.1 \downarrow$ <b>Histological analysis:</b> <b>Osteoclast</b> CG: $6.5 \pm 1.3$ PG: $22.2 \pm 3.7 \uparrow$ NTAPG: $10.2 \pm 1.8 \downarrow$ <b>Inflammatory Score:</b> CG: $0.3 \pm 0.5$ PG: $2.4 \pm 0.5 \uparrow$ NTAPG: $1.1 \pm 0.6 \downarrow$ <b>Immunohistochemical analysis:</b> <b>OCN (H-score)</b> CG: $343 \pm 25.4$ PG: $162.7 \pm 21.3 \downarrow\downarrow$ NTAPG: $319.5 \pm 18.5 \uparrow$ <b>ALP (H-score)</b> CG: $311.3 \pm 13$ PG: $197.4 \pm 20.6 \downarrow\downarrow$ NTAPG: $313.1 \pm 14.6 \uparrow$

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Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
Lima et al., <sup>39</sup>	Case – control	Periodontitis	Cold Atmospheric Plasma as adjuvant therapy for periodontal disease CG: not induction of periodontal disease (n=5); TG: - RP (n = 10); - RP + P1 (n = 10); - RP + P2 (n = 10)	Thirty C57Bl/6 female mice weighing approximately 20 g	Left mandibles: <b>histological analyses</b> (24 h after application)→ furcation JEC-JE distance % of collagen Right mandibles: <b>CT</b> (24 h after application) → BV BV/TV Tb.Th Tb.N	<b>Histomorphometric analysis:</b> <b>Furcation</b> (μm) RP: 148 ± 44.5 P1: 142 ± 42.6 P2: 147 ± 42.3 <b>JEC-JE distance</b> (μm) RP: 235.6 ± 60.2 P1: 242.1 ± 96.9 P2: 225.9 ± 61.4 <b>% of collagen</b> RP: 12.9 ± 5.1 P1: 12.7 ± 5.9 P2: 13.3 ± 5.2 Not significant differences among the groups <b>Micro-CT:</b> <b>BV</b> (μm <sup>3</sup> ); <b>BV/TV</b> (%); <b>Tb.Th</b> (μm); <b>Tb.N</b> (1/μm) There was no difference among RP, P1 and P2 groups -CAP generated with Helium had no cytotoxic effects and no physiological and histological alterations were induced
Park et al., <sup>47</sup>	Case – control	Periodontitis	Rats were divided into 3 groups: CG = PBS injection G1 = PG-LPS injection G2 = PG-LPS injection + NCP treatment	5-week-old male SD rats	<b>Micro-CT:</b> tooth and periodontium space measurement <b>RNA Extraction and RT-PCR:</b> IL-1β, TNFα <b>HE Staining:</b> Cell density in periodontal ligament <b>Immunohistochemistry:</b> CD4 and CD68 + cells <b>TRAP Assay:</b> Osteoclast activity	<b>Micro-CT:</b> G1: hole size ↑ G2: hole size ↓ <b>RNA Extraction and RT-PCR:</b> IL-1β G1: ↑ G2: ↓ TNFα G1: ↑ G2: ↓ <b>HE Staining:</b> G1: cell density ↑ G2: cell density ↓ <b>Immunohistochemistry:</b> T cell accumulation G1: ↑ G2: Similar to control Macrophage accumulation G1: ↑ G2: ↓ <b>TRAP Assay:</b> Osteoclast G1 ↑ G2 ↓

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Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
Shi et al., <sup>40</sup>	Case-control	Peri-implantitis	Two random groups CG: conventional techniques TG: conventional techniques + nonequilibrium plasma	Six adult beagles approximately 18 months old, 12-13 kg	<p><b>Clinical examination</b> at baseline (lig.-), and at month 1, 2 and 3 following ligature removal:</p> <p>SBI PD</p> <p><b>CT and micro-CT</b> at baseline and month 3 after ligature removal:</p> <p>BH<sub>(CT)</sub> <b>Histomorphometric analysis:</b> BH<sub>(HM)</sub> RH<sub>(HM)</sub> <b>Microbial examination:</b> Pg Aa Tf</p>	<p><b>Clinical examination: SBI</b> <i>Baseline</i> CG: 4 TG: 4 <i>3rd month:</i> CG: 1.5 ↓ TG: 1 ↓ <b>PD (mm)</b> <i>Baseline</i> CG: 4.27 ± 1.25 TG: 4.77 ± 1.45 <i>3rd month</i> CG: 3.29 ± 0.58 ↓ TG: 2.52 ± 0.70 ↓↓ <b>CT and micro-CT:</b> <b>BH<sub>(CT)</sub> (mm)</b> <i>Baseline</i> CG: 5.62 ± 0.70 TG: 5.89 ± 0.85 <i>3rd month</i> CG: 6.13 ± 0.69 TG: 7.22 ± 0.75 ↑ <b>Histomorphometric analysis</b> <b>BH<sub>(HM)</sub> (mm)</b> <i>3rd month</i> CG: 6.38 ± 0.55 mm TG: 7.52 ± 0.47 mm ↑ <b>RH<sub>(HM)</sub> (mm)</b> <i>3rd month</i> CG: 0.81 ± 0.37 TG: 1.52 ± 0.46 ↑ <b>Microbial examination</b> <b>Pg</b> <i>Baseline</i> CG: 0.67 TG: 0.67 <i>3rd month</i> CG: 0.50 TG: 0.33 ↓↓ <b>Aa</b> <i>Baseline</i> CG: 0.33 TG: 0.33 <i>3rd month</i> CG: 0.33 TG: 0.21 ↓ <b>Tf</b> <i>Baseline</i> CG: 0.83 TG: 0.83 <i>3rd month</i> CG: 0.67 TG: 0.33 ↓↓</p>

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Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
Tang et al., <sup>44</sup>	Randomized, controlled, split-mouth	Periodontitis	CG: RP + CHX TG: APP = RP + plasma	Six adult, male beagles (1-1.5 years, with an average weight of 10 kg)	At baseline, 4th week, 8th week and 12th week after treatment <b>Clinical examination:</b> PI SBI PD CAL <b>CBCT analysis:</b> BL <b>Histological examination:</b> HE staining to observe periodontium regeneration, in particular inflammatory cell infiltration	<b>Clinical examination</b> <b>PI</b> <i>Baseline</i> CG: 2.50 ± 0.67 TG: 2.33 ± 0.75 <i>Week 4</i> CG: 1.83 ± 0.73 ↓ TG: 1.80 ± 0.70 ↓ <i>Week 8</i> CG: 1.03 ± 0.55 ↓↓ TG: 0.87 ± 0.62 ↓↓ <i>Week 12</i> CG: 0.60 ± 0.49 ↓↓ TG: 0.43 ± 0.50 ↓↓ <b>SBI</b> <i>Baseline</i> CG: 2.80 ± 0.48 TG: 2.87 ± 0.34 <i>Week 4</i> CG: 2.00 ± 0.58 ↓ TG: 1.57 ± 0.62 ↓↓ <i>Week 8</i> CG: 0.87 ± 0.56 ↓↓ TG: 0.90 ± 0.60 ↓↓ <i>Week 12</i> CG: 0.87 ± 0.67 ↓↓ TG: 0.70 ± 0.64 ↓↓ <b>PD (mm)</b> <i>Baseline</i> CG: 3.74 ± 0.87 TG: 3.83 ± 1.00 <i>Week 4</i> CG: 3.67 ± 2.52 ↓ TG: 3.36 ± 1.13 ↓↓ <i>Week 8</i> CG: 3.17 ± 1.25 ↓↓ TG: 3.12 ± 1.36 ↓↓ <i>Week 12</i> CG: 2.03 ± 0.93 ↓↓ TG: 1.97 ± 0.96 ↓↓ <b>CAL (mm)</b> <i>Baseline</i> CG: 4.18 ± 0.28 TG: 4.24 ± 0.28 <i>Week 4</i> CG: 3.84 ± 0.11 ↓ TG: 3.86 ± 0.30 ↓ <i>Week 8</i> CG: 3.49 ± 0.21 ↓ TG: 3.14 ± 0.27 ↓ <i>12th week</i> CG: 2.84 ± 0.18 ↓ TG: 2.39 ± 0.23 ↓↓ <b>CBCT analysis</b> <b>BL (mm)</b> <i>Baseline</i> CG: 3.86 ± 0.23 TG: 3.87 ± 0.31 <i>Week 4</i> CG: 3.61 ± 0.19 ↓ TG: 3.58 ± 0.30 ↓

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Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
						<p>Week 8                      CG: 3.12 ± 0.19 ↓                      TG: 2.77 ± 0.21 ↓                      12th week                      CG: 2.81 ± 0.05 ↓                      TG: 2.32 ± 0.19 ↓↓  <b>Histological examination</b>                      -HE staining: complete periodontal ligament-cementum/bone complex structure observed in the APP group and the periodontium was denser in the APP group</p>
Zhang et al., <sup>35</sup>	Case – control	Periodontitis	<p>Four groups:  <b>CG:</b> (n = 12)  <b>TG:</b>                      Peri group (n = 12);                      SRP group (n = 12);                      SRP-NTP group (n = 12)                      All with experimental periodontitis</p>	<p>Forty-eight 4-week-old male Sprague-Dawley rats</p>	<p>7 days and 30 days post treatment, animals were euthanized, and maxillae were harvested  <b>Microbial analysis</b> on periodontal pockets:                      Pg                      Aa                      Tf  <b>Micro-CT analysis:</b>                      BV/TV  <b>Histomorphometric analysis and quantification of osteoclasts:</b>                      CAL                      Osteoclasts number  <b>Immunohistochemistry analysis:</b>                      RANKL and OPG (role in bone remodeling)  <b>Measurements of cytokines</b> on homogenate of gingival-mucosal tissues:                      TNF-a                      IL-10                      IL-1b</p>	<p><b>Microbial analysis:</b>  <b>Pg</b>                      Baseline                      CG: 0.25                      Peri Group: 0.83                      SRP: 0.83                      SRP-NTP: 0.92                      Day 7                      CG: 0.25                      Peri Group: 0.75 ↑                      SRP: 0.33 ↓                      SRP-NTP: 0.25 ↓↓                      Day 30                      CG: 0.33                      Peri Group: 0.83 ↑                      SRP: 0.58 ↓                      SRP-NTP: 0.33 ↓↓  <b>Aa</b>                      Baseline                      CG: 0.08                      Peri Group: 0.58                      SRP: 0.50                      SRP-NTP: 0.58                      Day 7                      CG: 0.17                      Peri Group: 0.50                      SRP: 0.27                      SRP-NTP: 0.08                      Day 30                      CG: 0.08                      Peri Group: 0.58                      SRP: 0.33                      SRP-NTP: 0.08  <b>Tf</b>                      Baseline                      CG: 0.08                      Peri Group: 0.83                      SRP: 0.92                      SRP-NTP: 0.83                      Day 7                      CG: 0.17                      Peri Group: 0.75                      SRP: 0.33                      SRP-NTP: 0.17</p>

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Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
						<p>Day 30 CG: 0.17 Peri Group: 0.92 SRP: 0.67 SRP-NTP: 0.25</p> <p><b>Micro-CT analysis:</b> <b>BV/TV (%)</b> 7 days SRP: no significant difference SRP-NTP: no significant difference</p> <p>30 days SRP: SRP-NTP: ↑ 40%</p> <p><b>Histomorphometric analysis and quantification of osteoclasts:</b> <b>CAL (mm)</b> 7 days and 30 days: CG: Peri Group: ↑ SRP: ↓ SRP-NTP: ↓↓</p> <p><b>Osteoclasts</b> (number/mm<sup>2</sup>) 7 days and 30 days: CG: Peri Group: ↑ (2 folds and 2.5 folds) SRP: ↓ SRP-NTP: ↓↓</p> <p><b>Immunohistochemistry analysis</b> (bone remodeling): <b>RANKL</b> Peri Group: ↑ SRP: ↓ SRP-NTP: ↓↓</p> <p><b>OPG</b> Peri Group: ↓ SRP: ↑ SRP-NTP: ↑↑</p> <p><b>Biochemical analysis:</b> <b>TNF-a (pg/ml)</b> SRP-NTP: ↓↓ <b>IL-10 (pg/ml)</b> SRP-NTP: ↑ <b>IL-1b (pg/ml)</b> SRP-NTP: ↓↓</p> <p>(continued on next page)</p>

Authors	Study design	Study model	Study groups	Animals' characteristics	Outcomes and experimental times	Results
Zhou et al., <sup>45</sup>	Case – control	Peri-implantitis	CG: surgical MD + irrigation with 2% CHX TG: plasma group treated with surgical MD + irradiation with the MCAP	Ten adult beagles weighing 12-13 kg	Clinical evaluation by CT and histological staining before and at 3 months: SBI PD BH BIC; Measurement on PISF of pro-inflammatory cytokines: IL-1β IL-17 IL-6	Histological, CT, and radiographical examination: BIC CG: ↑ TG: ↑↑ SBI Before treatment CG: no difference TG: no difference 3rd month CG: ↓ TG: ↓ PD (mm) Before treatment CG: no difference TG: no difference 3rd month: CG: ↓ TG: ↓↓ BH (mm) 3rd month CG: ↑ TG: ↑↑ Biochemical analysis: Before treatment IL-1β, IL-17, IL-6 (ng/ml) CG ≈ TG After treatment IL-1β, IL-17 (ng/ml) CG: ↓ TG: ↓↓ IL-6 (ng/ml) CG: ↓ TG: ↓

**Aa**, *Aggregatibacter actinomycetemcomitans*; **ALP**, alkaline phosphatase; **APP**, atmospheric pressure plasma; **BH**, bone height; **BIC**, bone-to-implant contact; **BL**, bone loss; **BoP**, bleeding on probing; **BV**, bone volume; **BV/TV**, bone volume fraction; **CAL**, clinical attachment level; **CBCT**, cone-beam computed tomography; **CG**, control group; **CHX**, chlorhexidine digluconate; **CT**, computerized tomography; **ELISA**, enzyme-linked immunosorbent assay; **GCF**, gingival crevicular fluid; **GI**, gingival index; **HE**, hematoxylin-eosin; **IL**, interleukin; **JEC-JE**, attachment loss; **MCAP**, modified cold-atmospheric pressure plasma; **MD**, mechanical debridement; **MPO**, myeloperoxidase; **NAG**, N-acetyl glucosaminidase; **NCP**, no-ozone cold plasma; **NAPP**, non-thermal atmospheric pressure plasma; **NTAPG**, non-thermal atmospheric plasma group; **OCN**, osteocalcin; **OPG**, osteoprotegerin; **P1**, plasma x 1; **P2**, plasma x 2; **PD**, probing depth; **Peri group**, untreated periodontitis group; **PG**, periodontitis group; **Pg**, *Porphyromonas gingivalis*; **pg/ml**, picogram/milliliter; **PG-LPS**, *Pgingivalis*-derived lipopolysaccharide; **PI**, plaque index; **PISF**, peri-implant sulcular fluid; **RANK**, receptor activator of nuclear factor kappa B; **RANKL**, RANK Ligand; **RH**, re-osseointegration height; **RP**, root planning; **RT-qPCR**, Quantitative Reverse Transcriptase-Polymerase Chain Reaction; **SBI**, sulcus bleeding index; **SD**, standard deviation; **SRP**, scaling and root planning; **SRP-NTP group**, SRP followed by plasma treatment group; **Tb.N**, Trabecular number; **Tb.Th**, Trabecular thickness; **Td**, *Treponema denticola*; **Tf**, *Tannerella forsythia*; **TG**, test group; **TNF-a**, tumor necrosis factor-a; **TRAP**, Tartrate-Resistant Acid Phosphatase Assay; **EGF**, vascular endothelial growth factor

detection rates significantly decreased only in the plasma-treated group ( $P < .05$ ). At 1, 2, and 3 months, the plasma-treated group consistently exhibited significantly lower detection rates of *P. gingivalis* and *T. forsythia* compared to the control group ( $P < .05$ ). By 3 months, the detection rates for *P. gingivalis* and *T. forsythia* in the plasma-treated group remained significantly lower compared to baseline ( $P < .05$ ), whereas in the control group, the reductions plateaued after 2 months.

In addition, Zhang et al.<sup>35</sup> conducted a study on the effect of SRP alone or in combination with CAP in rats with experimen-

tal induced periodontitis. After treatment, the SRP+CAP group showed a significant reduction in bacterial detection rates at 7 and 30 days, whilst in the SRP alone the reduction was temporary as recolonization occurred by 30 days.

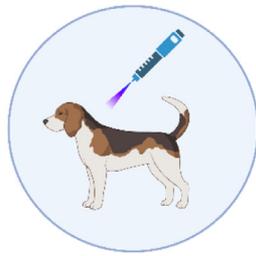
### Measurement of Inflammation

5 studies<sup>33,35,38,45,46</sup> analyzed inflammation by measuring cytokines with ELISA.

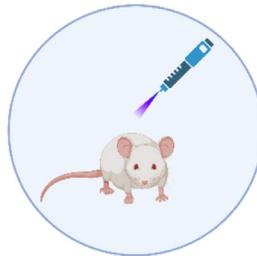
### Human Study

The study by Kùçük et al.<sup>33</sup> on a human model showed a statistically significant decrease in GCF levels of IL-1β and

Figure 2. Schematic representation of the different models used in the included studies: beagle dogs (n=3), rats (n=4), mice (n=1), human (n=1).



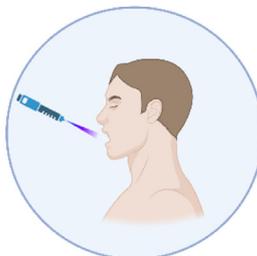
- Shi Q et al. 2015
- Tang XZ et al. 2022
- Zhou X et al. 2022



- Lima GMG et al. 2021



- de Oliveira IC et al. 2024
- Kusakci-Seker B et al. 2021
- Park KH et al. 2024
- Zhang Y et al. 2018



- Küçük D et al. 2020

VEGF in both the SRP and SRP + CAP groups at 1 and 3 months compared to baseline ( $P < .05$ ). However, no statistically significant differences were observed between the groups at any time point ( $P > .05$ ). Regarding IL-10 levels, neither significant changes over time within groups nor between groups were observed ( $P > .05$ ).

#### Animal Model Studies

In the study by Kusakci-Seker et al.<sup>38</sup> on rat model, a histological semi-quantitative inflammatory scoring system was employed, defined as follows: (1) absence or rare presence of inflammatory cells, (2) moderate cellular infiltration, (3) dense cellular inflammation, and (4) very dense cellular inflammation. The findings demonstrated that the untreated periodontitis group exhibited significantly higher inflammation compared to both the control group (healthy animals) and the periodontitis group treated with CAP.

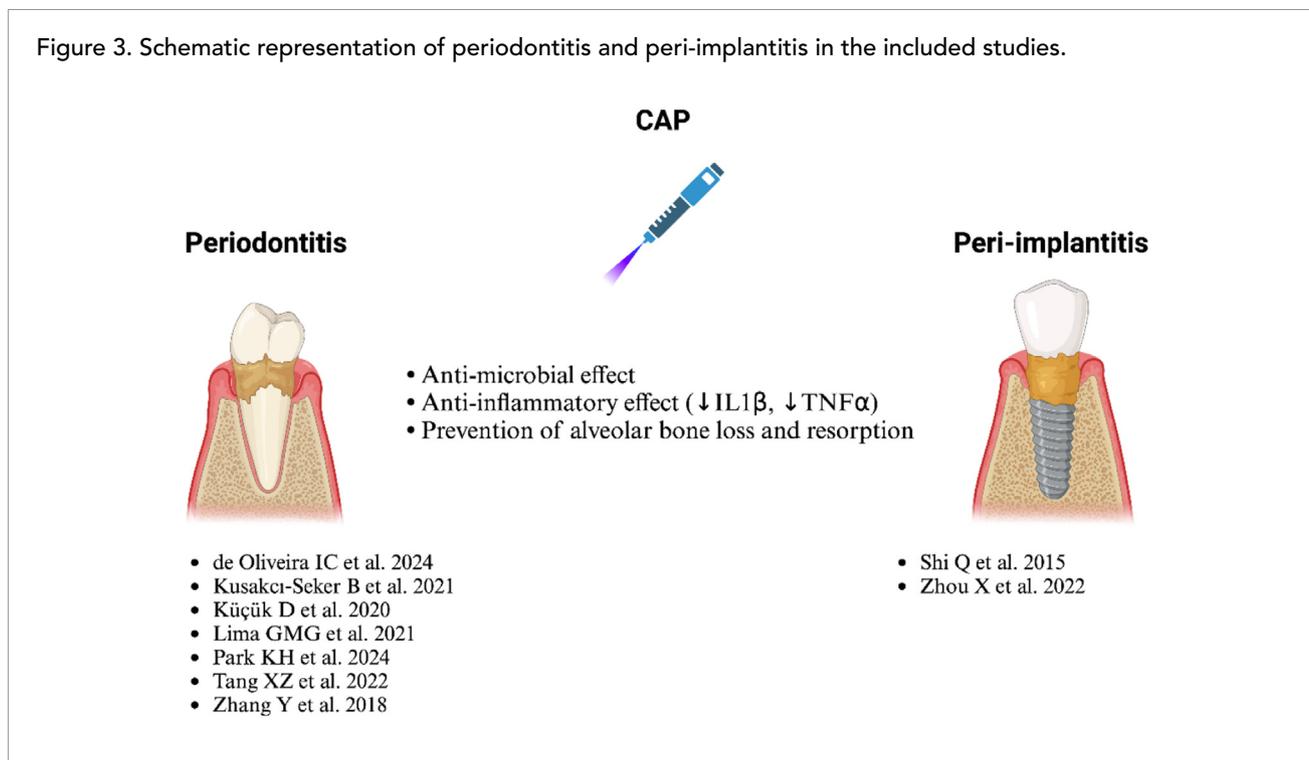
In another study, Zhang et al.<sup>35</sup> evaluated on rat the effects of SRP + CAP on the expression of inflammatory-related factors TNF- $\alpha$ , IL-1 $\beta$ , and IL-10 in homogenate of gingival-mucosal tissues. Compared to the control group, TNF- $\alpha$  and IL-1 $\beta$  levels were significantly elevated in periodontitis-induced group. SRP + CAP treatment significantly reduced TNF- $\alpha$  levels to near control levels and markedly decreased IL-1 $\beta$  expression compared to the SRP alone. Additionally,

IL-10 was strongly upregulated in the combination group compared to the SRP group at both time points. Also, de Oliveira et al.<sup>46</sup> observed a significant reduction in TNF- $\alpha$  and IL-1 $\beta$  levels, coupled with an increase in IL-10, after 4 weeks in treated rats. Further, their findings revealed elevated myeloperoxidase (MPO) levels, a marker of neutrophil activity, and increased N-acetylglucosaminidase (NAG) levels, indicative of macrophage activity, in the treated group. Histological analysis of the periodontal tissues showed a reduction in inflammatory infiltrate and fibroblasts, accompanied by a notable increase in blood vessel density in the treated group.

Park et al.<sup>47</sup> investigated the effects of No-Ozone Cold Plasma (NCP) in rats. They found that the *P. gingivalis*-derived lipopolysaccharide (PG-LPS) + NCP-treated groups exhibited lower levels of IL-1 $\beta$  and TNF $\alpha$ , while the PG-LPS group showed higher levels of these markers. RNA was extracted from periodontal tissues, synthesized into cDNA, and subjected to RT-PCR for the gene expression of IL-1 $\beta$  and TNF $\alpha$ .

Finally, only one study in beagle dogs<sup>45</sup> investigated inflammation in a peri-implantitis model. A significant reduction in IL-1 $\beta$  and IL-17 (ng/ml) levels was observed in the peri-implant crevicular fluid of the group treated with surgical mechanical debridement (MD) followed by CAP irradiation,

Figure 3. Schematic representation of periodontitis and peri-implantitis in the included studies.



compared to the control group, where peri-implantitis was treated with surgical MD and 2% chlorhexidine digluconate.

### Periodontitis and Peri-Implantitis

A schematic representation of the included studies on periodontitis and peri-implantitis is presented in Figure 3.

#### Human Study

Regarding clinical parameters, a human study<sup>33</sup> showed that clinical attachment level (CAL) and probing pocket depth (PD) did not differ significantly when comparing the control group treated with SRP and the test group where CAP treatment was added to SRP as adjuvant therapy, although significant differences were found in relation to the group without any treatment. Regarding plaque index (PI), gingival index (GI), and bleeding on probing (BoP), a significant decrease was found in the patients treated with SRP and in the ones treated with SRP + CAP, in relation to the untreated group, at 1 and 3-month follow-ups.<sup>33</sup>

#### Animal Model Studies

Three articles<sup>35,38,47</sup> used radiographic and histological analysis to examine alveolar bone loss in rats with experimental induced periodontitis. Micro-computed tomography (micro-CT) revealed that CAP showed protective effects against alveolar bone loss. When compared to the periodontitis group, the nonthermal atmospheric plasma group (NTAPG) exhibited a significant decrease in alveolar resorption and cementum damage<sup>38</sup> Zhang et al.<sup>35</sup> found that the group

treated with SRP + CAP had a higher bone volume fraction, which suggests a high rate of new bone production. Histomorphometric analysis, which showed a notable improvement in clinical attachment level (CAL), provided additional support for these findings. Variations in osteoclasts' activity were noted in all investigations on rats model.<sup>35,38,47</sup> Specifically, the number of osteoclasts was significantly higher in the periodontitis group compared to the control group without disease and to the test group with periodontitis treated with CAP<sup>38</sup> Moreover, Kusakci-Seker et al.<sup>38</sup> through immunohistochemistry, found that osteocalcin (OCN) and alkaline phosphatase (ALP) levels were lower in the periodontitis group compared to the CAP group. In Park et al.<sup>47</sup> the control group had periodontitis but was not subjected to treatment, while Group 1, treated with PG-LPS, showed significantly higher osteoclast activity, detected by TRAP staining, compared to Group 2, treated with PG-LPS + NCP. Similarly, Zhang et al.<sup>35</sup> found a lower number of osteoclasts in the group treated with SRP, with a further significant decrease when CAP was added. Regarding bone remodeling, a significant decrease in the Receptor Activator of Nuclear Ligand (RANKL) and an increase in osteoprotegerin (OPG) in the plasma group compared to the periodontitis group was found.<sup>35</sup> While OPG levels were unchanged, de Oliveira et al.<sup>46</sup> noted a notable decline in receptor activator of nuclear factor kappa B (RANK) and RANKL. Because OPG functions as a regulator by suppressing RANKL and preventing excessive bone resorption, and because RANKL stimulates osteoclast activity to promote bone resorption, the balance

between the 2 is essential for maintaining good bone remodeling and bone density. Indeed, RANKL promotes bone resorption by stimulating osteoclast activity, while OPG acts as a regulator by inhibiting the action of RANKL and preventing excessive bone resorption. The anti-inflammatory effects of NCP in periodontitis-induced oral tissues were examined by Park *et al.*<sup>47</sup> According to 3D micro-CT imaging, the hole between the first molar and the alveolar bone grew larger during PG-LPS treatment, while it shrank in the PG-LPS + NCP group.

Lima *et al.*<sup>39</sup> used a mice model to investigate the effects of CAP produced using helium; specifically, the lack of cytotoxic effects or physiological or histological alteration were evaluated. The SRP and plasma groups did not, however, differ significantly in histomorphometry (furcation, attachment loss, collagen %) or micro-CT results (bone volume, bone volume fraction, trabecular number, trabecular thickness).

Finally, 3 articles<sup>40,44,45</sup> investigated clinical indices in experimental periodontitis and peri-implantitis models in Beagle dogs. Shi *et al.*<sup>40</sup> observed a significantly greater decrease in PD and sulcus bleeding index (SBI) in the third month after plasma treatment, compared to the control group treated with conventional techniques (surgical intervention, manual scaling, antibiotic irrigation with 0.2% chlorhexidine). Moreover, bone height (BH) and re-osseointegration height (RH) increased after 3 months in the plasma group compared to the control group. Similarly, Zhou *et al.*<sup>45</sup> observed a significant decrease in PD and a significant increase in BH and bone-to-implant contact (BIC) 3 months after plasma treatment. A decrease in SBI was also observed, but the differences with the control group were not significant. Tang *et al.*<sup>44</sup> observed PD, CAL, PI, and SBI at baseline, and at weeks 4, 8, and 12, with all significantly decreased at week 12, in both the control group treated with root surface debridement (RP) + chlorhexidine digluconate and in the test group treated with RP + plasma. Finally, bone loss significantly decreased, and histologically, a complete periodontal ligament-cementum/bone complex structure was observed in the test group.

### Types of CAP Devices and CAP Treatment Parameters

In all the studies included in this review, regardless of the model -humans or animals - were treated by direct CAP application. Devices differed among studies, with 1 study<sup>46</sup> using SURFACE-SAP01 (SURFACE – Engineering and Solutions by Plasma Ltda., Campinas, SP, Brazil), another study<sup>38</sup> employing the kINPen Med® (kINPen 11 plasma jet Leibniz Institute for Plasma Science and Technology, Greifswald, Germany), 1 study<sup>39</sup> using GBS Elektronik (GmbH, Radeberg, Germany), 1 study<sup>33</sup> using Plasma 1 (plasma MEDICAL SYSTEMS, GmbH, Germany), and finally one<sup>35</sup> applying Nanova (Columbia, MO, USA). The other 4 articles<sup>40,44,45,47</sup> did not

provide the manufacturer of the device, probably because they used experimental ones. All the investigations used a plasma jet, with the exception of Küçük *et al.*,<sup>33</sup> who used a floating electrode dielectric barrier discharge (FE-DBD), and Park *et al.*<sup>47</sup> who employed a DBD device.

Concerning the feeding gases, 2 articles<sup>40,44</sup> used a mixture of Helium with 0.1% Oxygen, 3 studies<sup>38,46,47</sup> used pure Argon, and 1 study<sup>39</sup> pure Helium, while 1 study<sup>35</sup> used a mixture of Argon with 1% of Oxygen. One study<sup>45</sup> used helium as a working gas pure or mixed with 0.1% oxygen and bubbled in chlorhexidine di-gluconate (CHX) to feed the jet. Küçük *et al.*<sup>33</sup> converted atmospheric gasses in the environment into low temperature plasmas or cold plasma.

All devices described above operate in open air thus humid air actively contributes to the formation of reactive species during plasma processing. One study was carried out without a carrier gas switching on the discharge with the air surrounding the HV electrode.<sup>33</sup>

The plasma protocol widely varied among studies, including the setting features. The pulse frequency ranged from 420 Hz to 31kHz, and the application distance and time ranged between 5 mm to 15 mm, and 45 seconds to 5 minutes, respectively. In only 5 studies,<sup>38,40,44,46,47</sup> the applied voltage was indicated, and in 2 studies,<sup>33,40</sup> pulse with or without power values were reported. Furthermore, some of the information about the plasma setting was lacking in most articles, such as the energy, power, and pulsing parameters of the plasma device. All the parameters used in these different protocols are summarized and outlined below (Table 2).

These findings suggest that CAP has significant potential as an adjunctive approach for the treatment of periodontitis and peri-implantitis; however, a consistent protocol was not employed across the studies.

## DISCUSSION

The success of translational applications in the fields of periodontology and implantology is contingent upon the meticulous selection and optimization of CAP sources.<sup>48-50</sup> A detailed understanding of the various plasma configurations and settings that affect biological tissues is necessary to meet the specific needs of managing dental implants and treating periodontal disease. The accuracy of CAP in locating and eliminating bacteria in periodontal pockets while preserving healthy tissue becomes critical for treating periodontal issues.<sup>51</sup>

Figure 4 illustrates the plasma configurations applied in periodontology and implantology, as discussed in this review: 2 different plasma jet configurations (Figures 4A-B), FE-DBD

Table 2. Characteristics of plasma device.

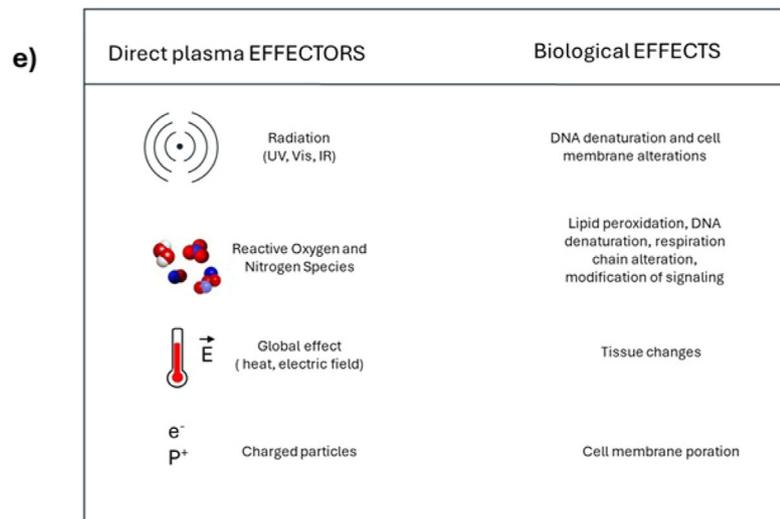
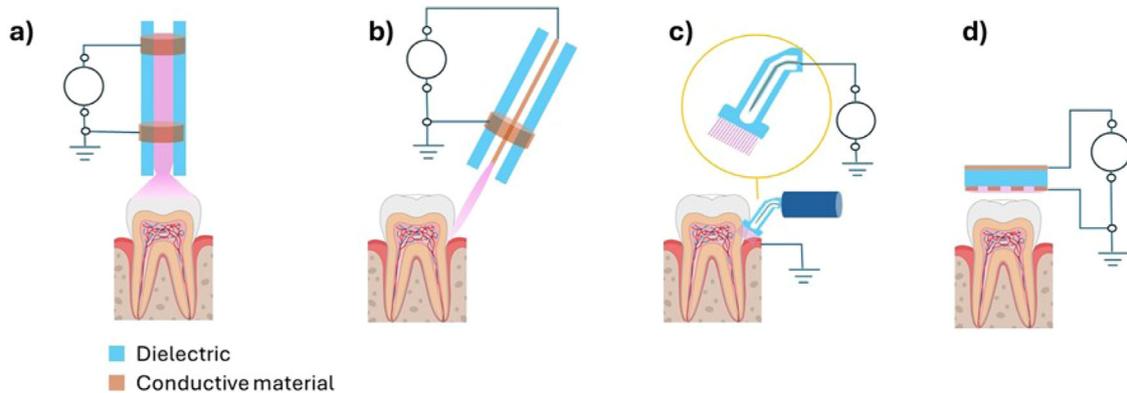
Authors	Human or animal study	Cap device	Application distance	Treatment duration	Feeding gas	Flow rate	Pulse	Pulse frequency	Voltage	Total energy	Power	Manufacturer
Küçük <i>et al.</i> <sup>33</sup>	Human	FE-DBD	N/P	2.5 min/tooth	atmospheric gasses	N/P	5–10 $\mu$ s	420 Hz–1220 Hz	N/P	N/P	4-5 power levels: maximal power 5 W	Plasma One, plasma MEDICAL SYSTEMS, GmbH, Germany
de Oliveira <i>et al.</i> <sup>46</sup>	Animal	Plasma jet	10mm	N/P	Ar	1 L/min	N/P	30kHz	5000 V		50 W	SURFACE-SAP01 (SURFACE – Engineering and Solutions by Plasma Ltda., Campinas, SP, Brazil).
Lima <i>et al.</i> <sup>39</sup>	Animal	Plasma jet	15 mm	5 min	He	1.0 slm*	N/P	31 kHz	N/P	N/P	0.6 W	GBS Elektronik GmbH, Radeberg, Germany
Kusakçı-Seker <i>et al.</i> <sup>38</sup>	Animal	Plasma jet	5 mm	60 s	Ar	5 L/min at 2.5 bar	N/P	21 kHz	5 kV	N/P	N/P	kINPen 11 plasma jet Leibniz Institute for Plasma Science and Technology, Greifswald, Germany
Park <i>et al.</i> <sup>47</sup>	Animal	DBD	N/P	5 min	Ar	1 L/min	N/P	20 kHz	3 kVpp	N/P	N/P	N/P
Shi <i>et al.</i> <sup>40</sup>	Animal	Plasma jet	5 mm	3 min	0.1% O <sub>2</sub> /He	2 L/min	1600 ns	8 kHz	8 kV	N/P	N/P	N/P
Tang <i>et al.</i> <sup>44</sup>	Animal	Plasma jet	5 mm	N/P	0.1% O <sub>2</sub> /He	2 L/min	N/P	23.5 kHz	12.5 kV	N/P	N/P	N/P
Zhang <i>et al.</i> <sup>35</sup>	Animal	Plasma jet	N/P	2 min	1% O <sub>2</sub> /Ar	Ar: 3000 sccm** O <sub>2</sub> : 30 sccm	N/P	N/P	N/P	N/P	N/P	Nanova (Columbia, MO, USA)
Zhou <i>et al.</i> <sup>45</sup>	Animal	Plasma jet	5 mm (at less)	45 s	0.1% O <sub>2</sub> /He + vapors of 2% CHX solution	2 L/min (the flow of CHX is unknown)	N/P	8 kHz	N/P	N/P	N/P	N/P

Ar, Argon; DBD, dielectric barrier discharge; He, Helium; kVpp, KiloVolt peak-to-peak; N/P, not provided; O<sub>2</sub>, Oxygen.

\* slm: standard liter per minute.

\*\* sccm: standard cube centimeter per minute.

Figure 4. Schematic diagram of various cold atmospheric plasma (CAP) used in periodontology and implantology. (A) Ring-ring electrodes plasma jet, (B) needle-ring electrodes plasma jet, (C) FE-dielectric barrier discharge plasma (DBD) source, (D) surface DBD plasma source, (E) Plasma-generated effectors and their effects on eukaryotic and prokaryotic cells.



(Figure 4C), and surface DBD (Figure 4D). The configurations reported in the studies analyzed in this review exhibit inherent physical differences and a variety of application approaches, which make it challenging to draw common conclusions. These differences ultimately hinder the standardization of clinical protocols. Through this review, we aim to identify a common thread that could help the scientific community establish a clearer correlation between plasma application and clinical outcomes. Specifically, when plasma comes into contact with tissue, regardless of the configuration used, similar biological effects can be induced by comparable plasma effectors, as shown in Figure 4E. These effects are closely dependent on the experimental parameters, such as dissipated power, treatment time, frequency, duty cycle, among others.

We tried to highlight the advantages in working with 1 configuration respect to the other. As an example, the ability to switch-on the plasma between ring-ring (Figure 4A) and needle-ring (Figure 4B) electrodes in plasma jets provides significant advantages for periodontology and implantology. Therapy can be more easily tailored to the complex anatomy of periodontal tissues, with ring-ring configurations providing wider coverage for larger tissue areas and needle-ring configurations allowing accuracy in small areas like periodontal pockets.<sup>7</sup> Furthermore, power is essential to achieve a careful balance between optimizing therapeutic efficacy and ensuring tissue safety. The versatility of the apparatus along with the ability to adjust plasma parameters (e.g. input power, applied voltage, and gas feed used), could be advantageous in periodontology and implantology by low-

ering the risk of injury and enabling targeted therapy for specific tissue types. Floating Electrode-Direct dielectric barrier discharge (FE-DBD) (Figure 4C) and surface DBD plasma sources (Figure 4D) offer unique benefits in implantology, where the health of peri-implant tissues is crucial due to their "soft" approach. The 3 plasma sources investigated (Figures 4A-D) when in contact with the tissue enable flexible treatment of irregular surfaces and living tissues.

In the FE-DBD configuration (Figure 4C), there is a powered, high voltage (HV) electrode and a floating electrode that is not grounded and can be any object placed near (<3 mm) the HV electrode (e.g. human skin, a sample, and even an organ). FE-DBD is different from plasma jet and surface DBD because the plasma switches on only when the plasma source is near the target.

Similarly to plasma jet, both FE-DBD and surface DBD facilitate surface modification, cleaning, and bacterial inactivation, ensuring a bioactive and infection-resistant implant surface. The nonthermal nature of all the plasma sources up to now used in periodontology and implantology reduces inflammatory responses and improves peri-implant tissue health, allowing selective cell activation and a controlled release of active species.<sup>52</sup> Due to their direct interactions with surrounding tissues, FE-DBD and plasma jet sources are useful tools for enhancing the performance and longevity of dental implants. This is thanks to the high levels of short-lived species and UV radiation, which are responsible for their documented efficacy in decontamination. These technologies enable targeted treatment of implant surfaces to promote osseointegration and reduce the risk of peri-implantitis through a chemical surface treatment and/or cleaning of the materials used for the implant.<sup>53,54</sup> The plasma jet configuration is a special type of DBD that consists of 2 coaxial electrodes through which a feed gas (e.g. He, Ar, O<sub>2</sub> and mixtures of them) flows at high gas rate (e.g. L/m). The reactive species produced in the plasma phase - and, under particular conditions, the plasma zone itself - exit the nozzle at high velocity, producing a plasma plume that may or may not come into contact with the target tissue. Regardless of the DBD configuration used, when the plasma zone contacts the tissue at least 3 CAP-related factors may impact periodontology and implantology: the short-lived reactive species, the long-lived reactive species, and the physical factors (e.g. electromagnetic field, UV-vis, local heating). Surface-DBD differs from plasma Jet and FE-DBD because it is switched on remotely. However, due to short work distances during applications in implantology and periodontology, also in case of surface DBD the effect of electromagnetic field and UV-radiation, as long as short-lived species cannot be completely excluded.

For all the mentioned plasma approaches both chemical and physical factors are responsible for the biological phenomena reported in literature. As an example, plasma-generated

biological active components such as ROS, both short and long-lived ones, are crucial for antimicrobial effects due to their ability to promote lipid peroxidation, DNA damage, cell membrane modifications and alterations in the cell respiration chain.

Targeting microbial infections in periodontal pockets and on implant surfaces requires creating a biologically active environment via primary reactive species, which are produced when feed gas ionizes in the plasma phase.<sup>55</sup> Reactive oxygen and nitrogen species (RONS) are secondary reactive species generated when ambient gas diffuses into the liquid environment along with plasma. Since the implant and peri-implant regions are often wet (e.g. with saliva or blood) (Figures 4A-D), these species play a key role.<sup>7,56,57</sup> RONS influence the redox balance and exhibit antibacterial properties. By reducing inflammation and creating an environment conducive to tissue regeneration, these secondary species are essential in periodontal applications.<sup>8,58</sup> In implantology, they enhance biofilm removal from implant surfaces and facilitate osseointegration. Due to plasma-target interactions, tertiary reactive species are produced. These species influence the chemistry and molecular biology of the liquid (e.g. saliva) covering the application site, thereby affecting host responses and enhancing the therapeutic benefits of CAP in periodontology and implantology. The nature of the gas feed and, in case of wet environments, the liquid in contact with the plasma can promote the formation of specific species over others. One important aspect that must be not underestimated is the formation of ozone during the process, which can be inhaled by patients and practitioners inhale during clinical procedures. Finally, among the papers listed in Table 2, Zhou et al.<sup>45</sup> Describe using vapors of CHX solutions transported by O<sub>2</sub> and Ar used as gas feed, suggesting a possible combined action of RONS produced by the plasma and CHX or its derivatives, an aspect deserving further investigation

Plasma produced UV radiation is well known for its mutagenic and cytotoxic potential, while plasma generated charged particles are involved in cell membrane poration. Finally, undesirable effects from direct contact with local tissue warming or with the electric field can generally be avoided by using well customized plasma sources. As an example, with plasma devices featuring an internal electrode configuration (Figures 4B-C), no or low current flows through the biological target. On the other hand, if the recommended guidelines (e.g. plasma-target distance, plasma device movement during the application) are followed, thermal damage due to hyperthermia of biological targets (tissues, organs, cells) can be excluded.

The complex interactions between different reactive species emphasize the versatility of CAP as a tool to treat microbial infections, promote tissue repair, and enhance the integra-

tion of dental implants with surrounding tissues. Ongoing research aims to further elucidate specific mechanisms and effects, facilitating the translation of CAP into targeted and effective clinical applications in dental practice. Scientists are exploring different gas compositions and discharge parameters in order to maximize the characteristics of the implant surface, guaranteeing improved biocompatibility and sustained stability. The variability in protocols - such as gas composition, flow rates, and treatment durations - demands standardization to provide consistency among in vivo investigations in the fields of periodontology and implantology. In order to formulate comprehensive conclusions on the safety and efficacy of CAP in dental applications, a systematic review should carefully assess and synthesize trial outcomes while taking this protocol variety into account. To facilitate the seamless incorporation of these technologies into routine clinical practice for periodontics and implant applications, future research efforts in the field of CAP should concentrate on standardizing methods, optimizing settings, and enhancing device designs. Cost of the NTP device and its maintenance are prime concern for application in dentistry. The use of noble gases like Ar and even more He due to their costs can discourage the application of most plasma jets in dentistry, highlighting the advantage in using the FE-DBD and surface DBD as alternative. Portability of the NTP device is also 1 of the concerns in dental care<sup>59</sup> but the configurations present in the market up to now offer devices easily handled and suitable for the purpose because have the great potential to be vibration-free leading to lesser pain perception by the patient. By adopting an approach, CAP opens the door to more personalized and targeted oral health therapy measures, as well as helping us better understand the potential of CAP in these specific situations.

Periodontitis treatment traditionally focuses on removing or controlling bacterial biofilm, halting the disease's progression, and restoring tooth support. Recent advancements in using CAP for microbial decontamination, Haemostasis, and enhancing cell behavior highlights its potential as an additional approach in treating periodontitis alongside conventional methods.<sup>35</sup> This systematic review aimed to evaluate the potential of CAP as a novel therapeutic option in dentistry, specifically focusing on its ability to disrupt biofilms associated with periodontitis and peri-implantitis. The included studies, comprising both human and animal models, provided valuable insights into the antimicrobial, anti-inflammatory, and regenerative effects of CAP treatment. The possibility of using CAP as a effective tool to counteract bacterial biofilms raises interest given that biofilms represent a serious health issue due to their recalcitrance. Biofilms sustained by pathogens in the oral cavity are difficult to remove due to the EPS matrix which protect bacteria from both immune system and antimicrobials, thereby driving the progression of periodontal diseases.

In periodontal therapy, SRP plays a role of primary importance and is able to close 69%-72% of all initial pockets,<sup>59</sup> being able to represent, without the need for surgery, the definitive treatment of periodontitis in a significant part of patients.<sup>60</sup> However, SRP may present some limitations linked to situations of poor accessibility, as in the case of deep pockets,<sup>61</sup> root furcation,<sup>62</sup> root concavities, penetration of bacteria into dentinal tubules<sup>63</sup> and soft tissues.<sup>60</sup> Particularly in these situations, the addition of antibacterial agents may be desirable. There is a large literature on the additional therapeutic effects exerted by antiseptics and antibiotics<sup>64</sup> in periodontology. Often this chemotherapeutics, used locally, demonstrate that they improve the clinical results of SRP, in terms of CAL gain and PD reduction to a statistically significant extent; however, in absolute terms, the CAL gain advantage is on average 0.3-0.6 mm.<sup>62,64</sup> Such limited added benefits raise the doubt that the advantage, although statistically significant, is not also clinically significant. This doubt also remains in the case of CAP, as demonstrated by the only human study<sup>33</sup> which reported an added benefit of CAP + SRP on SRP of 0.6 mm. This limitation, common to all topical antimicrobials, finds its explanation in the great effectiveness of SRP used as the only therapy which thus partially masks the effect of the topical antibacterial agent.<sup>38</sup> In this context, the use of antimicrobials must be generally avoided, especially if unnecessary and useless for the clinical outcome, in order to reduce the overall issue of AMR. The world health organization has already declared AMR as 1 of the top ten global public health threats facing humanity and the use of plasma against pathogens of the oral cavity could represent a solution, from the moment that CAP mechanism of action is different from that of antimicrobial drugs currently used, thus preventing the capability of pathogens to develop resistance.

Regarding the treatment of peri-implantitis, the role of CAP as an antibacterial agent could be significant given that no universally recognized method for effective implant surface decontamination currently exists.<sup>63</sup> Furthermore, unlike in periodontology, SRP alone has shown that it cannot be a definitive treatment of peri-implantitis without subsequent surgical treatment.<sup>63</sup> This is primarily because the implant surface presents macro- and micro-irregularities that favor bacterial colonization and make the removal of the subgingival biofilm difficult. In this regard, studies conducted on animal peri-implantitis<sup>35</sup> seem to support this hypothesis.

Pre-treatment with non-equilibrium plasma in vitro showed the potential to enhance bone formation in vivo on titanium implants or abutments.<sup>32</sup> Shi *et al.*<sup>40</sup> demonstrated that non-equilibrium plasma promotes bone healing and exhibits antimicrobial efficacy against peri-implant pathogens, especially the red complex. Zhang *et al.*<sup>35</sup> reported that NTP, when used in conjunction with conventional therapy, has a comprehensive and long-lasting effect on pe-

riodontal lesions. Plasma effectively controls major periodontal pathogens, influences bone repair, and inhibits inflammation. Küçük *et al.*<sup>33</sup> confirmed that adjunctive NAPP treatment provides additional clinical attachment level gain and effectively reduces the recolonization of periodontal pathogens in periodontitis patients. Although the differences in CAL (deep pockets: 3.90 mm; pockets  $\geq$ 5 mm: 2.72 mm) compared to the control group (deep pockets: 3.40 mm; pockets  $\geq$ 5 mm: 2.58 mm) reached statistical significance, their clinical relevance remains questionable. It has also been suggested that biofilm removal with CAP can be more beneficial than conventional therapy alone,<sup>40</sup> and in support of this, other studies such as Soeroso *et al.*<sup>65</sup> have confirmed that SRP alone is ineffective in controlling *Pg*, *Tf* and *Td* colonization.

Conventional therapies cannot recover lost periodontium; however, CAP does show bio-stimulatory effects as an adjunct to periodontal therapy.<sup>39</sup> CAP treatment also demonstrated regenerative potential, with improvements in clinical parameters and support for alveolar bone regeneration. Kusakci-Seker *et al.*<sup>38</sup> demonstrated an increase in ALP antibodies in the NTAP group. ALP is an early indicator of osteoblastic activity<sup>66</sup> and helps in the bone mineralization process.<sup>67</sup> Some studies have also shown formation of new attachments and healing of bacterial contaminated wounds.<sup>68</sup> Attachment formation has been studied by Tang *et al.*<sup>44</sup> as well with promising results.

Despite encouraging results, the heterogeneity in CAP treatment protocols across studies underscores the need for standardization. Variability in parameters such as application distance, treatment time, gas sources, and power settings may contribute to inconsistent outcomes and hinder the development of clinical protocols. For instance, the potential discomfort caused by the electric current when no anesthesia is administered represents a procedural risk, emphasizing the importance of clear guidelines for applying the electrical field safely. Future research should prioritize the establishment of standardized protocols for CAP application in periodontal and peri-implant therapies to ensure reproducibility and comparability of results.

There is a clear need for more well-designed clinical studies to validate the effectiveness of CAP in human subjects. Standardized protocols derived from preclinical studies can provide a foundation for clinical trials, facilitating the safe and effective integration of CAP into chair-side dental practices. The promising results indicate that CAP has the potential to be a valuable addition to existing dental therapies. However, its translation into routine clinical practice requires further investigation through rigorous clinical trials and the establishment of standardized protocols to ensure consistent and reproducible outcomes in human subjects.

## CONCLUSIONS

While the current evidence suggests that CAP shows promise as an adjunctive therapy for periodontitis and peri-implantitis, offering potential clinical, microbiological, and anti-inflammatory benefits, these findings remain preliminary. The conclusions are primarily based on 1 clinical split-mouth study with a limited sample size of 25 patients and several animal studies with different species and artificially induced diseases. Therefore, further well-designed clinical trials with larger patient cohorts and standardized protocols are needed to confirm the efficacy, safety, and translational potential of CAP. These additional studies will help solidify CAP's role in periodontal and peri-implant therapy and facilitate its implementation in clinical practice.

## ETHICS APPROVAL STATEMENT

Not Applicable.

## INFORMED CONSENT

For this type of study, formal consent is not required.

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## DECLARATION OF COMPETING INTEREST

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript. The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript

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## SUPPLEMENTARY MATERIALS

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