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Effects of cold atmospheric plasma therapy on chronic wounds: A systematic review and meta-analysis

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ABSTRACT

Background: Chronic wounds, including diabetic foot ulcers (DFUs) and pressure ulcers (PUs), present major therapeutic challenges due to delayed healing, high recurrence, and persistent microbial colonization. Cold Atmospheric Plasma (CAP) has emerged as a potential adjunctive therapy, but its clinical efficacy has not been comprehensively evaluated.

Methods: This systematic review and meta-analysis, registered with PROSPERO (CRD42025640913), analyzed randomized controlled trials (RCTs) published from 2010 to 2025. A comprehensive search across PubMed, EMBASE, Cochrane Library, and Web of Science identified studies comparing CAP with standard care. Data synthesis employed random- or fixed-effects models. Risk of bias and certainty of evidence were assessed with the Cochrane Risk of Bias Tool and the GRADE framework. Subgroup analyses were conducted based on plasma generation technology, treatment frequency, and study quality.

Results: 12 RCTs (477 participants) were included. CAP significantly reduced wound area (MD = −1.36; 95% CI: −2.09 to −0.62; $p = 0.0011$) and DFU area (MD = −1.48; 95% CI: −2.81 to −0.15; $p = 0.0296$). CAP also improved healing rates (RR = 2.90; 95% CI: 1.45 to 5.80; $p = 0.0052$) and reduced bacterial load (MD = −0.36; 95% CI: −0.64 to −0.08; $p = 0.0126$). Subgroup analysis revealed variability by plasma technology (Dielectric Barrier Discharge vs Plasma Jet) and methodological quality.

Conclusion: CAP therapy significantly improves wound healing and bacterial load reduction in chronic wounds, particularly DFUs. However, significant heterogeneity and limitations in study quality underscore the need for larger, high-quality trials with standardized protocols to validate long-term benefits.

1. Introduction

Chronic wounds are defined as injuries that fail to proceed through the normal, orderly phases of healing and remain unhealed for more than four weeks [1]. Their healing involves a complex cascade of cellular and molecular processes, and disruption at any stage can lead to impaired or stalled repair [2]. The major clinical categories of chronic wounds—diabetic foot ulcers (DFUs), pressure ulcers (PUs), venous leg ulcers (VLUs), and arterial ulcers (AUs)—are particularly prevalent in elderly patients with multiple comorbidities [3]. Among these, DFUs have become a global concern due to the increasing prevalence of diabetes and its associated microvascular and neuropathic complications [4]. Chronic wounds substantially diminish patients' quality of life and increase the risks of disability and mortality. They also impose heavy economic burdens on healthcare systems worldwide [5]. Therefore, developing effective and accessible therapeutic strategies for chronic wounds is a pressing global priority [6].

Over the past decades, substantial progress has been made in developing innovative wound management strategies, such as tissue-engineered skin grafts, stem cell–based therapies, exosome-mediated regenerative treatments, platelet-rich plasma (PRP) therapy, and advanced wound dressings systems [7]. However, despite these advances, clinical translation remains limited by narrow indications, inconsistent efficacy across wound types, high treatment costs, and technical complexity. These constraints underscore the imperative for innovative, cost-effective, and broadly applicable interventions [8]. In this context, cold atmospheric plasma (CAP) has recently emerged as a promising adjunctive therapeutic modality, effectively bridging biological innovation with clinical applicability.

CAP is a quasi-neutral ionized gas composed of electrons, ions, and

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neutral particles, generated at atmospheric pressure and near-physiological temperatures [9]. It produces a complex mixture of reactive oxygen and nitrogen species (ROS and RNS), including hydroxyl radicals (•OH), nitric oxide (NO•), and ozone (O_3), which are critically involved in modulating biological and antimicrobial processes [10]. Owing to its non-thermal nature, CAP can interact safe interaction with biological tissues, thereby enabling its application across various medical domains, such as oncology, infection control, and dermatology [11].

Microbial colonization and biofilm formation are key impediments to wound healing. CAP exerts broad-spectrum antimicrobial activity with minimal potential for resistance, effectively disrupting microbial biofilms and supporting a balanced wound microenvironment [12]. Beyond its antimicrobial properties, CAP promotes keratinocyte proliferation, enhances fibroblast migration, and stimulates angiogenesis through redox-sensitive signaling pathways [13]. Preclinical studies have demonstrated accelerated epithelial regeneration and collagen deposition following CAP treatment [14]. Clinically, randomized controlled trials (RCTs) have shown significant improvements in wound closure rates, bacterial clearance, and pain reduction in patients receiving CAP therapy compared to those receiving standard care [15].

However, earlier systematic reviews yielded inconclusive results, primarily due to limited sample sizes, methodological heterogeneity, and the use of first-generation plasma devices [16]. With recent technological advancements and an expanding body of high-quality clinical evidence, the therapeutic potential of CAP in managing chronic wound has become increasingly evident [17].

This meta-analysis aims to deliver an updated and comprehensive evaluation of CAP therapy for chronic wounds by synthesizing the most recent randomized controlled trials. Through a systematic assessment, this study seeks to elucidate the efficacy of CAP in enhancing wound healing and reducing microbial load, thereby supporting evidence-based clinical decision-making and guiding future research directions.

2. Methods

2.1. Registration information

This systematic review and meta-analysis was registered with PROSPERO (registration number: CRD 42025640913). As the study did not involve primary data collection or human participants, ethical approval and informed consent were not required. The methodology and reporting of this review were conducted in full accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.

2.2. Search strategy and inclusion criteria

A systematic literature search was conducted across four electronic databases: Web of Science, Cochrane Library, PubMed, and EMBASE. The literature search included studies published between January 1, 2010, and January 31, 2025. During the database search, specific filters were applied to restrict results to the defined time period, English language publications, and peer-reviewed journal articles. The search strategy was developed using the following key terms: ulcer, chronic ulcer, wound, chronic wound, diabetic foot, CAP, atmospheric pressure plasma, cold plasma, and non-thermal atmospheric plasma. The search strategy was developed using Boolean operators to logically combine search terms. Synonymous keywords within each conceptual category (e.g., 'ulcer' OR 'chronic ulcer') were combined using the 'OR' operator, while the distinct conceptual categories related to wounds and plasma therapy were linked using the 'AND' operator. Additionally, methodological filters, such as the human studies filter in PubMed, were applied to improve the precision of the search results. A comprehensive search strategy, including specific search terms and syntax for each database, is provided in Table A.1 in the appendices.

The literature search was independently conducted by reviewers N.Y and C.T, who also resolved any discrepancies encountered during the process. Subsequently, title and abstract screening was conducted collaboratively by the same reviewers, with all excluded articles thoroughly discussed and justified. Two investigators (Y.Y and J.C.) independently selected the full-texts. Discrepancies in the selection of studies and data extraction were resolved by consensus or following discussions with third reviewer (X.R.). References of the included articles were also reviewed to identify potentially relevant articles. If two studies had the same population source, the study with a larger sample size would be included. Duplicate publications were identified and removed using EndNote version 2.0 (Clarivate Analytics, Philadelphia, PA, USA), with subsequent manual verification performed by the review team to ensure the accuracy of duplicate removal.

Studies were included if they met the following criteria: (1) participants were adults diagnosed with chronic wounds; (2) the intervention involved the application of CAP therapy; (3) the control group received conventional or standard wound care; and (4) outcomes included primary measures such as wound area reduction, as well as secondary outcomes including wound healing rate and bacterial load reduction. Studies focusing on acute wounds, malignant wounds, and skin implantation wounds, as well as those employing non-RCTs, observational or retrospective study designs, utilizing animal models, or presenting case reports, review articles, or conference abstracts were excluded.

2.3. Data analysis

Data extraction was performed independently by two reviewers (Y.Y. and J.C.) using a standardized data extraction form that encompassed the following variables: study design, country, sample size, age, wound type, intervention, intervention parameters, outcomes, treatment endpoints, and duration. Any discrepancies identified during the independent extraction process were resolved through discussion to reach consensus; in cases where agreement could not be achieved, a third reviewer (X.R.) was consulted. This collaborative approach ensured the accuracy and consistency of the extracted data, thereby improving the comparability of results across studies and facilitating meta-analysis pooling.

The primary outcomes included overall wound area reduction, as well as reduction in wound area specifically in diabetic patients. Secondary outcomes included wound healing rate and bacterial load reduction. These outcome measures were selected to provide a comprehensive evaluation of CAP's efficacy. The primary outcomes reflected the direct effects of CAP therapy, while the secondary outcomes offered further insights into the mechanisms underlying the healing process.

For outcome assessments, wound area was measured in cm^2 using standardized methodologies, including planimetry or digital imaging, with evaluations performed at baseline and upon completion of the treatment period (ranging from 2 to 12 weeks across studies, as detailed in Table 1). Wound healing was defined as complete re-epithelialization of the wound without the need for drainage or dressings. Bacterial load was assessed using microbial cultures or molecular techniques and subsequently transformed into the log ratio (log Nf/Ni) to standardize diverse outcome metrics (e.g., CFU, semi-quantitative scores, or percentages) across studies. Assessments were conducted at post-treatment and follow-up time points. This analytical strategy improved comparability across studies and facilitated reliable pooling of effect estimates in the meta-analysis.

Data synthesis was performed using R software (version 4.3.2). A random-effects model was applied when heterogeneity was detected; otherwise, a fixed-effect model was used. The effect sizes were expressed as mean differences (MDs) and risk ratios (RRs), accompanied by their respective 95% confidence intervals (CIs). Data transformations were applied when necessary to ensure the accuracy and reliability of pooled results.

Heterogeneity was assessed using the Cochran's Q test and the I^2

Table 1
General characteristics of studies.

Author(year)	Design	Country	Sample size (E/C)	Age (range or mean ± SD)	Type of wound	Intervention	CAP technical parameters	Control group	Treatment duration	Outcomes
Isbary G-2010 [18]	RCT	Germany	38/38	40-94y	VU, AU, DFU, TU	5 min treatment for everyday	Microwave 2.46 GHz, 50-100 V, 86 W, Ar 2.2 slm.	ST	negative bacterial swabs/ nitrocellulose filters, the wound was healed or the patient decided to stop	(c)
Isbary G-2012 [19]	RCT	Germany	α:14/14 β:10/10	40-85y	VU, AU, TU	2 min treatment for everyday	Microwave 2.46 GHz, 50-100 V, 86 W, Ar 2.2 slm.	ST	negative bacterial swabs/ nitrocellulose filters, the wound was healed or the patient decided to stop	(c)
Brehmer F-2015 [20]	RCT	Germany	7/7	E:51-85y C:51-83y	VU	45 s/cm^2 treatment, 3 times a week	DBD	ST	8 weeks	(a),(b), (c),(d)
Chuangsuwanich A-2016 [21]	RCT	Thailand	23/19	E:70:75 ± 17:5y C:70:7±1y	PU	once a week	Argon gas; frequency 15–20 Hz; peak voltage was around 6 to 7 kV	ST	8 weeks	(a), (e)
Mirpour S-2020 [22]	RCT	Iran	22/22	E: 60.2 ± 5.5y C: 54.6 ± 8.8y	DFU	3 times a week	Helium gas; voltage 10 kV; frequency 6 kHz	ST	3 weeks	(a),(c)
Moelleken M – 2020 [23]	RCT	Germany	14/13/10	E1:65.21y E2:66.82y C:73.74y	VLU, PU, DFU, etc	2 min treatment, group1:once a week Group2: 3 times a week.	Argon gas;	Placebo	12 weeks	(a),(d)
Amini M – 2020 [24]	RCT	Iran	22/22	18-80y	DFU	3 times a week	helium gas; voltage 10 kV; frequency 6 kHz	ST	3 weeks	(c), (f)
Stratmann B-2020 [25]	RCT	Germany	27/27	E:68:3 ± 9:5y C:68:7 ± 8:8y	DFU	8 times within 2 weeks	Argon gas	ST	2 weeks	(a), (e)
Samsavar S-2021 [26]	RCT	Iran	10/10	E: 62 ± 6.06y C: 59.9 ± 6.23y	DFU	2 times a week	Helium gas; voltage 4.5 kV; frequency 22 kHz	ST	6 weeks	(a)
Strohal R-2022 [27]	RCT	Austria	39/39	E: 42.58–93.91y C: 42.58–93.91y	VLU, AU, DFU, PU	3 times in the 1st week, twice in the 2 nd week, and once for 4 weeks	Argon gas	Best practice wound dressings	6 weeks	(a)(b)
Abu Rached N.-2023 [15]	RCT	Germany	25/22	E:72.2 ± 10.9y C:66.5 ± 11.2y	AU, VU	2 min treatment, 3 times a week	DBD	ST	4 weeks	(b), (d)
Bakker O.-2024 [28]	RCT	Netherland	17/14/15	E1:75y E2:75y C:77y	VLU	2 min treatment, group1:once a week group2: 2 times a week	DBD	ST	12 weeks	(a), (b)

Abbreviations: CAP: cold atmospheric plasma; RCT: randomized clinical trial; E: CAP group; C: control group; y-years; ST: standard treatment; DFU: diabetic foot ulcer; VLU: venous leg ulcer; VU: venous ulcer; AU: arterial ulcer; PU: pressure ulcer; TU: traumatic ulcer. DBD: dielectric barrier discharge. Outcomes: (a) reduction of wound area (cm^2); (b) rate of wound healing; (c)reduction of the bacterial load; (d) assessment of pain (visual analogue scale (VAS) from 0 (no pain) to 10 (most severe pain)); (e) bacterial load score; (f) inflammatory factors.

statistic. A P-values less than 0.05 were considered statistically significant; and I^2 values of 25%, 50%, and 75% were interpreted as indicators of low, moderate, and high heterogeneity, respectively [29,30]. Sensitivity analyses were conducted to further explore potential sources of heterogeneity among the included studies.

The risk of bias was independently assessed by two reviewers (N.Y. and C.T.) using the Cochrane Risk of Bias tool (RoB 1.0), which evaluates seven key domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential sources of bias. Any discrepancies in the assessment of bias risk were resolved through consensus discussions among the research team. The risk levels were categorized as low, unclear, or high [31]. To examine potential publication bias, funnel plots were employed [32], supplemented by the Fail-safe N test [33], and adjusted using the trim-and-fill method [34].

The certainty of the evidence was evaluated using the GRADE approach. This evaluation was based on factors such as risk of bias, inconsistency, indirectness, imprecision, and publication bias [35]. The quality of evidence was classified as high, moderate, low, or very low. This systematic evaluation ensured a rigorous and reliable appraisal of the evidence supporting the study's conclusions.

2.4. Role of the funding source

The funding source for this study was not involved in the study design, data collection, analysis, or interpretation, nor preparation of the manuscript. The corresponding author had full access to all data collected throughout the study and takes full responsibility for the final decision to submit the manuscript for publication.

2.5. Subgroup analysis

Subgroup analyses were conducted to explore potential sources of heterogeneity and assess the consistency of treatment effects across various clinical and methodological dimensions, thereby providing more refined insights into the factors influencing the efficacy of CAP therapy. These analyses were prospectively defined based on the following key variables: plasma generation technology, categorized as Dielectric Barrier Discharge (DBD), Indirect Atmospheric Plasma, or Plasma Jet; carrier gas type, classified as Argon-based CAP, Helium-based CAP, or studies that did not specify or utilize a carrier gas; treatment frequency, stratified into high frequency ($\geq$3 times per week) and low frequency ($\leq$2 times per week); methodological quality, evaluated using the Cochrane Risk of Bias tool and categorized as low risk (no domains rated as high-risk, with unclear risks occurring less frequently than low risks) or high risk (presence of at least one domain rated as high risk); follow-up duration, grouped as short-term ($\leq$4 weeks), medium-term (5–8 weeks), or long-term (>8 weeks); and mean patient age, divided into elderly (mean age $\geq$65 years) and non-elderly (mean age <65 years) subgroups. Pooled effect estimates for each subgroup were calculated using random-effects models in the presence of heterogeneity($I^2 > 50\%$) and fixed-effects models otherwise. Between-subgroup differences were evaluated through interaction tests or meta-regression analyses where appropriate, with statistical significance defined as $p < 0.05$. This analytical approach facilitated a comprehensive evaluation of how specific CAP parameters and study characteristics might influence therapeutic outcomes, thereby enhancing the robustness and clinical relevance of the findings.

3. Results

3.1. Study selection and characteristics of included studies

The database search yielded 395 records: 231 from PubMed, 102 from EMBASE, 53 from the Cochrane Library, and 9 from Web of Science. After removing duplicates, 325 unique records remained for title and abstract screening. Of these, 303 were excluded: 6 letters, 72

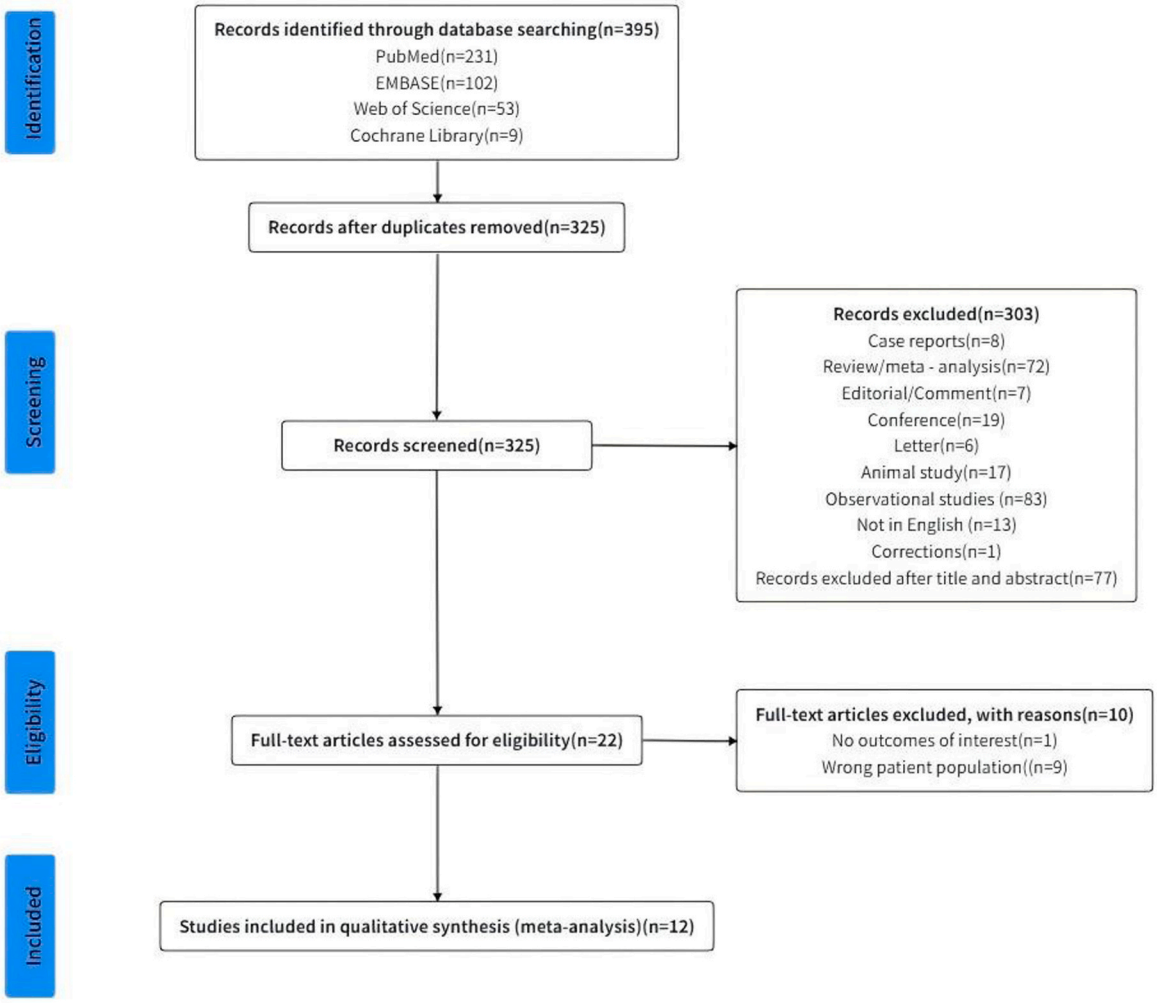


Fig. 1. The study selection process for the included studies.

reviews or meta-analyses, 7 editorials or comments, 77 records excluded after title and abstract screening, 8 case reports, 13 ongoing or unpublished trials, 1 correction, 19 conference abstracts, 83 observational studies, and 17 animal studies. 22 full-text articles were assessed for eligibility, and 10 were excluded based on the predefined criteria. 12 studies were finally included in the qualitative synthesis and quantitative meta-analysis (Fig. 1).

Overall, the meta-analysis included 477 participants, comprising 295 males (61.84%) and 182 females (38.16%). The mean age across studies was 66.76 years (SD = 14.84). Key study characteristics are presented in Table 1. Although the mean follow-up duration was not consistently reported, comorbidities known to affect wound healing, particularly diabetes mellitus and vascular insufficiency, were regularly documented across the included trials.

3.2. Outcomes

The primary outcome was reduction in wound area. The pooled analysis showed a statistically significant MD of −1.36 (95% CI: −2.09 to −0.62, $p = 0.0011$, Fig. 2). No significant heterogeneity observed ($I^2 = 0\%$, $p = 0.6623$). A second primary analysis focused on patients with DFUs. In this outcome, CAP was also associated with a statistically significant MD of −1.48 (95% CI: −2.81 to −0.15; $p = 0.0296$, Fig. 3), again with no evidence of heterogeneity ($I^2 = 0\%$, $p = 0.5533$). These findings indicate a consistent beneficial effect of CAP on wound size reduction, both in the overall chronic wound population and specifically in patients with DFUs.

Secondary outcomes included wound healing rate and the reduction in bacterial load. The analysis reveaded that the intervention was associated with a pooled RR of wound healing of 2.90 (95% CI: 1.45 to 5.80, $p = 0.0052$, Fig. 4). Moderate heterogeneity was observed ($I^2 = 50\%$), although the Cochran's Q test p-value did not achieve statistical significance ($p = 0.0812$). This pattern—of a non-significant Q statistic coupled with a moderate I^2 value—is commonly encountered in meta-analysis involving a small number of studies, wher limited statistical power may impede the detection of existing heterogeneity, while the I^2 metric still indicates appreciable between-study variability. Similarly, for the reduction in bacterial load, a statistically significant MD of −0.36 was found (95% CI: −0.64 to −0.08, $p = 0.0126$, Fig. 5), with moderate heterogeneity ($I^2 = 34\%$, $p = 0.1799$).

Subgroup analyses were conducted to investigate potential sources of heterogeneity in relation to the secondary outcomes. With respect to the wound healing rate, notable differences in effect estimates were observed based on plasma generation technology and methodological quality (Fig. 6). Studies using DBD technology reported a pooled effect size of 1.98 (95% CI: 1.39 to 2.80; $p < 0.01$) with no indication of heterogeneity ($I^2 = 0\%$). In contrast, studies employing plasma jet technology demonstrated a considerably larger effect size of 11.50 (95% CI: 2.91 to 45.49; $p < 0.01$), along with moderate heterogeneity ($I^2 = 50\%$). When stratified by methodological quality, a consistent trend emerged: studies judged to have a low risk of bias exhibited a higher effect size (11.50; 95% CI: 2.91 to 45.49; $p < 0.01$) compared to those with a high risk of bias (1.98; 95% CI: 1.39 to 2.80; $p < 0.01$). However, no statistically significant subgroup differences were detected when analyses were stratified by carrier gas, treatment frequency, or duration of follow-up.

Subgroup analyses indicated significant variations in bacterial load reduction according to plasma generation technology, carrier gas type, follow-up duration, and mean patient age (Fig. 7). Indirect atmospheric plasma was associated with a statistically significant reduction in bacterial load (effect size: −0.72; 95% CI: −1.17 to −0.28; $p < 0.01$; $I^2 = 0\%$), whereas plasma jet technology did not yield a statistically significant effect (effect size: −0.12; 95% CI: −0.48 to 0.24; $p = 0.53$; $I^2 = 32\%$). A similar pattern emerged with respect to carrier gas type: argon-based CAP demonstrated a significant reduction in bacterial load (effect size: −0.72; 95% CI: −1.17 to −0.28; $p < 0.01$; $I^2 = 0\%$), while helium-based CAP showed no statistically significant benefit. Significant reductions were also observed in studies with long-term follow-up (>8 weeks; effect size: −0.72; 95% CI: −1.17 to −0.28; $p < 0.01$; $I^2 = 0\%$), but not in those with short-term follow-up. Additionally, studies involving predominantly elderly patients (mean age ≥65 years) reported a significant reduction in bacterial load (effect size: −0.72; 95% CI: −1.17 to −0.28; $p < 0.01$; $I^2 = 0\%$), whereas studies with younger populations did not show such an effect. No significant differences across subgroups were observed when stratified by methodological quality. Furthermore, analysis of treatment frequency was constrained by the inclusion of only one study per subgroup, limiting the ability to draw comparative comparisons.

The sensitivity analysis confirmed the robustness of the overall findings, as sequentially excluding individual studies did not significantly affect the results. No sources of instability were identified, further reinforcing the consistency of the outcomes. Detailed sensitivity analyses for all outcomes are provided in the appendices (Figure A.1).

Funnel plot analysis for the primary outcome revealed asymmetry, suggesting the potential risk of publication bias (Figure A.2). Although fail-safe N analysis indicated that 33 unpublished studies with null effects would be required to render the observed effect statistically non-significant — thereby supporting the robustness of the findings—the observed asymmetry may reflect an underrepresentation of smaller studies reporting non-significant or negative results. Application of the trim-and-fill method imputed two theoretically missing studies, yielding an adjusted MD of −1.36 (95% CI: −2.09 to −0.62; $p = 0.0069$). While the treatment effect remained both statistically significant and clinically meaningful, this adjusted estimate offers a more conservative interpretation, indicating that the original meta-analytic effect size may have been moderately inflated due to selective reporting or publication bias. Similarly, for wound area reduction in DFUs, fail-safe N analysis suggested relative stability of the results; however, the persistent funnel plot

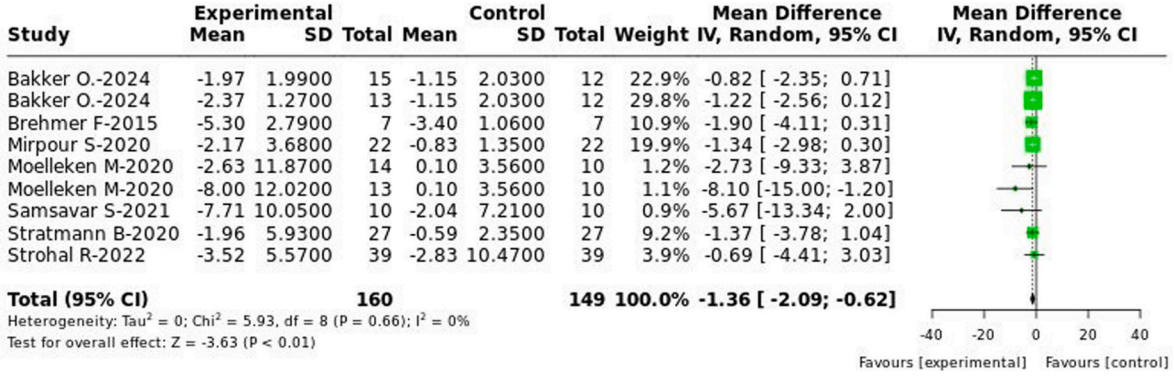


Fig. 2. Reduction in wound surface area.

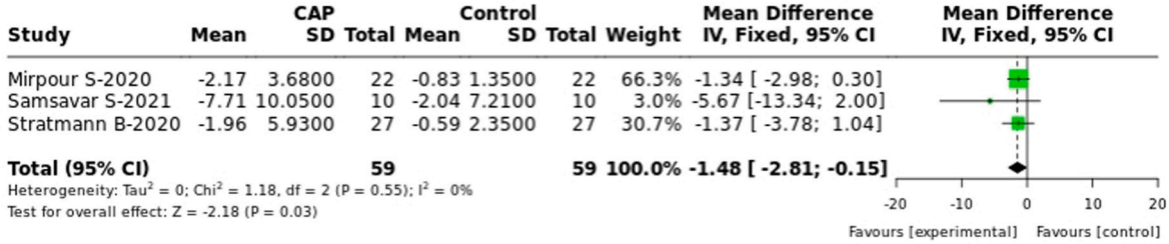


Fig. 3. Reduction in wound surface area in patients with DFUs.

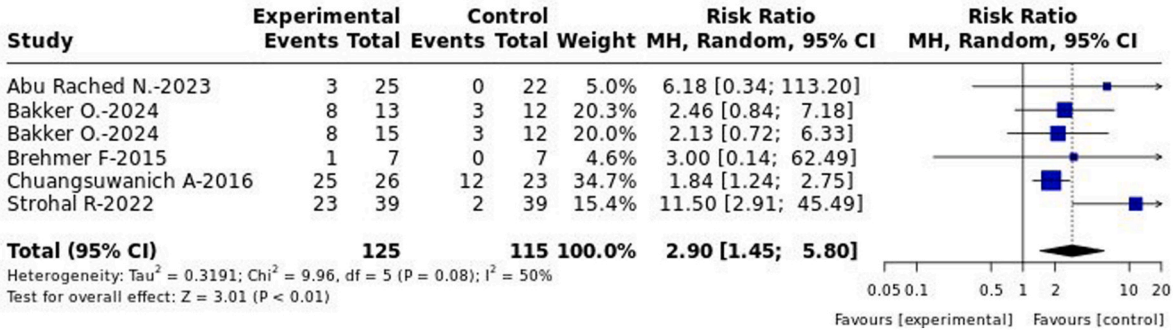


Fig. 4. The wound healing rate observed during the various follow-up periods.

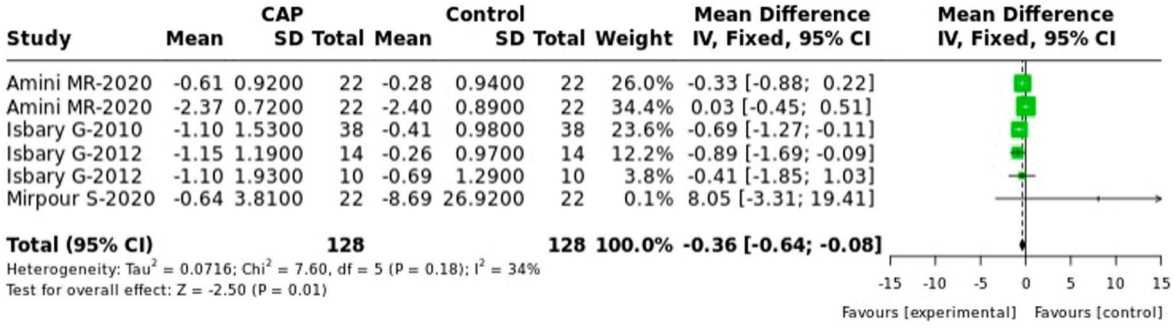


Fig. 5. Reduction of bacterial load.

asymmetry highlights the need for cautious interpretation. Collectively, these findings indicate that although the direction of benefit associated with CAP is consistent across studies, the magnitude of the estimate treatment effect may be influenced by publication bias. Therefore, future large-scale, prospectively registered trials are warranted to mitigate such biases and validate the current evidence. Trim-and-fill analyses for all outcomes are presented in the appendices (Figure A.3), providing a comprehensive basis for this assessment. In addition to publication bias, potential methodological limitions within individual included studies should also be taken into account when interpreting the results.

The methodological quality of the 12 included studies is summarized in Figs. 6 and 7. A critical appraisal using the Cochrane Risk of Bias tool revealed significant variability across the studies, with several—such as Abu Rached N. et al. and Brehmer F. et al.—exhibiting a high risk of bias in critical domains, including allocation concealment and blinding of participants and personnel (Figs. 8–9). These biases could lead to inflated estimates of treatment effects due to performance or detection bias. To assess their impact on the overall findings, sensitivity analyses were performed by sequentially excluding studies with a high risk of bias in these domains. The results demonstrated that the pooled effect estimates for primary outcomes— such as wound area reduction—remained statistically significant and directionally consistent, with only minimal changes in magnitude (see Appendix Figure A.1). This consistency suggests that, although individual studies at high risk of bias may contribute to some degree of overestimation, the overall conclusions are robust. The use of random-effects models further accounted for between-study heterogeneity, thereby reducing the disproportionate influence of any single study. Nevertheless, the presence of potential bias highlights the need for cautious interpretation of the reported effect sizes.

The certainty assessment using GRADE indicated that CAP therapy for chronic skin wounds showed moderate certainty for reducing wound area and bacterial load. However, it showed low certainty for improving the healing rate. The evidence was downgraded primarily due to the risk of bias. Detailed results are provided in the appendices (Figure A.4).

4. Discussion

Chronic ulcers, particularly DFUs, are significant contributors to morbidity, limb amputation, and mortality in the aging population. This review and meta-analysis evaluated 12 studies assessing the efficacy, safety, and antimicrobial effects of CAP therapy in the management of chronic wound. Our analysis demonstrated that CAP significantly accelerates wound closure and promotes healing, with antimicrobial efficacy comparable to existing therapies.

The quantitative analysis of seven RCTs focusing on wound size

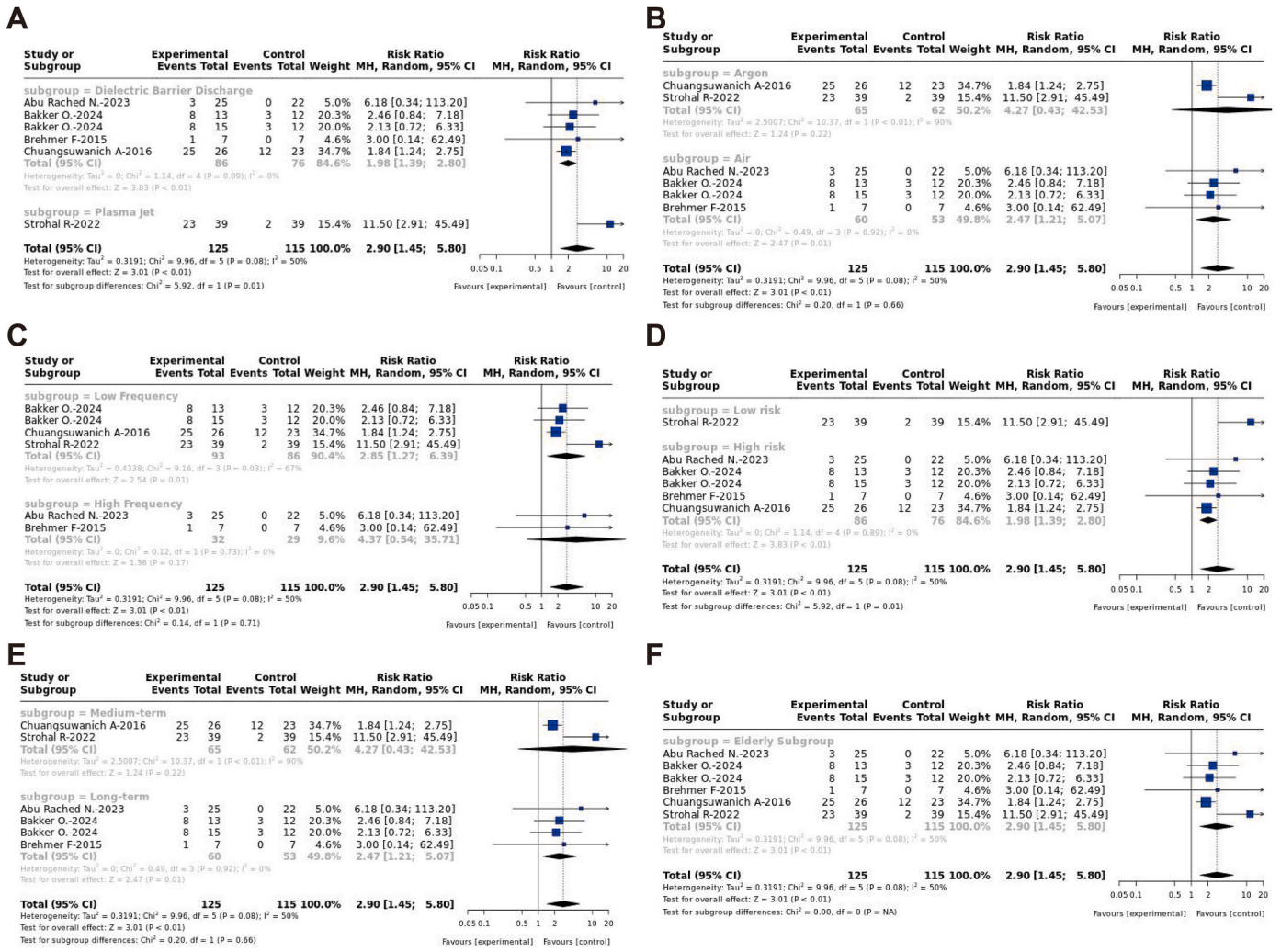


Fig. 6. Subgroup analyses of wound healing rate
A. Plasma Generation Technology; B. Carrier Gas Type; C. Treatment Frequency; D. Methodological Quality; E. Follow-up Duration; F. Mean Patient Age.

reduction revealed that CAP outperforms conventional treatments. Traditional wound dressings, such as gauze and hydrocolloids, serve primarily as physical barriers or exudate absorbers. However, they fail to address the complex biological needs of chronic wound healing, which often involves microbial persistence, microvascular insufficiency, and impaired immune responses [7]. This highlights the need for more advanced therapeutic strategies, such as CAP, that can target these underlying factors.

CAP has emerged as a promising adjunctive therapy due to its multimodal biological effects, including antimicrobial activity, promotion of neovascularization, and the release of immunomodulatory growth factors. These effects enable CAP to target multiple stages of the wound healing process [17]. By delivering a reactive cocktail of ROS, RNS, electrons, UV light, and charged particles, CAP not only disinfects wounds but also modulates inflammation, angiogenesis, and tissue regeneration.

However, previous reviews and meta-analyses have yielded inconsistent conclusions. Assadian et al. (2019) [16] found no clinical benefit of CAP in chronic wounds, based on earlier studies and less advanced plasma technologies. In contrast, recent evidence has highlighted CAP's therapeutic potential. Li et al. (2025) [36] updated the evidence and suggested that CAP improves chronic wound healing, although their analysis included both non-RCTs and non-chronic wounds. In our study, we incorporated high-quality, updated evidence and confirmed that CAP significantly improves wound closure rates and enhances healing in chronic wounds and DFUs. A detailed comparative analysis is presented in the appendices (Table B.1).

Our subgroup analyses revealed important sources of heterogeneity. For wound healing, the effect size was significantly larger in Plasma Jet studies compared to DBD studies. Similarly, studies with low risk of bias showed greater treatment efficacy compared to high-risk studies. These findings suggest that the technology used in CAP devices and the methodological quality of studies play a crucial role in determining therapeutic outcomes. The differences in device configuration likely influence the composition and delivery of reactive species, which in turn affect CAP's therapeutic efficacy. The robustness of trials with a low risk of bias further strengthens the credibility of CAP's benefits.

Several studies have highlighted the need to optimize CAP's clinical application [37]. Variations in plasma generation technology, carrier gases, and treatment protocols can influence the composition and efficacy of plasma, ultimately affecting therapeutic outcomes [38]. Technical factors such as dose, treatment duration, and frequency also play a significant role in modulating biological responses, including cell proliferation, migration, and apoptosis [39]. Future innovations, including portable plasma devices and optimized treatment protocols, are expected to enhance clinical outcomes for chronic wound healing [40].

DFUs represent a particularly burdensome subtype of chronic wounds. Approximately 18.6 million people worldwide suffer from DFUs, and these ulcers are associated with high rates of amputation and death [4]. The lifetime risk of foot ulcers in diabetic individuals ranges

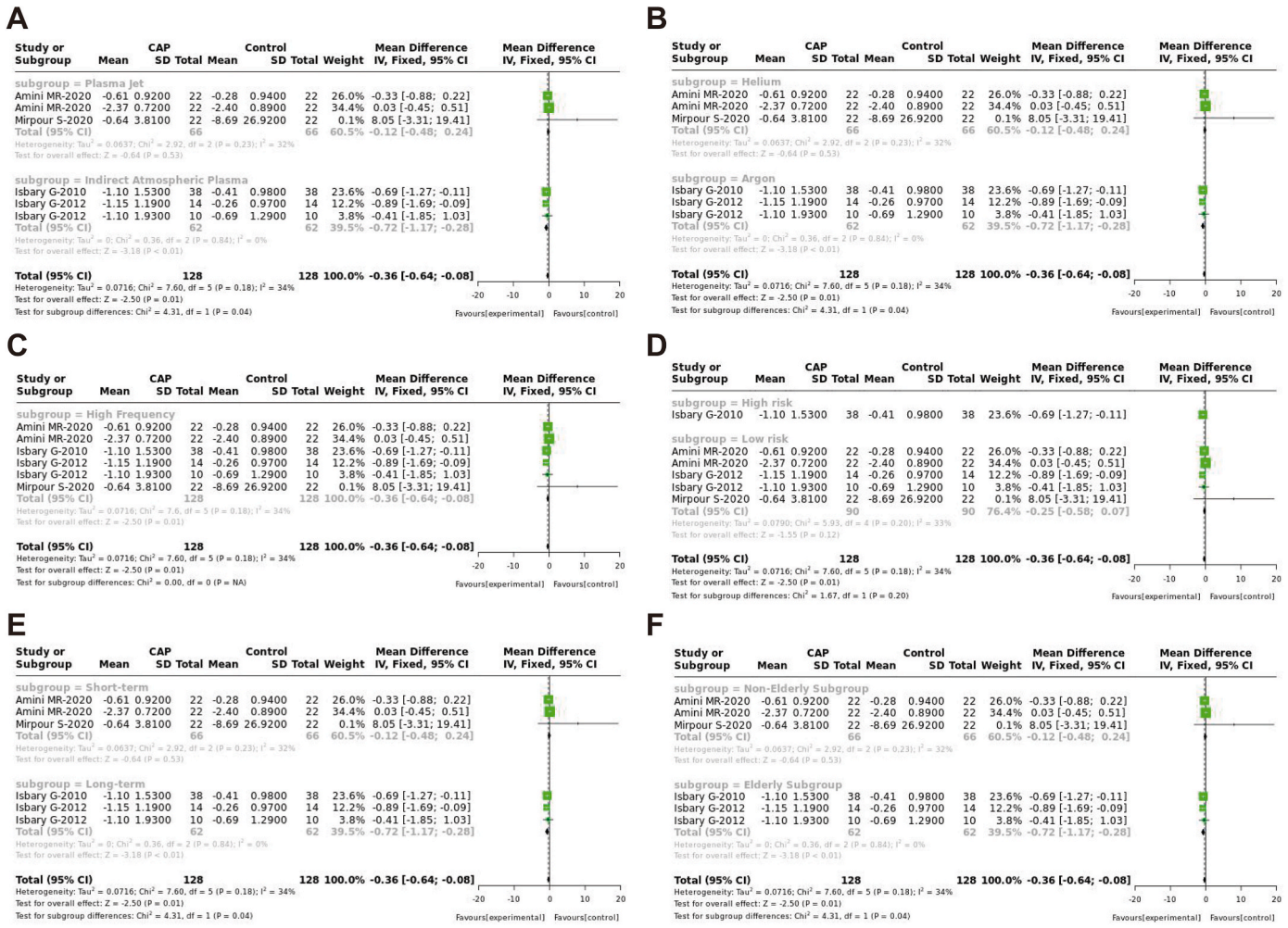


Fig. 7. Bacterial load reduction by subgroup analyses
A. Plasma Generation Technology; B. Carrier Gas Used; C. Treatment Frequency; D. Methodological Quality; E. Follow-up Duration; F. Mean Patient Age.

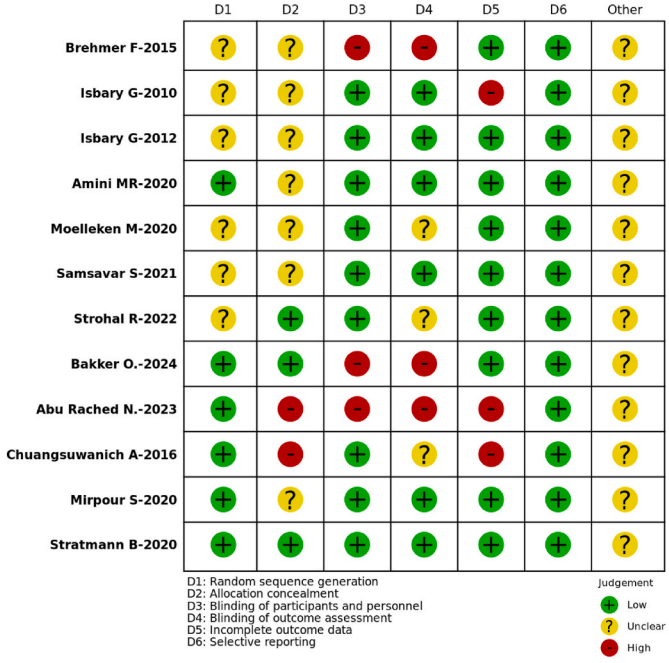


Fig. 8. Risk of bias summary.

from 19% to 34%, with recurrence rates exceeding 65% within five years and a five-year mortality rate up to 70% [5]. CAP has demonstrated significant potential in improving DFU healing rates compared to conventional treatments. ROS and RNS are the key mediators of CAP's therapeutic activity, modulating redox signaling, reducing oxidative stress, and regulating immune responses [41]. CAP also helps resolve inflammation by downregulating pro-inflammatory cytokines and altering immune cell phenotypes, promoting wound resolution [42]. Studies have shown that CAP can stimulate collagen synthesis, upregulate key growth factors, and support tissue regeneration, which are critical for healing DFUs [43,44].

A critical aspect of CAP's efficacy is its ability to reduce bacterial load, a major barrier to wound healing. Our analysis confirmed that CAP significantly reduces bacterial burden, with a marked effect in studies using Indirect Atmospheric Plasma and argon as the carrier gas. In contrast, Plasma Jet and helium-based systems did not show a significant antimicrobial effect. This suggests that the antimicrobial mechanism may be highly dependent on the specific reactive species generated by different plasma technologies and gases. Long-term follow-up and the inclusion of elderly patients further indicate that CAP can offer sustained antimicrobial benefits, which is crucial for the management of chronic wounds in older populations. The antimicrobial effects of CAP were observed across a range of bacterial pathogens, including both gram-positive (e.g., *Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative (e.g., *Pseudomonas aeruginosa*, *Proteus mirabilis*) bacteria. CAP was particularly effective at disrupting bacterial biofilms and increasing

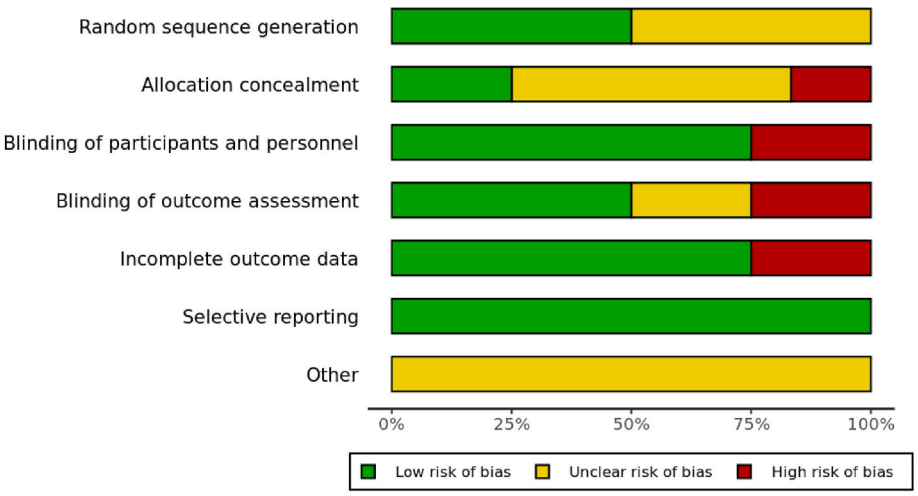


Fig. 9. Risk of Bias graph.

free bacterial DNA levels, indicating effective microbial lysis without damaging host lymphocytes. These findings, which align with prior research, suggest that CAP's broad-spectrum antimicrobial activity can bypass traditional resistance mechanisms [45].

Although the findings of this meta-analysis are promising, several significant limitations merit careful consideration. The observed heterogeneity in secondary outcomes ($I^2 = 50\%$ for healing rate, $I^2 = 34\%$ for bacterial load), coupled with non-significant Q test p-values, warrants cautious interpretation. This degree of heterogeneity is frequently encountered in meta-analyses that include a limited number of studies, as the Q test has low statistical power to detect heterogeneity when the number of studies is small or sample sizes are balanced [46,47]. The moderate I^2 values likely reflect genuine clinical and methodological diversity across the included trials, such as variations in CAP application parameters (e.g., device type, gas composition, treatment duration, and frequency), wound etiology, and baseline patient characteristics. Notably, subgroup analyses demonstrated significant differences in effect sizes according to plasma technology, methodological quality, carrier gas, and follow-up duration, providing a plausible explanation for the observed statistic heterogeneity. These factors may substantially influence treatment effect on wound healing dynamics and microbial reduction. In light of this variability, the use of a random-effects model for these outcomes is appropriate, as it accounts for between-study heterogeneity and yields a more conservative and generalizable estimate of the pooled effect. To minimize heterogeneity and improve comparability across studies, further research should emphasize the adoption of standardized protocols.

A major challenge in implementing CAP therapy is the lack of standardized treatment protocols. This is reflected in the considerable variability in CAP devices, carrier gases (e.g., argon, helium), treatment frequencies, and application parameters across the included studies. Our subgroup findings, which show that these very parameters (like technology and gas) significantly modulate outcomes, directly highlight the consequences of this lack of standardization. Such heterogeneity hinders direct comparisons and consensus on optimal regimens. Moreover, although our analysis is based on RCTs, the number of high-quality, rigorously designed RCTs remains limited. Many existing studies exhibit methodological weaknesses, including small sample sizes, inadequate randomization or blinding, and potential risks of bias. The subgroup analysis for healing rate, which found a substantially larger effect size in low-risk-of-bias studies, underscores the potential for methodological limitations to underestimate the true treatment effect. The possibility of publication bias, suggested by funnel plot asymmetry, also calls for cautious interpretation of the results. The variability in follow-up duration across the included studies—ranging from short-term (≤4 weeks) to long-term (>8 weeks)—may have influenced the observed outcomes, particularly with respect to secondary endpoints such as bacterial load reduction and complete healing rates. Subgroup analyses indicated that long-term follow-up (>8 weeks) was associated with a statistically significant reduction in bacterial load (effect size: −0.72; 95% CI: −1.17 to −0.28; $p < 0.01$), whereas no significant effect was observed in studies with short-term follow-up. This findings suggest that extended observation periods may be necessary to fully capture the therapeutic benefits of CAP, especially for outcomes reliant that depend on cumulative antimicrobial or tissue-regenerative effects. In contrast, shorter follow-up durations may underestimate treatment efficacy and contribute to heterogeneity in effect estimates. Although subgroup stratification by follow-up duration was performed to address this issue, the lack of standardized outcome measures remains a limitation that could affect the comparability and generalizability of the findings. Therefore, definitive evaluation of the efficacy and safety of CAP will require future large-scale, multicenter, rigorously designed RCTs with standardized endpoints, harmonized treatment protocols, and consistent long-term follow-up across diverse ulcer types.

Given the rising global prevalence of chronic wounds and the limitations of current therapies, CAP represents a promising intervention with significant clinical implications. Its demonstrated ability to enhance wound closure kinetics and improve healing outcomes—particularly in challenging cases like DFUs—supports its potential integration into standardized wound care protocols. Future research should focus on how specific CAP parameters (e.g., technology, gas composition, and dosing) interact with wound type and patient factors to achieve optimal, reproducible outcomes. Definitive validation of CAP's therapeutic profile requires rigorously designed, multicenter RCTs with standardized endpoints and harmonized data across diverse ulcer types.

5. Conclusion

This systematic review and meta-analysis further substantiate the therapeutic potential of CAP in the management of chronic wounds, particularly DFUs. CAP is associated with significantly improved wound closure rates and enhanced healing outcomes compared to conventional treatments, which may be primarily attributed to its antimicrobial properties and ability to reduce bacterial bioburden. Nevertheless, these findings are constrained by substantial heterogeneity among the included studies, variability in treatment protocols, and the potential presence of publication bias. The overall quality of evidence is rated as moderate to low, largely due to methodological limitations, including risk of bias in certain RCTs. Therefore, future large-scale, multicenter,

RCTs utilizing standardized intervention protocols are warranted to confirm the long-term efficacy of CAP and to support its adoption as a reliable, evidence-based treatment modality for chronic wounds.

Author contributions

The contributions of the authors are as follows: N.Y. conceptualized the research and oversaw project administration, and conducted formal analysis; Y.Y. was responsible for data curation and investigation, and performed analytical validation; J.C. contributed to resource acquisition and data management, and developed software tools; C.T. secured funding, conducted investigation, and participated in validation; X.R. provided supervision throughout the research process, contributed resources, and was actively involved in manuscript review & editing.

Institutional review board statement

This study performed a systematic search of four biomedical literature databases: Web of Science, Cochrane Library, PubMed, and EMBASE, utilizing publicly accessible summary-level data. In accordance with Articles 32(1) and 32(2) of China's Ethical Review Measures for Life Sciences and Medical Research Involving Humans (National Health Commission et al., 2023), secondary analyses based on legally acquired, de-identified public datasets are exempt from additional ethical review. All original studies included in these databases had previously obtained ethical approval from their respective Institutional Review Boards, with documented informed consent from participants permitting the sharing and reuse of data for research purposes.

Data availability statement

All raw data and code are available reasonable request.

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Conflict of interest statement

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtv.2026.101017.

Glossary

Cold Atmospheric Plasma (CAP) A partially ionized gas generated at atmospheric pressure and near-physiological temperatures, capable of delivering reactive species (e.g., reactive oxygen/nitrogen species) for biomedical applications in wound management

Diabetic Foot Ulcer (DFU) A chronic, full-thickness wound occurring below the ankle in diabetic patients, commonly associated with peripheral neuropathy and/or peripheral arterial disease

Reactive Oxygen/Nitrogen Species (ROS/RNS) Chemically reactive species, such as hydroxyl radicals (•OH), nitric oxide radicals (NO•), and ozone (O_3), generated by CAP, contribute to antimicrobial activity and modulate cellular signaling pathways associated with wound healing

Randomized Controlled Trial (RCT) A randomized controlled trial is an experimental study design in which participants are randomly assigned to either intervention or control groups to minimize selection bias and enhance the internal validity of the results

Heterogeneity (I^2 statistic) I^2 is a quantitative measure of heterogeneity across studies, ranging from 0% to 100%, which reflects variability beyond that expected by chance. I^2 values of 25%, 50%, and 75% are conventionally interpreted as indicating low, moderate, and high levels of heterogeneity, respectively

Grade of Recommendations Assessment, Development and Evaluation (GRADE) A systematic framework for evaluating the certainty of evidence, classified as high, moderate, low, very low, based on assessments of risk of bias, inconsistency, and indirectness

Mean Difference (MD) The weighted absolute difference in continuous outcomes (e.g., wound area) between the intervention and control groups across studies was calculated to assess the magnitude of treatment effects

Risk Ratio (RR) The ratio of the probabilities of occurrence of a specific event (e.g., complete healing) between the intervention group and the control group.

Trim-and-fill Analysis A statistical approach specifically developed to address publication bias by imputing hypothetical missing studies into funnel plots

Dielectric Barrier Discharge (DBD) A widely employed technique for CAP generation involves the use of insulating barriers interposed between electrodes to produce non-thermal plasma

Bacterial Load Reduction A quantitative reduction in microbial colonization density, such as measured in $\log_{10}$ CFU/g tissue, constitutes a key mechanism underlying the therapeutic efficacy of CAP in the management of biofilm-associated chronic wounds

Standard Treatment (ST) Conventional wound care encompasses debridement, the use of moisture-balancing dressings, and compliance with established clinical guidelines for infection prevention and control

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