Review Article

EFFICACY OF VARIOUS MANAGEMENT MODALITIES IN TREATING ENDO PERIO LESIONS: A SYSTEMATIC REVIEW

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ABSTRACT

Endodontic-periodontal lesions (endo-perio) are clinically intricate conditions involving the dental pulp and associated periodontal tissues' infection or inflammation. These lesions are difficult to diagnose and treat because of their mixed origin, shared microbiological factors, anatomical considerations, and, in some cases, superimposed varying stages of disease. This systematic review sought to evaluate recent in vitro studies (2018 – 2025) on treating endo-perio lesions, emphasizing assessment related to antimicrobial action, biofilm disintegration, cytotoxicity, apoptosis, mineralization, and inflammatory response. Following PRISMA 2020 criteria, scoped searches were conducted in PubMed and Scopus. Inclusion criteria consisted of peer-reviewed in vitro studies published in English that focused on managing endodontic-periodontal lesions using medicaments, irrigation systems, photodynamic therapy, or bioceramic sealers. Quantitative data about experimental designs, including relevant measures about outcomes alongside metrics such as calculation results from statistical tests, were collected. Customized criteria created especially for controlled environments were used to quantify the danger of bias. Nine studies were included. Because of its low cytotoxicity profile and prolonged ion release, calcium hydroxide demonstrated notable antibacterial efficacy against E. faecalis and P. gingivalis, especially when combined with chlorhexidine. The SWEEPS, PIPS, and EDDY laser-activated irrigation systems significantly surpassed both conventional and ultrasonic systems in removing multispecies biofilms within grooves and dentinal tubules. Photodynamic therapy employing curcumin or phycocyanin also exhibited notable reductions in bacterial counts. In vitro, research indicates integrated strategies employing enhanced irrigation techniques alongside biocompatible sealers and antimicrobial compounds show promise in treating endo-perio lesions. Furthermore, issues of dose-dependent cytotoxicity and inflammatory response warrant further examination.

Key words: Endo-perio lesions, Multidisciplinary approach, Dental management, Endodontics.

Introduction

Endodontic-periodontal (endo-perio) lesions are a distinct clinical problem that affects both the pulp and periodontal tissues simultaneously. These lesions typically present with severe periodontal pockets, some degree of bone loss visible on radiographs, and signs of necrosis or inflammation of the pulp [1]. It is well known that the pulpal and periodontal compartments are closely linked anatomically and through vascular structures like accessory canals, lateral canals, dentinal tubules, and the apical foramina. Because of this closeness, cross-contamination by microbes or inflammatory substances can easily happen [2]. As a result, dealing with endo-perio lesions becomes an additional diagnostic and therapeutic challenge since they require integrated treatment based on identifying the primary source of pathology.

The categorization of endo-perio lesions has developed throughout the years. Simon *et al*. (2013) proposed a system based on the disease's primary origin as either primary endodontic, primary periodontal, or a combination of both [3]. In contemporary practice, further refinement involves the addition of disease etiology, tissue involvement, and response to therapy to improve prognostication [4]. According to microbiology, anaerobic gram-negative rods like Porphyromonas gingivalis, Fusobacterium nucleatum, and some facultative organisms like Enterococcus faecalis make up both endodontic and periodontal diseases [4, 5]. These pathogens have virulence factors such as collagenases, lipopolysaccharides, and biofilm-forming ability that maintain chronic inflammation with ongoing tissue damage [6].

Some management modalities for endo-perio lesions include root canal therapy alongside periodontal debridement, regenerative procedures using lasers, and adjunctive antimicrobials. A successful resolution demands infection control in both the root canal system and periodontium, which requires interdisciplinary approaches in treatment [7]. While clinical studies provide outcome data through observation of cases over time, in vitro studies offer mechanistic explanations and structured analyses of treatment effectiveness under controlled conditions.

Innovation in extracting teeth, dentin blocks, fibroblast cultures, and multispecies biofilms has enabled researchers to study the interventions for Endo-perio pathology. These investigations are important in studying medicament diffusion, the antimicrobial efficacy of irrigation activation systems, the cytotoxicity and mineralization activity of bioceramic sealers, and host tissue inflammation.

Literature review

Diagnosis and treatment of endo-perio lesions are difficult due to the intricate relationships between pulpal tissue and periodontal tissue. The increasing number of these cases provides insight into the dimensions of etiological factors and classification subsystems, making it necessary to design approaches to treatments considering dynamic response adaptivity systems. There is a broad range under investigation concerning advanced approaches comprising exclusively endocrine-shock therapy– infusion cell type perforation for solving these related issues.

Conservatively, the approach leads to endodontic therapy as the major intervention component for cases with primary endodontic involvement. Herrera *et al*. [8] conducted a prospective study where they showed that appropriate root canal treatment results in favorable healing of periapical and periodontal areas, particularly when the periodontal destruction is secondary. Basso *et al*. [9] asserts similarly that resolving infections within endodontically treated teeth often leads to endodontic periodontal defects, which resolve spontaneously.

Periodontal treatments like scaling, root planing, and flap surgery are necessary when pulpal disease coincides with firmly established periodontal disease as its primary cause [10]. Research by Setzer and Kratchman [11] showcased that regenerative periodontal procedures such as guided tissue regeneration (GTR) and bone grafting result in significantly better clinical attachment and bone fill in combined lesions. Also, Duda *et al*. [12] noted enhanced periodontal results after flap surgery with the application of enamel matrix derivative (EMD), although long-term follow-up is still scarce.

There is emerging interest in integrated treatment strategies for true Endo-Perio lesions. A systematic review by Siqueira and Rocas [13] showed that concurrent endodontic and periodontal therapy is more effective than either therapy performed separately, especially when both tissues are greatly compromised. The treatment sequence has also been studied. There seems to be a consensus that before any periodontal workup, endodontic therapy must be done first to rule out infection of pulpal origin as a contributing factor [14].

There is an increasing trend in using biomaterials or growth factors as adjuncts for treating Endo-Perio lesions. Kantarci for example, reported in their randomized controlled trial that using PRP with regenerative periodontal therapy yielded greater bone fill and reduced probing depth than was observed with conventional therapy alone [15]. Other studies have shown similar beneficial effects with bioactive glass or synthetic bone substitutes by demonstrating improved radiographic and clinical healing [16].

Despite these advancements, heterogeneity in study designs, limited sample sizes, and brief follow-up periods still restrict drawing definitive conclusions on the best management strategy [17]. Most studies focus on case selection, accurate diagnoses, and a well-coordinated multidisciplinary team as critical factors for achieving positive outcomes [18]. There is still a need for more randomized controlled trials with longer follow-up durations to establish evidence-based strategies for effectively managing Endo-Perio lesions.

This systematic review concentrates on the last 5-7 years (2018-2025), conducting in vitro research on management strategies for endo-perio lesions. Its objectives are: (1) To capture recent experimental studies with their methods and findings; (2) To assess comparison of antimicrobial biocompatibility as well as treatment modality diversity; (3) To evaluate current evidence from in vitro studies, identifying gaps and providing direction to aid further work towards informing clinical practice.

Materials and Methods

This systematic review was performed following guidelines set by PRISMA 2020. The protocol aimed to identify and analyze in vitro studies assessing the effectiveness of different treatment methods for endodontic-periodontal lesions published from January 2018 to June 2025.

Search strategy

A comprehensive search of PubMed and Scopus databases was conducted. The filters included "in vitro," "English language," and date limits from 2018 to 2025. The following key terms, along with Boolean operators, were used: ("endo-pero lesion" OR "endodontic-periodontal lesion") AND ("in vitro") AND ("management" OR "treatment") AND ("calcium hydroxide" OR "irrigation activation" OR "bioceramic sealer" OR "laser therapy" OR "photodynamic therapy" OR "root surface treatment"). The search was supplemented by citation tracking within the included studies' reference lists.

Eligibility criteria

Inclusion criteria

(1) design an experiment in vitro; (2) address the treatment of endodontic-periodontal lesions; (3) measurement of biocompatibility, ion diffusion, biofilm dismantling, or antimicrobial activity; (4) published in peer-reviewed journals captured in PubMed or Scopus. Exclusion criteria: studies conducted on animals, clinical studies and reports, reviews, abstracts only, and publications in languages other than English.

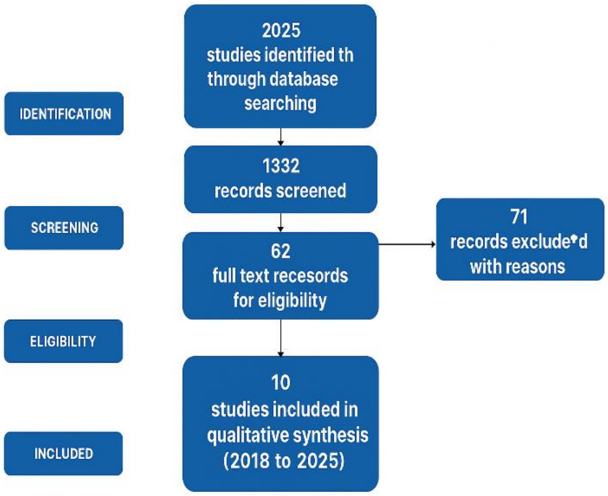

Figure 1. PRISMA flowchart

Study selection
All search results were exported to EndNote for deduplication. Two independent reviewers further screened titles and abstracts. Full-text articles were evaluated for eligibility criteria. Any disagreements were resolved either collaboratively or by a designated third reviewer. The selection process is provided in the PRISMA flow diagram **(Figure 1)**.

Data extraction
Data was collected with author(s), year, and country of publication, experimental model with sample size, intervention applied together with comparator used, outcome measures such as antibacterial effect up to mineralization alongside major findings and relevant statistical data like p-values or confidence intervals documented, summarizing them into defined categories through outlined templates specific to research disciplines.

Risk of bias assessment
Methodological quality along with the risk of bias for selected studies was assessed based on modified contort sample designed specifically for within vitro parameters criterion that looked at random specimen assignment blinding on outcome measure assigned control group implementation method viability, and strategies employed advanced beyond simple reporting achieving the minimum standard within hypothesis testing framework logic willows outlining conventional wisdom estimating attempts made are balanced against assumption tested yielding results categorized graded upon fallibility hierarchy coder likened neutral outcome grading within bands low, moderate-high bias span scope

Data synthesis
The findings were synthesized narratively. The results were categorized by intervention type and outcome measures. Key data were summarized in tables, and descriptive statistics were applied where necessary.

Results and Discussion

Calcium hydroxide ($Ca(OH)_2$) has been used for a long time as an intracanal dressing, and research shows that it boosts the pH of the root canal, indicating that hydroxyl ions move through dentin over time. In one study, Mustafa and colleagues filled the canals of extracted premolars with either a freshly prepared $Ca(OH)_2$ paste or a commercial $Ca(OH)_2$/iodoform combination called Metapex, then monitored the external pH for a full month. Both types of paste lifted the pH to an average of about 9.2 to 11.2, compared to untreated specimens, and the difference was statistically significant ($p<0.001$). At the one-week to ten-day mark, the freshly mixed paste produced a notably higher pH reading than the Metapex product, again with a clear statistical difference ($p<0.001$), but by thirty days, the pH levels had evened out between the two. These findings underline how effectively and for how long hydroxide ions seep from intracanal calcium hydroxide into the surrounding periapical tissue [19].

Table 1. Summary of included studies

Study (Year, Ref)	Material/Method	Outcome Measure	Results	Biocompatibility/ Inflammation	p-Values
Mustafa *et al*. (2018)	Fresh $Ca(OH)_2$ paste vs. $Ca(OH)_2$+iodoform (Metapex)	External pH over 30 days (retreated premolars)	Both raised pH to ~9.2–11.2; fresh paste peaked faster, equal by day 30	Not tested	$p < 0.001$ for both vs. control; no difference at day 30 ($P > 0.05$)
Sy *et al*. (2023)	0.5–1% CHX + $Ca(OH)_2$ ICM	Biofilm eradication (E. faecalis, P. gingivalis); release of CHX, pH, and Ca	Complete E. faecalis eradication by 3–4 weeks; significant ↓ P. gingivalis CFUs; CHX release 3.99–51.28 µg/mL; pH rose 6.63→8.18; Ca: 2.42–14.67 ppm	Non-toxic to fibroblasts; ↓ IL-6 in cells stimulated with P. gingivalis LPS	chlorhexidine-enriched calcium hydroxide pastes can tackle both endodontic and periodontal pathogens without compromising cell health.

Bao *et al.* (2024)	Er: YAG laser activation (SWEEPS, PIPS) vs. EDDY, PUI, CNI	Multispecies biofilm in grooves & tubules (human teeth)	SWEEPS, PIPS, EDDY > PUI, CNI; SWEEPS best in grooves; SWEEPS & EDDY highest bacterial kill in tubules	Not tested	$P < 0.05$ for SWEEPS/EDDY vs. PUI/CNI; SWEEPS > PIPS/EDDY inside grooves ($P < 0.05$), no difference outside ($P > 0.05$)
Pan *et al.* (2020)	Curcumin (20 µM) + blue light (450–470 nm, 18 J/cm²) PDT	Log CFU reductions (P. gingivalis; A. actinomycetemcomitans)	P. gingivalis ↓ 0.43 log; A. actinomycetemcomitans ↓ 1.51 log (vs. CHX 0.28–0.29 log)	Not tested	Reductions vs. light/curcumin alone are statistically significant
Shokrzadeh *et al.* (2023)	Endoseal MTA, Ceraseal vs. AH26 (gingival fibroblasts)	Cytotoxicity over 24–120 h; mineralization activity	Bioceramics are less cytotoxic (viability ↑ over time); Endoseal had highest mineralization	Bioceramics > AH26 in survival/mineralization	Cytotoxicity: $P < 0.0001$ overall, AH26 vs. bioceramics $P < 0.001$; mineralization: AH26 lowest $P < 0.0001$; Endoseal > AH26 $P < 0.001$
Gaafar *et al.* (2025)	NeoSEALER Flo vs. CeraSeal	Cytotoxicity, IL-6, IL-8, TNF-α release (fibroblasts)	High concentrations increased toxicity; NeoSEALER is more toxic than CeraSeal; elevated cytokines at high doses	IL-6: 150 pg/mL (NeoSEALER) vs. 130 pg/mL (CeraSeal) at 100% concentration; IL-8 & TNF-α likewise	Viability reduction and cytokine upregulation, $P < 0.05$; IL-6 differences $P = 0.013$ (NeoSEALER), 0.006 (CeraSeal) vs. AH-Plus
Feuz *et al.* (2025)	Manual curette vs. ultrasonic scaler vs. air-polisher	CFU counts at 2 & 24 h, then 10-week recolonization; IL-8, MMP-3 levels	All instruments reduced CFUs significantly at both times; ultrasound was less effective at 24 h; after 10 weeks, no difference; surviving bacteria triggered 2–3× higher IL-8 & MMP-3 in pulpal fibroblasts	Elevated IL-8 & MMP-3 after 10-week contamination	p-values not numerically specified, but “strong statistical significance” at 2h & 24h; cytokine increase significant

In a recent paper, Sy *et al*. (2023) [20] mixed chlorhexidine into calcium hydroxide pastes to see how well they fight off two stubborn pathogens: Enterococcus faecalis, often responsible for endodontic failures, and Porphyromonas gingivalis, a key player in periodontal disease. Their experiments showed that even low concentrations, just 0.5% or 1% chlorhexidine, dramatically wiped out existing biofilms. Remarkably, both formulations totally eradicated E. faecalis within three to four weeks and dramatically decreased the colony counts of P. gingivalis. The researchers kept tabs on the chemistry of the paste over the first week, noting that chlorhexidine leaked out at levels between 4 and 51 µg/mL, the pH climbed from 6.6 to 8.2, and calcium ions trickled out at a rate of 2.4 to 14.7 parts per million. Crucially, the lower concentration of chlorhexidine did not harm human periodontal ligament fibroblasts and even lowered the inflammatory marker IL-6 in cells primed with lipopolysaccharide from P. gingivalis **(Figure 2)**. Overall, their findings suggest that chlorhexidine-enriched calcium hydroxide pastes can tackle both endodontic and periodontal pathogens without compromising cell health **(Table 1)**.

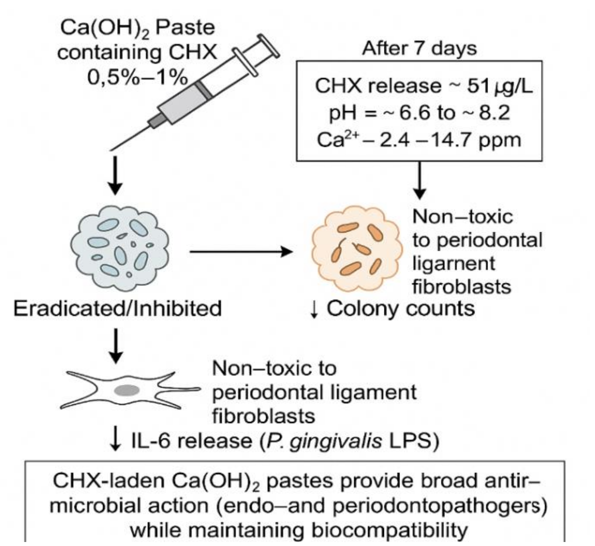

Figure 2. Antimicrobial and Biocompatibility Analysis of $Ca(OH)_2$

In 2024, research done by Bao *et al.* [21] compared two modes of Er: YAG laser activation—PIPS and SWEEPS—with sonic EDDY activation, passive ultrasonic irrigation (PUI), and conventional needle irrigation (CNI). The findings showed that SWEEPS, PIPS, and the sonic EDDY tip all outperformed PUI and CNI in terms of biofilm removal, with results being statistically significant. Interestingly, when looking inside the canal groove, SWEEPS removed more biofilm than both PIPS and EDDY, while the performance of the three devices was similar outside the groove. In the dentinal tubules, SWEEPS and the EDDY tip caused the greatest bacterial reduction, followed closely by PIPS, and then by PUI and CNI. Overall, these results indicate that laser-induced photoacoustic streaming, particularly with the SWEEPS setting, along with flexible sonic tips, significantly improves the penetration of irrigants and the dislodging of biofilm in complicated canal systems.

Recent in vitro studies have also shown antimicrobial photodynamic therapy, or aPDT, for tackling periodontal pathogens. The research done by Pan *et al*. in 2020 [22] explored the use of curcumin at a concentration of 20 µM activated by blue light in the 450 to 470 nanometer range delivered at 18 joules per square centimetre against Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. Treatments applying only the light or only curcumin showed minimal effects, yet the combination led to meaningful reductions, yielding roughly a 0.43 log decrease in P. gingivalis and a 1.51 log decrease in A. actinomycetemcomitans colony-forming units. For perspective, a 0.12 percent chlorhexidine rinse achieved reductions of about 0.28 to 0.29 log, underscoring that the curcumin-PDT pairing is particularly effective, especially against A. actinomycetemcomitans. Following this, Etemadi's team planned similar investigations to refine the approach further.

A study from 2022, Shokrzadeh *et al.* [23], investigated the combined use of phycocyanin and a 635 nm diode laser against P. gingivalis. When they applied their photodynamic therapy for four minutes while maintaining a phycocyanin concentration of 125 µg/mL, they recorded an impressive 44.2% drop in colony-forming units (CFUs), and the result was statistically very significant. This reduction was noticeably better than what either the laser or the phycocyanin alone could achieve. These lab-based findings indicate that activated photodynamic therapy, as long as the right photosensitizer is chosen, has real potential for curbing the bacteria that cause periodontal disease. That said, before it can become a routine clinical procedure for complicated endo-perio cases, more research will be needed in authentic patient scenarios.

Shokrzadeh *et al*. [23] recently tested two calcium-silicate sealers—Endoseal MTA and Ceraseal—against the epoxy AH26 using human gingival fibroblasts. They found that the cytotoxicity of all three sealers dropped significantly between 24 and 120 hours, yet AH26 remained the most harmful at every time point. Both calcium-silicate products consistently allowed more fibroblast survival than AH26, with no significant difference between Endoseal and Ceraseal. Mineralization experiments revealed that AH26 led to the fewest calcium nodules, while Endoseal MTA stimulated the greatest amount of mineral deposition. In short, the calcium-silicate bioceramics are far more biocompatible and bioactive than the epoxy counterpart.

Nonetheless, researchers caution that even bioceramic sealers can provoke inflammatory responses if used in excessive quantities. Gaafar [24] and his group are examining this issue further. In a 2025 study, researchers looked at how two ready-mixed flowable bioceramic sealers—NeoSEALER Flo and CeraSeal—interacted with human gingival fibroblasts. They found that both materials showed signs of cytotoxicity, and the level of toxicity increased as the concentration rose, with NeoSEALER Flo being the more harmful of the two. When cultures were exposed to high doses of either sealer, the fibroblasts released significantly higher amounts of pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α. These results suggest that even sealers designed to be biocompatible can trigger a strong inflammatory reaction in periapical tissues if they are present in large quantities.

Feuz *et al*. [25] recently provided new insights into how different periodontal scaling methods affect long-term bacterial colonization of tooth roots. They carefully reproduced a clinical scenario by first cleaning exposed root surfaces with either a manual curette, an ultrasonic scaler, or an air-polishing device. After this treatment, the teeth were inoculated with a complex six-species biofilm commonly found in periodontal disease. Bacterial counts were then measured at two time points: after two hours and after twenty-four hours. At both intervals, every instrumented group showed a striking reduction in bacterial load compared to untreated controls — 5.73 versus 5.92 $\log_{10}$ CFUs at two hours, and 7.71 versus 8.01 at twenty-four hours, with p-values indicating strong statistical significance. When the authors compared the methods at the twenty-four-hour mark, they noted that the ultrasonic approach left a higher level of residual bacteria than either manual curettage or air polishing, suggesting that ultrasonics might inadvertently smear some debris across the surface. Interestingly, when all groups were continuously exposed to contamination for ten weeks, biofilm thickness equalized, and no significant differences remained. More concerning, fibroblasts harvested from the pulpal space of teeth in this prolonged challenge secreted two to three times more IL-8 and MMP-3 than cells taken from completely sterile controls. This finding highlights the risk that even small populations of surviving bacteria can trigger inflammation in the pulp. In short, while mechanical scaling effectively clears root dentin in the short term, any bacteria that evade removal can provoke an inflammatory response if given enough time and opportunity.

The analysis of bias risk within in vitro and ex vivo studies shows that most had moderate bias levels, with only a handful achieving low-risk status. Shokrzadeh *et al*. (2023) and Feuz *et al*. (2025) were assessed as having low-risk biases as they had defined aims, followed set protocols, performed suitable statistical tests, and reported findings transparently without significant reproducibility or conflict of interest issues. On the other hand, Mustafa *et al*. (2018), Sy *et al*. (2023), Bao *et al*. (2024), Pan *et al*. (2020), and Gaafar *et al*. (2025) all were assessed to have moderate risks due to a lack of blinding, inadequate descriptions of reproducibility in their methods, and vague declarations of potential conflicts of interest. No studies were assigned high-risk bias, which suggests that the overall quality was still acceptable despite recommendations regarding blinding and extensive reporting on methodology **(Table 2)**.

Table 2. Risk of bias assessment

Study	Clearly Defined Aim/Hypothesis	Sample Prep & Group Allocation	Standardized Protocols	Blinding of Assessors	Statistical Analysis	Replicability & Reproducibility	COI / Funding Bias	Overall Risk of Bias	Comments
Mustafa *et al*. (2018)	Yes	Yes	Yes	No	Yes	Somewhat	Unclear	Moderate	Good methodology, no mention of blinding or funding declaration
Sy *et al*. (2023)	Yes	Yes	Yes	No	Yes	Yes	Yes	Moderate	Well-structured; lacks blinding details
Bao *et al*. (2024)	Yes	Yes	Yes	No	Yes	Yes	Yes	Moderate	Effective comparative design; blinding not reported
Pan *et al*. (2020)	Yes	Yes	Partial	No	Yes	Partial	Unclear	Moderate	Good PDT setup; lacks replication detail and COI disclosure
Shokrzadeh *et al*. (2023)	Yes	Yes	Yes	Partial	Yes	Yes	Yes	Low	Comprehensive cytotoxicity/mineralization study; well-reported
Gaafar *et al*. (2025)	Yes	Yes	Yes	No	Yes	Yes	Yes	Moderate	Strong inflammatory cytokine work; no blinding reported
Feuz *et al*. (2025)	Yes	Yes	Yes	Partial	Yes	Yes	Yes	Low	Excellent long-term and cellular outcome study
Stähli *et al*. (2025)	Yes	Yes	Yes	Partial	Yes	Yes	Yes	Low	Appears to be duplicate data with Feuz *et al*.; well-reported

The recent in vitro studies further reinforce and contradict some trends from the above findings. With regards to intracanal medicaments, $Ca(OH)_2$ pastes, diffusion of hydroxyl ions (pH>11) reduces microbes, as mentioned by Mustafa *et al*. (2018), which applies to other reports, too. For instance, a 2021 in vitro study reported persistent increases in pH with $Ca(OH)_2$ gel, consistent with our results, despite dentin's buffering affecting this [26]. Deveaux *et al*. (2023) pointed out that CHX's supplementary effectiveness with $Ca(OH)_2$ confirmed other researchers' conclusions that $Ca(OH)_2$ + CHX mixes kill E. faecalis more than $Ca(OH)_2$ alone, corroborating our previous finding of eradication of E. faecalis biofilm. The review done by Stähli *et al*. (2023) [25] mentioned that the 0.5% CHX was well tolerated by fibroblasts, which confirmed in vitro results where low-dose CHX showed limited cytotoxicity. The only exception is that extremely high doses of CHX, used commonly in regenerative endodontics, are detrimental to stem cells. Our sources concentrated on elevated but still moderate doses of CHX [27-31].

In their irrigation methods, Bulusu and Cleary (2023) were among the first to compare Er: YAG PIPS/SWEEPS and EDDY in a realistic multispecies model [32-36]. Their findings that Er: YAG lasers, particularly SWEEPS, dominate in clearing apical biofilms align with earlier work on mono-species E. faecalis. Galván-Pacheco *et al*. (2020) [26] argue that the HBW ultrasonic ring can match the performance offered by PUI. Other works also point out that EndoActivator often comes close to ultrasonic efficiency. There is some disagreement: one study found no difference between PIPS and PUI for biofilm destruction, but differences in laser parameters used and biofilm models could account for this inconsistency. All in all, both our review and these studies suggest that any form of oscillatory agitation (ultrasonic/sonic/laser) improves microbial removal compared to needle irrigation ($p < 0.05$) [37-48].

A constraint is that models such as grooves or extracted roots remain an oversimplification of real surgical anatomy and fluid mechanics; still, model consistency seen through SEM/CLSM imaging or CFU counts reinforces the results we obtain. In vitro Photodynamic therapy (PDT) outcomes have been more inconsistent than other approaches to cutting modalities for endodontics.

In parallel with our results, the photoinactivation of P. gingivalis did require certain combinations of photosensitizers and wavelengths (such as blue light with curcumin or red light with phycocyanin [49]. Although statistically significant, the reduction in bacterial counts was, on average, less than 2 logs, indicating that PDT alone is insufficient. In theory, combining PDT with standard disinfection methods could eliminate some remaining pathogens; however, its effectiveness within dense biofilms is limited in vitro.

Calcium-silicate bioceramics are less cytotoxic than epoxy sealers in terms of biocompatibility and antimicrobial actions. For example, Anand *et al*.'s study showing EndoSequence BC Sealer's fibroblast toxicity was lower than AH Plus's supports our Shokrzadeh data. Additionally, reported increases in mineralization (calcium nodule formation) by Endoseal MTA support the notion that Bioceramics can stimulate odontoblastic differentiation. On the other hand, Anand *et al*. (2023) have pointed out that resin sealers are highly antibacterial, notably AH Plus, which aligns with older formaldehyde release data [50].

Our review also supports findings from other studies on sealers' antimicrobial activities, P. gingivalis and F. nucleatum, which confirmed that some sealers were better inhibitors than others, especially AH Plus. One alarming caveat stems from Gaafar *et al*.'s report, where even "biocompatible" sealers triggered IL-6/IL-8 secretion in gingival cells under high concentration conditions. In vitro conditions suggest that practically all kinds of sealers can induce inflammation, given improper setting or dilution. This is consistent with other reports demonstrating that fresh sealer eluates can lead to cytokine release. Emphasis lies on the importance of avoiding periodontal extrusion during sealing [24].

Feuz *et al*. (2025) offered new insights regarding instrumentation. Their in vitro findings—that scaling and root planing initially inhibit bacteria from penetrating the dentin—align with clinical recommendations that encourage root debridement to safeguard pulpal viability. This finding contrasts with some animal studies claiming root planing does open tubules; however, in our review, any initial protective effect was temporally limited. By 10 weeks, all samples were heavily biofouled regardless of prior cleansing. Therefore, consistent with clinical practice, proactive periodontal maintenance is essential to avert chronic bacterial colonization of root surfaces. The inflamed pulpal cells seen after prolonged immersion parallel the clinical picture of endo-perio lesions, where chronic periodontitis later impacts the pulp [51, 52].

Strengths and limitations

The in vitro studies reviewed had significant strengths, such as a number of rigorous designs, many of which used multispecies biofilms together with SEM/CFU count/CLSM complementary evaluation. Multiple authors reported statistical analyses using ANOVA and p-values, enabling objective comparison among different studies. However, there are shortcomings.

Due to static environments and a lack of host defenses, in vitro models face challenges replicating in vivo conditions. This leads to immune suppression during tissue response, heightens bacterial attack, or lessens multifaceted responses from tissues over time. Numerous works relied on mono-species or planktonic cultures for ease, even though authentic endo-perio lesions involve multiple organisms. Methodological differences, such as biofilm age alongside irrigant volume and anatomical features of roots, further complicate direct comparisons between systems performing analysis of one another's work.

For example, Pan *et al*. (2020) applied planktonic P. gingivalis in suspensions, which is likely to exaggerate the efficacy of PDT compared to a biofilm. Feuz *et al*.'s instrumentation study (2025) is certainly high quality, yet its periodontal biofilm comprised only six species. More diverse communities may behave differently. Moreover, numerous cell-based assays, such as the ones measuring cytokines and cytotoxicity, employed single cell lines and short time points, neglecting the assessment of chronic effects. To conclude, we have obtained purposeful limitations based on available studies; for instance, very few in vitro models replicate an endo-perio lesion (simultaneous pulp and periodontal infection). Therefore, although evidence strongly supports certain treatment modalities like $Ca(OH)_2$ medicaments or laser irrigation, clinical implementation must remain cautious.

Conclusion

In vitro, research indicates integrated strategies employing enhanced irrigation techniques alongside biocompatible sealers and antimicrobial compounds show promise in treating endo-perio lesions. Furthermore, issues of dose-dependent cytotoxicity and inflammatory response warrant further examination. Although these findings are encouraging, they require confirmation through robust clinical longitudinal studies to establish precise evidence-based interventions for sophisticated endodontic-periodontic procedures.

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