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Review Article

Enzyme-Based Mouthwashes for Oral Wound Healing and Xerostomia

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Abstract

Saliva plays an essential role in maintaining oral health by providing antimicrobial protection, regulating inflammation, and supporting tissue repair. Salivary enzymes such as lactoperoxidase, lysozyme, and lactoferrin are central to these protective functions. Conditions associated with reduced salivary flow, including xerostomia and postoperative states, impair these mechanisms and may result in delayed wound healing, microbial imbalance, discomfort, and increased susceptibility to infection. Conventional antiseptic mouthwashes, particularly chlorhexidine, are effective in controlling oral microorganisms but are frequently associated with adverse effects, including mucosal irritation, taste alteration, tooth discoloration, and concerns about long-term use. This structured review summarizes current evidence on natural enzyme-based mouthwashes, focusing on their mechanisms of action, potential benefits for oral wound healing and xerostomia management, and antimicrobial effects, and compares their efficacy and safety with conventional antiseptic agents.

A focused literature search was conducted using PubMed, Scopus, and Web of Science from database inception to December 2025, supplemented by manual screening of reference lists. Peer-reviewed English-language studies, including clinical, experimental, observational, and in vitro research addressing enzyme-based mouthwashes, were considered.

The available literature suggests that enzyme-based mouthwashes exert selective antimicrobial effects by reducing pathogenic microorganisms and biofilm formation while largely preserving the commensal oral microbiota. Lactoperoxidase contributes to antimicrobial activity through hypothiocyanite generation, lysozyme disrupts bacterial cell walls, lactoferrin limits microbial growth through iron sequestration and immunomodulatory effects, and glucose oxidase supports sustained enzymatic

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- antimicrobial mouthwash
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- oral microbiome

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activity. Clinical studies report improvements in oral wound healing, relief of xerostomia-related symptoms, enhanced oral comfort, and good tolerability when compared with conventional antiseptic mouthwashes. However, limitations include variability in enzyme stability, a narrower antimicrobial spectrum, and a limited number of long-term clinical trials.

In conclusion, enzyme-based mouthwashes appear to be safe and biocompatible adjuncts for supporting oral wound healing and managing xerostomia. By mimicking natural salivary defense mechanisms, they offer a microbiome-friendly, non-antibiotic alternative to conventional antiseptics in selected clinical situations. Further well-designed randomized clinical trials with standardized formulations and long-term follow-up are required to clarify their effectiveness and optimal clinical indications.

Introduction

The oral cavity harbors a complex microenvironment. Saliva plays an important role in maintaining mucosal integrity, microbial balance, and overall oral health. It plays a crucial role in maintaining oral homeostasis by regulating pH, buffering capacity, and antimicrobial activity within the oral cavity.[1] It contains a natural defense system composed of antimicrobial proteins and enzymes such as lysozyme, lactoferrin, and the lactoperoxidase (LPO) system, which collectively protect against bacterial and fungal overgrowth, contribute to wound healing, and support tissue homeostasis. Besides its protective function, it also aids in speech, lubrication, and digestion.[2,3] In conditions where salivary secretion is reduced or when oral tissues are under surgical or pathological stress, the disruption of this natural defense system leads to xerostomia, delayed wound healing, and opportunistic infections.[4]

Mouthrinses are widely used in dentistry to promote oral hygiene and support healing after dental procedures. Among these, chlorhexidine (CHX) remains the gold standard because of its broad-spectrum antimicrobial activity.[5] However, long-term or repeated use of CHX is associated with several drawbacks, including tooth staining, altered taste sensation, mucosal irritation, and emerging concerns of microbial resistance.[5,6] These limitations have prompted the search for natural and biocompatible alternatives that can mimic the protective functions of saliva without the adverse effects of synthetic antiseptics.

Natural enzyme–based mouthwashes, such as those containing the LPO system, lysozyme, and lactoferrin, have gained attention as saliva substitutes and antimicrobial agents.[2,7] Commercially available formulations are designed to replicate salivary defense mechanisms, offering benefits in the management of xerostomia, wound healing following oral surgery, and infection control in denture wearers or immunocompromised patients.[4,8] Examples of such formulations are Oral7 and Biotène. Emerging clinical trials and in vitro studies suggest that enzyme-containing rinses can reduce microbial biofilm formation, improve patient comfort, and accelerate mucosal healing.[9,10] Previous studies have also demonstrated that natural antimicrobial agents can inhibit oral biofilm formation and periodontal pathogens.[11] Mouthrinse formulations have also been shown to influence salivary parameters and inflammatory biomarkers in the oral cavity.[12]

Recent literature further reinforces the comparative value of enzyme-based and conventional mouthwashes in postoperative oral wound care. CHX significantly improves early wound healing after oral surgery; however, its long-term use is limited by staining and mucosal irritation.[13] In contrast, enzyme- and protein-containing oral hygiene products have been reported to benefit gingival health and reduce plaque, supporting the concept that augmenting saliva's natural defenses can promote oral tissue stability and aid in wound healing.[14] While CHX supports initial tissue healing in periodontal surgical wounds, enzyme-based alternatives may offer better tolerance and fewer adverse effects during prolonged use.[15] These emerging findings support growing interest in enzyme-based formulations as microbiome-sparing, patient-friendly adjuncts in contemporary dental practice.

Given the increasing emphasis on reducing antimicrobial misuse and promoting One Health–aligned strategies, enzyme-based mouthwashes represent a promising adjunct in dental practice. This structured review aims to summarize the current evidence on the role of natural enzyme–containing mouthwashes in oral wound healing, xerostomia management, and infection control, highlighting their mechanisms of action, clinical outcomes, and potential as alternatives to conventional chemical antiseptics.

Methods

Search Strategy

This review was conducted using a structured literature selection approach to enhance transparency and reproducibility. A focused literature search was conducted in PubMed, Scopus, and Web of Science from database inception to December 2025. The search strategy combined relevant keywords and MeSH terms using Boolean operators as follows: ("enzyme-based mouthwash" OR "salivary enzymes") AND ("oral wound healing" OR "xerostomia") AND ("antimicrobial mouthwash" OR "oral microbiome").

The search was limited to English-language, peer-reviewed publications. Additional relevant studies were identified through manual screening of the reference lists of selected articles.

Inclusion Criteria

Studies were included if they:

1. Investigated enzyme-containing mouthwashes or oral rinses.
2. Reported on mechanisms of action, antimicrobial activity, oral wound healing, xerostomia, or oral microbiome effects.
3. Were clinical studies, randomized controlled trials (RCT), observational studies, in vitro experiments, or narrative/systematic reviews.
4. Provided sufficient methodological detail to allow interpretation of findings.

Exclusion Criteria

Studies were excluded if they:

1. Were editorials, commentaries, conference abstracts, letters to the editor, or opinion pieces.
2. Were non-English publications.
3. Focused solely on nonenzymatic mouthwashes without relevance to salivary enzyme systems.
4. Reported incomplete data or unclear methodology.

Study Selection

The screening process involved an initial title and abstract evaluation followed by a full-text assessment of potentially eligible articles. Studies were assessed for relevance to enzyme-based mouthwash formulations and their clinical or microbiological effects on oral wound healing and xerostomia. Particular attention was given to RCTs, controlled studies, and mechanistic laboratory investigations. Although this review follows a narrative format, the selection process was structured to ensure transparency and reproducibility of the included literature.

Result

A total of 46 studies evaluating enzyme-based mouthwashes were reviewed, including in vitro experiments, clinical trials, and pilot studies. To provide a clear overview, 15 representative studies were selected and summarized in ►**Table 1**, highlighting the mechanisms of action, clinical outcomes, and comparisons with conventional mouthwashes.

Mechanisms of Action

Enzyme-based mouthwashes consistently demonstrated antimicrobial activity through natural salivary enzymes:

- **LPO:** Catalyzes the oxidation of thiocyanate by hydrogen peroxide, producing hypothiocyanite, which selectively inhibits bacterial metabolism and plaque formation.
- **Lysozyme:** Hydrolyzes bacterial cell walls, reducing microbial load and limiting the proliferation of pathogenic species.

Table 1 Representative studies related to enzyme-based oral therapeutics and xerostomia management

No.	Author (year)	Study type	Population (*n*)	Intervention	Comparator	Outcome(s)	Key findings	Quality/bias notes
1	Chiam et al, 2024	RCT	Adults, $n = 48$	Natural enzyme mouthwash	Benzydamine mouthwash	Xerostomia Inventory, Clinical Oral Dryness Score (CODS), resting and stimulated salivary flow rate, functional symptoms (chewing, swallowing, speech)	Natural enzyme mouthwash significantly improved xerostomia symptoms, cognitive perception of dry mouth, oromotor function, CODS, and resting salivary flow rate compared with control	Small sample size; short follow-up period (2 wk)
2	Gil-Montoya et al, 2008	Pilot clinical	Elderly, $n = 20$	Mouthwash + oral gel with lactoperoxidase, lysozyme, lactoferrin	Placebo/crossover	Subjective dry mouth sensation, OHIP scores, signs of dry mouth, sialometry, *Candida albicans* culture	Improvement in OHIP values, dry mouth signs, and fluid intake; placebo effect possible	Pilot, small sample, crossover design, some outcomes improved with placebo
3	Siddiqui et al, 2025	Experimental study	In vitro / ex vivo oral biofilms (human-derived, multispecies)	Naturopathic mouthwash	Chlorhexidine 0.12% (CHX), Listerine®, PBS	Biofilm prevention, selective antimicrobial activity, cytocompatibility lower cytotoxicity vs. CHX and Listerine	Reduced pathogenic bacteria (*F. nucleatum*, *P. gingivalis*) while preserving commensals (*S. oralis*, *S. gordonii*, *V. parvula*); lower cytotoxicity vs. CHX and Listerine	Pilot, in vitro/ex vivo, no human clinical validation yet
4	Schlafer et al, 2024	Randomized controlled pilot trial (parallel arms)	Healthy adults, $n = 24$ (active = 12, placebo = 12)	Enzyme lozenge	Placebo lozenge	De novo plaque formation, gingival index, and oral microbiome composition	Enzyme lozenge significantly reduced plaque accumulation and slowed the increase in salivary bacterial species	Small sample size, short intervention period, experimental

(*Continued*)

Table 1 (*Continued*)

No.	Author (year)	Study type	Population (*n*)	Intervention	Comparator	Outcome(s)	Key findings	Quality/bias notes
5	Porangaba et al, 2024	RCT	Head and neck cancer survivors postradiotherapy, $n = 40$	Salivary substitute spray containing an enzymatic system	Placebo spray	Xerostomia severity, unstimulated salivary flow, stimulated salivary flow, quality of life	Enzymatic spray showed improvement in xerostomia complaints	Small sample size, short follow-up, placebo crossover design, limited statistical significance
6	Marimuthu et al, 2020	Prospective RCT	Nasopharyngeal cancer survivors with xerostomia, $n = 94$	Immunologically active saliva substitute mouthwash	Non-immunologically active mouthwash	Summated xerostomia Inventory, unstimulated whole saliva flow rate	IASS mouthwash significantly reduced subjective xerostomia scores and improved salivary flow	Short follow-up; single-center study
7	Hoffstedt et al, 2023	Randomized double-blind placebo-controlled pilot trial	Adolescents with fixed orthodontic appliances, $n = 28$ completed	Enzyme mouthwash	Placebo without enzyme	Orthodontic Plaque Index; salivary microbiome composition	Significant reduction in plaque accumulation in the enzyme mouthwash group; no significant changes in microbiome composition	Small sample size, short intervention period, pilot trial
8	Tjahajawati et al, 2025	Clinical observational	Pre- and postmenopausal women	N/A	Pre- vs postmenopausal comparison	Oral health status, salivary pH, salivary flow rate, phosphate levels, dietary phosphorus intake	Postmenopausal women showed alterations in salivary parameters and oral conditions	Observational design; no intervention; potential confounding factors
9	Mahgoub et al, 2025	In vitro experimental study	Not applicable (laboratory study)	Lysozyme–dequalinium encapsulated in Zn–Fe LDH–chia seed matrix	Free lysozyme / non-encapsulated formulation	Antimicrobial activity against oral pathogens; drug delivery efficiency	Encapsulation enhanced the antimicrobial activity and stability of lysozyme,	In vitro study; no clinical validation
10	Graziani et al, 2024	RCT	Periodontitis patients undergoing periodontal surgery ($n = 33$)	CHX + ADS + hyaluronic acid (HA) mouthwash; CHX + ADS mouthwash	CHX + ADS + hyaluronic acid mouthwash; CHX + ADS mouthwash	No mouthwash (control)	CHX-ADS mouth rinses improved early wound healing and reduced plaque and gingival inflammation	Small sample size; short follow-up
11	Tonguc Altin et al, 2021	In vitro experimental study	Not applicable (cell culture and laboratory model)	Saliva substitutes containing lysozyme or lactoferrin	Human saliva and saliva substitute without added enzymes	Antibacterial activity against *Streptococcus mutans*, bacterial adhesion on hydroxyapatite discs, and fibroblast wound-healing assay	Lysozyme- and lactoferrin-containing saliva substitutes significantly inhibited *S. mutans* growth and adhesion and enhanced fibroblast wound closure in vitro	Laboratory study; no clinical validation
12	Takemura et al, 2023	Double-blind crossover clinical trial	Xerostomia patients ($n = 37$	Oral rinse containing sodium hyaluronate and functional ingredients (GUM HYDRAL)	Placebo rinse and Biotene oral rinse (active control)	Xerostomia symptoms, Oral Health Impact Profile-14, unstimulated salivary flow rate, Revised Oral Assessment Guide	Improved subjective xerostomia symptoms and salivary flow rate;	Moderate sample size; short treatment period; crossover design
13	Alqutub et al, 2023	RCT	Peri-implant mucositis patients ($n = 60$)	Herbal mouthwash and chlorhexidine mouthwash after mechanical debridement	Saline rinse	Inflammatory parameters, plaque levels, peri-implant clinical indices	Both chlorhexidine and herbal mouthwashes significantly reduced inflammation and improved peri-implant clinical parameters compared with saline	Moderate sample; short follow-up; adjunct to mechanical debridement
14	Welk et al, 2010	In vitro experimental study	Not applicable (laboratory suspension test)	Lactoperoxidase with thiocyanate–hydrogen peroxide antimicrobial system	Thiocyanate–hydrogen peroxide system without lactoperoxidase	Antimicrobial activity against oral microorganisms in quantitative suspension test	Addition of lactoperoxidase enhanced antimicrobial effectiveness of the thiocyanate–hydrogen peroxide system	Laboratory study; no clinical validation
15	Carvalho et al, 2024	In vivo animal study	Mice, $n = 48$	Oral chlorhexidine + Western diet	Western diet only	Oral microbiome, gut microbiome, weight gain, body fat, liver steatosis, plasma insulin, nutrient absorption	Chlorhexidine reduced oral bacterial load, decreased gut microbial diversity; reduced weight gain, body fat, steatosis, plasma insulin; increased colon triglycerides and proteins indicating reduced nutrient absorption	Animal model; short-term intervention; may not fully translate to humans; mechanistic pathways not completely elucidated

- **Lactoferrin:** Sequesters iron, disrupts bacterial membranes, and modulates host immune responses, contributing to antimicrobial and anti-inflammatory effects.
- **Glucose oxidase:** Generates hydrogen peroxide to fuel LPO activity, enhancing the overall antimicrobial potential.

Clinical Outcomes

Representative studies summarized in ►**Table 1** reported that enzyme-based mouthwashes:

- Reduced oral biofilm and plaque accumulation.
- Supported oral wound healing by mitigating inflammation, promoting epithelialization, and accelerating tissue repair.
- Improved symptoms of xerostomia and enhanced oral comfort, particularly in patients with reduced salivary flow.
- Maintained a balanced oral microbiome more effectively than broad-spectrum antiseptic mouthwashes.

Safety and Tolerability

The included studies indicated a favorable safety profile for enzyme-based mouthwashes. Minimal adverse effects were reported, including a low incidence of mucosal irritation, cytotoxicity, or taste alteration. The products were well-tolerated for short-term use, and high patient compliance was consistently observed.

Comparative Observations

Compared with conventional mouthwashes, such as CHX or alcohol-based formulations, enzyme-containing rinses demonstrated similar antimicrobial efficacy while offering:

- Improved biocompatibility.
- Reduced cytotoxicity.
- Better patient acceptability and comfort.

Discussion

Mechanisms of Action of Enzyme-Based Mouthwashes

Lactoperoxidase System and Antimicrobial Activity

The LPO system plays an important role in the defense mechanism of the oral cavity. It catalyzes the oxidation of thiocyanate ions (SCN^-) by hydrogen peroxide (H_2O_2) to form hypothiocyanite ($OSCN^-$), a potent antimicrobial agent. This reaction selectively targets microbial thiol groups, inhibiting bacterial metabolism and compromising cell wall integrity, thereby reducing microbial load and plaque formation, and so LPO-based mouthwashes are efficient in reducing dental biofilm accumulation.[2,16,17]

Lysozyme and Bacterial Cell Wall Hydrolysis

Lysozyme exerts antimicrobial activity by hydrolyzing the β-1,4-glycosidic bonds connecting N-acetylglucosamine and N-acetylmuramic acid in bacterial cell walls, causing bacterial cell death. When incorporated into mouthwash formulations, lysozyme increases the efficacy of their antibacterial action and contributes to lowering the number of harmful microbes in the mouth, thus preventing gingivitis and periodontitis. Recent studies have emphasized the antimicrobial potential of lysozyme, highlighting its role in oral hygiene products.[18,19]

Lactoferrin and Iron Sequestration

Lactoferrin is an iron-binding glycoprotein that shows antimicrobial activity by sequestration of free iron, which is an important nutrient for bacterial growth. It binds to bacterial cell membranes, disrupting membrane integrity and modulating host immune responses. This combination contributes to the inhibition of microbial activity and the modulation of inflammation in the oral cavity and thus keeps the population of microorganisms in control. Recent studies have shown that the efficacy of lactoferrin-containing mouthwashes in reducing oral microbial load and inflammation.[20,21]

Glucose Oxidase and Hydrogen Peroxide Generation

Glucose oxidase helps in the oxidation of glucose to gluconic acid, producing H_2O_2 (hydrogen peroxide) as a byproduct. H_2O_2 serves as a substrate for the LPO system, increasing the production of $OSCN^-$. This cascade of enzymes improves the antimicrobial efficacy of mouthwashes by providing a continuous source of H_2O_2. This enzymatic cascade enhances antimicrobial efficacy and limits oral microbial proliferation.[22]

A schematic representation of the enzymatic interactions and downstream effects of the enzyme-based mouthwash is shown in ►**Fig. 1**.

Clinical Implications and Formulation Considerations

The clinical effectiveness of enzyme-based mouthwashes depends on several factors, including the stability of the enzymes, the availability of their required substrates, the pH of the mouth, and the formulation's ability to remain active on oral surfaces. Appropriate storage conditions are required to maintain enzyme stability and activity. Patient compliance and the taste of the mouthwash also play important roles in the overall efficacy of these products. Therefore, recent emphasis has been given to the importance of formulation considerations in the clinical effectiveness of enzyme-based mouthwashes.[22,23]

The effect of enzyme-based mouthwash on oral wound healing is illustrated in ►**Fig. 2**. Following tissue injury, application of the mouthwash reduces microbial load, mitigates inflammation, and promotes tissue repair, which together accelerate epithelialization, reduce pain and swelling, and restore mucosal integrity.

From a materials science perspective, maintaining enzyme stability within oral formulations remains a critical challenge. Enzymes are sensitive biomolecules that may lose catalytic activity when exposed to temperature fluctuations, oxidative conditions, or unfavorable pH environments. Recent advances in formulation science have explored protective delivery systems such as polymeric carriers, liposomal encapsulation, and nanostructured matrices to improve enzyme stability and prolong shelf life. Encapsulation techniques can protect enzymes from degradation while

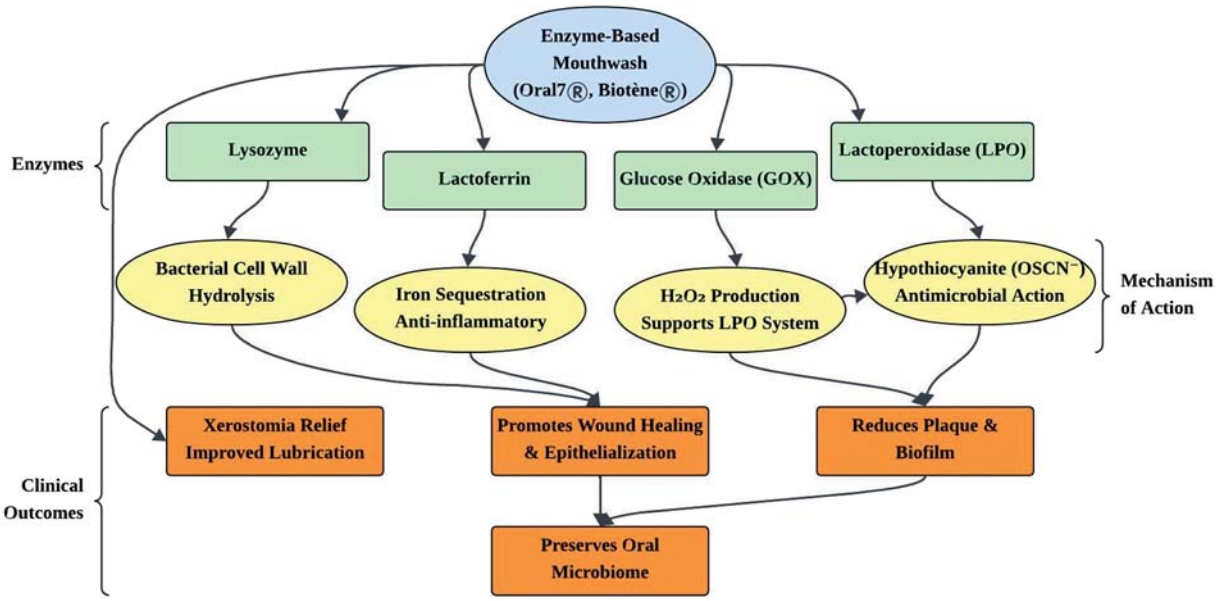

Fig. 1 Mechanism of action of an enzyme-based mouthwash. Schematic illustration of the mechanism of action of an enzyme-based mouthwash containing LPO, lysozyme, lactoferrin, and glucose oxidase. These enzymes enhance the natural salivary defense system through complementary pathways. Glucose oxidase generates H_2O_2, which activates the LPO system to produce $OSCN^-$ with broad antimicrobial activity. Lysozyme hydrolyzes bacterial cell walls, while lactoferrin sequesters iron, limiting microbial growth and contributing to anti-inflammatory effects. Together, these pathways reduce plaque and biofilm formation, promote wound healing and epithelial regeneration, relieve xerostomia symptoms, and help maintain a balanced oral microbiome.

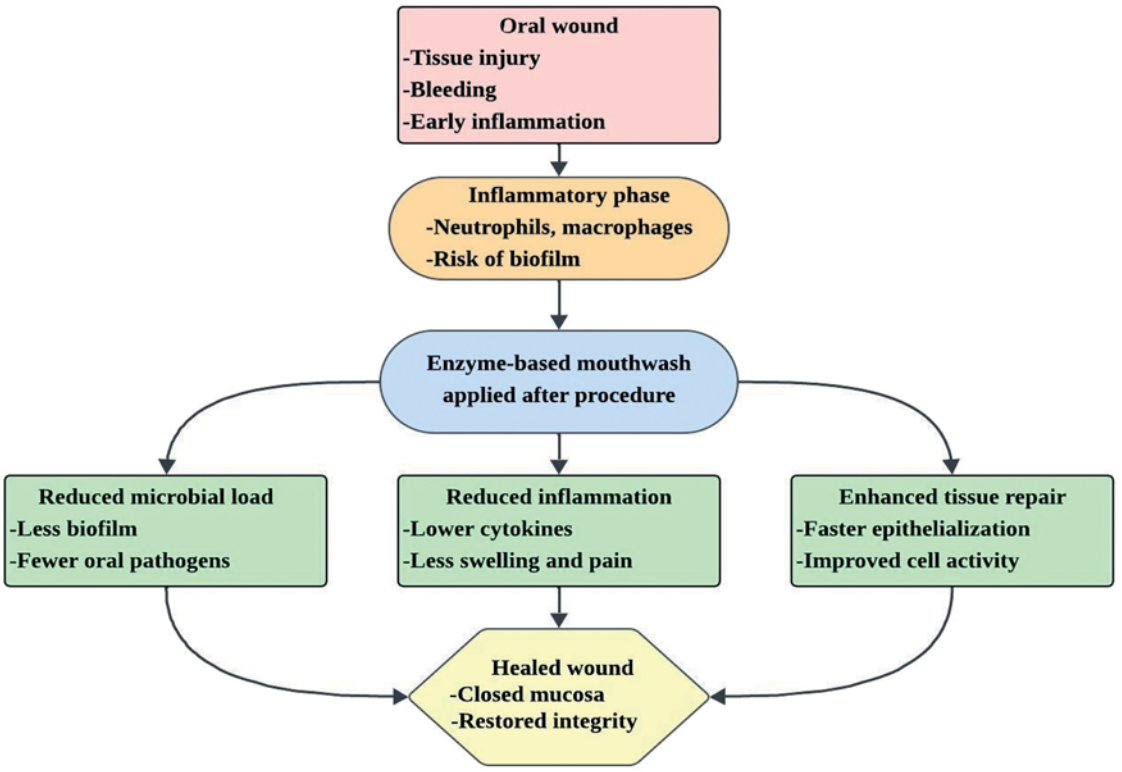

Fig. 2 Oral wound healing enhanced by enzyme-based mouthwash. Representation of oral wound healing with the application of enzyme-based mouthwash. After tissue injury, the wound enters an inflammatory phase with potential microbial colonization. The mouthwash reduces microbial load, alleviates inflammation, and promotes tissue repair, leading to accelerated epithelialization, reduced pain and swelling, and complete restoration of mucosal integrity.

Table 2 Components of enzyme-based mouthwashes and their antimicrobial mechanisms against oral pathogens

Enzyme	Substrate/ co-factor	Primary antimicrobial target	Mechanism of action	Clinical relevance/outcome	Key references
Lactoperoxidase	$SCN^- + H_2O_2$	*Streptococcus mutans*, *Candida albicans*	Catalyzes the oxidation of SCN^- to $OSCN^-$, which disrupts microbial metabolism and membrane function	Reduces dental plaque, promotes wound healing, and inhibits opportunistic pathogens	2,38
Lysozyme	N-acetylmuramic acid residues in peptidoglycan	Gram-positive bacteria (*Streptococcus*, *Staphylococcus*)	Hydrolyzes β-1,4-glycosidic bonds in bacterial cell walls peptidoglycan	Causes bacterial lysis; reduces bacterial load and inflammation	26,35
Lactoferrin	Iron (Fe^{3+}) binding	*Porphyromonas gingivalis*, *Candida albicans*	Sequesters iron required for microbial growth; disrupts bacterial adhesion and biofilm formation	Inhibits periodontopathogens; reduces inflammation and mucosal irritation	36,37
Glucose oxidase	Glucose → H_2O_2	Broad-spectrum bacterial inhibition	Generates low levels of H_2O_2 that activate LPO system	Enhances antimicrobial synergy and boosts oxidative defense	17,38
Amyloglucosidase	Starch/dextrin substrates	Indirect (via glucose release)	Produces glucose for the glucose oxidase system, enabling continuous $OSCN^-$ generation	Sustains antimicrobial activity and stabilizes formulation efficacy	4,7

enabling controlled release within the oral cavity. In addition, lyophilization and advanced stabilizing excipients are being investigated to maintain enzymatic activity during storage and transportation. These technologies may enhance the clinical reliability of enzyme-based mouthwashes and represent an important direction for future product development.

►**Table 2** summarizes the mechanisms of action of various natural enzymes commonly found in enzyme-based mouthwashes.

Applications for Oral Wound Healing

Natural enzyme-containing mouthwashes have shown potential in oral wound healing after dental procedures such as tooth extractions, periodontal surgeries, or trauma. LPO, lysozyme, and lactoferrin are natural enzymes that play important roles in reducing microbial load, modulating inflammation, and supporting tissue repair, supporting their potential role as adjuncts in routine clinical practice.

Antimicrobial Action

The LPO system, together with lysozyme and lactoferrin, helps with the antimicrobial properties of natural enzyme-containing mouthwashes. These enzymes inhibit bacterial growth and biofilm formation, reducing the risk of postsurgical infections and promoting a supportive environment for healing, as microorganisms delay healing by preventing the wound from progressing from the inflammatory phase to the proliferative phase. Mouthwashes containing these enzymes can effectively reduce the number of oral pathogens, which has critical importance in wound healing.[2,8]

Modulation of Inflammation

Inflammation is a natural response to tissue injury, but excessive or prolonged inflammation can delay healing. Lactoferrin, in particular, is notable for its anti-inflammatory properties, which help control the inflammatory response at wound sites, thus promoting healing. By modulating inflammation, enzyme-based mouthwashes can facilitate a more favorable environment for healing and reduce discomfort associated with oral wounds.[24]

Tissue Repair and Epithelialization

The enzymes in these mouthwashes also support tissue repair mechanisms. The ability of lysozyme to break down bacterial cell walls reduces the microbial load, aiding in the clearance of cellular debris and facilitating better tissue regeneration. The presence of these enzymes helps in epithelialization, which leads to faster healing and more efficient wound closure.[2,25]

Clinical Evidence

Recent clinical trials provide support for the efficacy of enzyme-based mouthwashes in oral wound healing after injury or surgery for infection control, as increasing focus is being given to the benefits of these mouthwashes. A 2024 RCT showed that a natural enzyme mouthwash improved signs and symptoms of xerostomia, a common condition

following oral surgery. This indicates its potential benefit in postoperative care. Another study highlighted the antibacterial effects of saliva substitutes containing lysozyme and lactoferrin, which inhibited the growth of Streptococcus mutans, a common bacterium associated with oral infections.[7,26]

Although the available clinical trials provide encouraging results, many studies are limited by relatively small sample sizes, short follow-up periods, and heterogeneity in outcome measurements. Some investigations are pilot studies or exploratory trials, which may introduce methodological bias and limit the generalizability of findings. Variability in enzyme formulations, dosage regimens, and patient populations further complicates direct comparisons across studies. Therefore, the current evidence should be interpreted cautiously until larger multicenter RCTs with standardized protocols become available.

Role in Xerostomia Management

Enzyme-based mouthwashes act as saliva substitutes by supplying protective salivary proteins and enzymes (e.g., LPO, lysozyme, and lactoferrin), which help restore lubrication and the biochemical defense functions of natural saliva.[8,25]

These formulations have been shown to reduce subjective dry-mouth symptoms and can improve oral comfort and swallowing in patients with chronic xerostomia, as shown in recent randomized trials of natural-enzyme mouthwashes.[7]

In head-and-neck radiotherapy survivors and other patients with severe salivary hypofunction, saliva substitutes containing enzyme systems or moisturizing agents have demonstrated short-term improvements in patient-reported xerostomia scores and quality-of-life measures compared with placebo or water.[27,28]

Comparative studies of commercially available saliva substitutes (e.g., Biotène/Oral7) report clinically meaningful relief of symptoms and improved oral comfort with repeated use; evidence comes from double-blind crossover trials and manufacturer-supported clinical evaluations.[29,30]

Enzyme-containing products may also support mucosal integrity by reducing opportunistic colonization (e.g., lowering *Streptococcus mutans* counts) and by modulating local inflammation—mechanisms that are biologically plausible and have preliminary in vitro and clinical support.[24,26]

Clinical efficacy is formulation-dependent and influenced by enzyme stability, substrate availability (SCN^-/H_2O_2), pH, and product substantivity; inconsistent formulations and short trial durations explain some variability across studies and underscore the need for larger, longer RCTs.[31]

Pharmacological stimulation of salivary secretion remains an established therapeutic strategy for the management of xerostomia. Agents such as Pilocarpine, a muscarinic receptor agonist, stimulate residual salivary gland function and are widely used in patients with radiation-induced xerostomia and Sjögren's syndrome. Clinical studies have demonstrated that pilocarpine can significantly increase salivary flow rates and improve subjective symptoms of oral dryness in affected patients.[32,33] In addition to pharmacological stimulants, emerging biomaterial-based therapies such as Nano-Bio fusion gingival gel have been explored to improve mucosal hydration and support tissue repair through advanced delivery systems. Although these approaches act through mechanisms different from enzyme-based mouthwashes, they illustrate the expanding range of therapeutic strategies aimed at restoring salivary function and improving oral comfort in xerostomic patients.

Infection Control and Oral Microbiome

Enzyme-based mouthwashes enhance innate salivary defense mechanisms by incorporating components such as LPO, lysozyme, and lactoferrin. These agents work synergistically to inhibit the growth of cariogenic and periodontopathogenic microorganisms, particularly Streptococcus mutans and Candida albicans, thereby reducing early biofilm formation and microbial adhesion.[2,26] Saliva also contains multiple biomarkers and immune mediators that contribute to host defense and periodontal health.[34]

Unlike broad-spectrum antiseptics such as CHX, enzyme-based formulations act more selectively and preserve the natural balance of the oral microbiome. They gently support the natural balance of bacteria in the mouth, helping to preserve the good microbes that keep the oral environment healthy and stable.[35,36]

Enzyme-based mouthwashes can lower harmful biofilm buildup while allowing beneficial bacteria to survive. This targeted activity controls infection while preserving overall microbial diversity, reducing the likelihood of antimicrobial resistance that can occur with broad-spectrum antiseptics.[17]

Disturbances in the oral microbiome caused by traditional antiseptic mouthwashes have been linked to alterations in salivary nitrate metabolism and even systemic microbial imbalances. This emphasizes the advantage of enzyme-based formulations that maintain microbial homeostasis.[2,37] Factors such as enzyme concentration, substrate availability (SCN^- and H_2O_2), and formulation stability alter the antimicrobial properties of the enzyme systems. Further long-term research is required to confirm the microbiome-sparing benefits and resistance outcomes associated with these products.[35,38]

Safety and Clinical Considerations

Enzyme-based mouthwashes are well-tolerated and have a favorable safety profile compared with conventional antiseptic mouthwashes like CHX. Their natural composition allows for effective antimicrobial activity without significant cytotoxicity or mucosal irritation.[38] Unlike CHX, which causes tooth staining, taste alteration, and mucosal desquamation with prolonged use, enzyme-containing formulations rarely produce such adverse effects.[7] Clinical evaluations of LPO and lysozyme-based mouthwashes have reported high patient compliance and minimal reports of burning sensations or tissue discomfort, indicating their suitability for long-term use in individuals with sensitive or xerostomic oral mucosa.[2] Additionally, they demonstrate good biocompatibility with gingival fibroblasts and epithelial cells, suggesting low cytotoxic potential in comparison with alcohol-based or CHX

Table 3 Comparison of enzyme-based and conventional mouthwashes in antimicrobial activity and microbiome preservation

Feature	Mechanistic basis		Clinical considerations	
	Enzyme-based mouthwashes	Conventional mouthwashes*	Enzyme-based mouthwashes	Conventional mouthwashes[a]
Antimicrobial action	Selective antimicrobial pathways; enzyme cascade produces OSCN; interferes with early biofilm	Broad, non-selective bactericidal effects (CHX membrane disruption; PVP-I oxidation; CPC surfactant action)	Preserves beneficial microbiota; low risk of dysbiosis	Effective but may cause dysbiosis; microbiome disruption
Biofilm effects	Targets early adhesion and initial colonizers	Disrupts mature biofilm (CHX) or oxidizes microbial structures (PVP-I)	Suitable for long-term use; minimal staining	Staining; taste alteration; mucosal irritation
Inflammation modulation	Lactoferrin reduces inflammation; gentle on the mucosa	No anti-inflammatory effect; may irritate tissues	Better mucosal comfort; less burning sensation	Possible burning, ulceration, and mucosal dryness
Resistance potential	Low—selective action does not drive resistance	Higher—broad-spectrum action increases selective pressure	Preferred for long-term or repeated use	Recommended for short-term therapeutic use
Safety profile	Very low cytotoxicity; enzyme levels mimic natural saliva	Higher cytotoxicity; potential soft tissue damage (CHX/PVP-I)	Safe for xerostomic or sensitive patients	Caution in sensitive mucosa or prolonged use
Evidence base	Growing evidence supports microbiome-sparing healing	Strong evidence; traditional gold standard for infection control	Emerging data support wound-healing benefits	Well-established but with notable side effects

[a]A conventional mouthwash includes nonenzyme formulations such as CHX, povidone-iodine (PVP-)I, and cetylpyridinium chloride (CPC), with CHX being the most commonly studied.

mouthwashes.[39] Because these formulations maintain physiological pH and lack alcohol, they are also suitable for postoperative care and for patients with mucosal sensitivity. They are therefore suitable for patients undergoing radiotherapy or those with Sjögren's syndrome.[37] Current evidence suggests that enzyme-containing mouthwashes are a safe and patient-friendly adjunct to oral hygiene, with a lower incidence of side effects and better long-term acceptability than conventional antiseptics.[35]

Comparison with Conventional Mouthwashes

Conventional mouthwashes like CHX, which is known as the gold standard, and other alcohol-based formulations are widely used for oral hygiene because of their antimicrobial properties. However, long-term use of CHX is associated with different side effects. Alcohol-based mouthwashes can cause burning sensations, mucosal irritation, and dryness, which may limit patient compliance.[5,40,41]

In contrast, enzyme-containing or natural mouthwashes, such as Oral7, demonstrate high biocompatibility with oral tissues, including gingival fibroblasts and epithelial cells, because of low cytotoxic potential.[39] Clinical evaluations report minimal reports of discomfort or burning sensation, indicating suitability for long-term use, particularly in patients with sensitive or xerostomic oral mucosa.[42] Moreover, these formulations replicate natural salivary defenses, offering antimicrobial and wound-healing benefits without contributing to microbial resistance and supporting oral health with improved patient compliance.

►**Table 3** summarizes mechanistic differences, microbiome impacts, and clinical considerations, highlighting the potential advantages of enzyme-based formulations for long-term use and microbiome preservation.

Challenges and Limitations of Enzyme-Based Mouthwashes

Limited Antimicrobial Spectrum

Enzyme-based mouthwashes have shown promising results, but several limitations still restrict their clinical application. One of the major challenges is their limited antimicrobial range, which is not broad-spectrum. Unlike broad-spectrum agents such as CHX, enzyme formulations tend to act on specific bacterial targets, which may reduce their overall plaque control potential and reduce efficacy in reducing overall oral microorganisms.[43]

Variability in Enzyme Activity

Another issue is the inconsistency in enzyme stability and activity among different commercial products. Enzymes are highly sensitive to storage temperature, pH, and formulation conditions, so their efficacy can deteriorate over time or under improper storage, leading to variable clinical results. Standardized storage is to be followed to maintain the overall quality of these mouthwashes.[44]

Short-term Clinical Trials

Most existing research and studies on enzyme-based mouthwashes have also been short-term, often lasting less than a week, which makes it difficult to assess their safety and therapeutic effectiveness. More extensive, long-term clinical trials are required to confirm their benefits for everyday use as a part of the maintenance of oral hygiene, and also after injury or surgical procedures.[45]

Potential for Oral Microbiome Disruption

Enzyme mouthwashes are designed to support the natural oral microbiome; they could still disturb microbial balance if used excessively or in combination with other antiseptic agents. Some studies suggest that even mild formulations may alter microbial diversity, which could affect the stability of the oral environment.[35]

Regulatory and Standardization Challenges

There are ongoing regulatory and standardization issues. Because these products are considered cosmeceutical in some regions and medical devices in others, manufacturers face inconsistent requirements for testing and approval. Developing harmonized quality control protocols and standardized formulations would help improve clinical reliability.[46]

Future Directions

The development of enzyme-based mouthwashes represents a combination of biotechnology and preventive dentistry. Optimization of enzyme kinetics and substrate specificity through bioengineering techniques, producing formulations that target specific pathogens while preserving beneficial microorganisms, should be focused on in future investigations. Advances in protein stabilization technology, including lyophilization and nano-carrier encapsulation, can further extend product shelf life and maintain enzyme integrity under variable storage conditions. Another direction is the integration of omics-based approaches—such as metagenomics and metabolomics—to evaluate the systemic and microbial effects of enzyme-based rinses beyond conventional culture studies. This would enable a more precise understanding of how these formulations affect host–microbe interactions, inflammatory responses, and healing dynamics following dental procedures.

Studies with long-term follow-up periods are important to assess efficacy, outcomes, and potential synergistic effects when combined with standard oral hygiene regimens. Including people with systemic conditions such as diabetes or xerostomia in the studies could provide insights into therapeutic adaptability. Sustainability and regulation should guide the next generation of mouthwashes. Biodegradable carriers, eco-friendly packaging, and harmonized quality standards could ensure that future enzyme-based mouthwashes align with the principles of the Global One Health framework to prevent antimicrobial resistance.

Limitations

The included studies showed heterogeneity in study design, enzyme formulations, and outcome assessment methods. In addition, many studies involved short-term follow-up periods, which limit the ability to assess long-term clinical outcomes.

Conclusion

Enzyme-based mouthwashes represent a promising evolution in oral hygiene as a biocompatible and targeted alternative to conventional antiseptic mouthrinses. Unlike CHX and alcohol-based formulations, enzyme-containing products follow the natural defense mechanisms of saliva, providing antimicrobial activity, supporting wound healing, and demonstrating high patient tolerability, particularly in sensitive or xerostomic populations, which is beneficial to oral health. Despite these advantages, several challenges remain, like variability in enzyme stability, limited antimicrobial spectrum, and a lack of long-term, extensive clinical trials. Addressing these limitations is necessary for broader clinical adoption and acceptance. Studies in the future should prioritize improving enzyme kinetics, identifying synergistic combinations with natural bioactive compounds, and applying advanced techniques of analysis to better understand their effects on the oral microbiome and overall oral health and environment. Patient compliance remains a critical determinant of clinical effectiveness as the efficacy of a mouthwash greatly depends on it.

By bridging the gap between biochemistry and preventive dentistry, enzyme-based mouthwashes have the potential to significantly alter oral care toward safer, patient-centered, and microbiome-conscious approaches. Continued research and long-term studies with clinical evaluation are essential to unlock their full potential and establish evidence-based guidelines for their routine use and maintenance of oral hygiene. Ultimately, the success of these formulations depends not only on biochemical efficacy but also on patient comfort and real-world adherence.

Conflict of Interest

None declared.

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