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A Supplement to
Compendium
of Continuing Education in Dentistry

September 2025
Volume 46 • Supplement 2

Beyond Brushing

The Case for an Alcohol-Free Antimicrobial Mouthwash for Superior Oral Health

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Support for the preparation and publication of this special issue was provided by Meghan A. Berryman, PhD, and Luciana Rinaudi-Marron, PhD, who served as scientific advisors from the Colgate-Palmolive Company.

Compendium of Continuing Education in Dentistry® and *Beyond Brushing* are published by Conexiant.

Printed in the U.S.A.

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Conexiant
400 N. Ashley Dr., Ste. 2600
Tampa, FL 33602

Bridging Evidence and Practice: The Role of Daily Cetylpyridinium Chloride Mouthrinses in Disease Prevention

Cristina Cunha Villar, DDS, MSc, PhD

Oral diseases affect nearly 3.7 billion people worldwide,[1] with conditions such as untreated dental caries and periodontal diseases impacting more than 2 billion and 1 billion individuals, respectively, thus imposing significant functional, social, and economic burdens.[1-3] Despite the dental profession's progress in understanding the etiopathogenesis of dental caries and periodontal diseases, the global burden of these disorders remains high, with case numbers increasing due to population growth and aging.[1,2,4] Unfortunately, this trend is expected to continue. Projections for the United Kingdom, for example, suggest that by 2050, over 60% of the adult population aged 60 or older will have untreated dental caries, and more than half will be affected by periodontal diseases.[4] The expected increase in these conditions poses a threat to dentists' ability to provide curative care to all affected patients, highlighting the need to focus on actions toward health promotion and disease prevention.

Cristina Cunha Villar, DDS, MSc, PhD

A Complex Interplay

The prevalence of dental caries and periodontal diseases reflects a complex interplay among biological, behavioral, and social determinants. Biologically, supragingival biofilm is the main etiological factor for both dental caries and periodontal diseases.[5] Contrary to earlier pathogen-specific models, current evidence indicates that the transition from oral health to dental caries or periodontal disease involves dysbiotic changes in the oral microbiome and overgrowth of commensal species with pathogenic potential, as opposed to the acquisition of exogenous pathogens.[5] This knowledge is key for health promotion and disease prevention, as it implies that regular supragingival biofilm control is paramount to prevent the maturation of supragingival biofilm, a condition required for microorganisms with pathogenic potential to overgrow and switch from a commensal to a pathogenic state.

Disease susceptibility also varies among patients. For instance, the classical "Experimental Gingivitis in Man" study demonstrated that 100% of the participants developed gingivitis in response to 21 days of undisturbed supragingival biofilm accumulation.[6] However, recent studies reveal individual variations in clinical response to supragingival biofilm accumulation, with some patients being hyperresponsive and others being hyporesponsive.[7] In addition to developing more intense clinical signs of gingival inflammation, hyperresponsive patients present higher supragingival biofilm formation rates and increased levels of proinflammatory mediators.[7] Adding to the scenario of individual variations in disease susceptibility, some current lifestyle behaviors, such as high sugar consumption, frequent snacking, and tobacco use, may further predispose to the development of dental caries and periodontal diseases.[8]

Toothbrushing remains the "gold standard" daily practice for supragingival biofilm control. However, even under the ideal and strict conditions of controlled clinical trials, toothbrushing alone removes on average only 42% of the supragingival biofilm and has limited effects on reaching interproximal spaces.[9,10] These limitations are further amplified by aging, disability, and low oral health literacy, which may compromise patient compliance.[11,12] Given these challenges, chemical supragingival biofilm control has emerged as an adjunctive approach to toothbrushing to enhance supragingival biofilm control, especially in patients with inadequate oral hygiene or who are susceptible to periodontal diseases.

Chemical supragingival biofilm control is primarily achieved through the use of antimicrobial mouthwashes or dentifrices. Although both formulations are effective in reducing supragingival biofilm and gingival inflammation, antimicrobial mouthwashes have demonstrated superior results over antimicrobial dentifrices in reducing supragingival biofilm and gingivitis,[13] making them the preferred option for susceptible patients. Moreover,

mouthwashes can access oral niches (eg, tongue, buccal mucosa, palate, and tonsils) that could serve as microbial reservoirs.[13] Among antimicrobial mouthwashes, chlorhexidine-based rinses are considered the "gold standard" for controlling supragingival biofilm, either as monotherapy when patients are unable to perform mechanical oral hygiene or as an adjunct to toothbrushing.[14] The duration of chlorhexidine use, however, is limited by its associated side-effects.[15] Therefore, alternative mouthwash formulations have been developed for daily use.

Reducing Biofilm Formation

Cetylpyridinium chloride (CPC), a monocationic quaternary ammonium salt, was first described in 1939.[16] Due to its positive charge, CPC is attracted to and binds nonspecifically to negatively charged phosphate groups on microbial cell membranes, disrupting the membrane physical integrity. In turn, this disruption results in increased cellular permeability and the leakage of low-weight microbial intracellular components, causing a reduction in metabolic activity—a bacteriostatic effect that lasts up to 5 hours.[17] At high concentrations, CPC has a bactericidal effect, causing microbial cell lysis, full extravasation of intracellular components, and microbial cell death. In addition to its disruptive effect on microbial cell membranes, CPC also reduces the adherence of early microbial colonizers to the salivary pellicle, an important early event in the process of biofilm formation,[18] thus contributing to a further reduction in biofilm formation.

CPC's broad-spectrum antimicrobial activity, wide margin of safety for topical use, and high solubility in both aqueous and alcohol solutions[16] have led to the development of aqueous-based CPC mouthwashes for daily use. Since the first study demonstrating the therapeutic effects of CPC mouthwashes was published in 1974, more than 140 clinical studies published in peer-reviewed journals indexed in PubMed have explored CPC effects in reducing the levels of intraoral pathogenic species, controlling supragingival biofilm, gingivitis, peri-implant mucositis, halitosis, and dentin hypersensitivity, preventing drug-influenced gingival enlargement, and promoting the disinfection of root canals, implant surfaces, and toothbrushes. Clinical studies on the use of CPC have also investigated its effects in limiting the generation of contaminated aerosols and symptoms of upper tract infections and its impact on patient safety and adherence profiles.

The impact of CPC in controlling supragingival biofilm and gingivitis has been extensively evaluated. Its effects on these two conditions, however, have shown substantial heterogeneity, with some studies reporting significant clinical improvements and others reporting minimal or no benefits.[13] A possible explanation for these discrepancies can be attributed to differences in CPC concentrations used across different studies. In support of this hypothesis, a systematic review and meta-analysis revealed that high-concentration CPC mouthwashes (≥0.07%) demonstrated superior outcomes in reducing supragingival biofilm and gingival inflammation compared to low-concentration CPC mouthwashes (≤0.05%).[13] Based on this evidence, CPC prescriptions must be restricted to high-concentration formulations.

In addition to CPC concentration, CPC bioavailability also impacts its antimicrobial and clinical efficacy. CPC exists in two major forms depending on the excipients in the formulation: free CPC (fCPC), which has the higher antimicrobial activity, and the less active micellized CPC (mCPC). Interplay with common mouthwash constituents, such as preservatives and certain block copolymer surfactants, can reduce the availability of fCPC molecules, limiting the antimicrobial effect of CPC mouthwashes.[19] While some surfactants, such as Cremophor, favor the formation of mCPC, others, like P407, allow the maintenance of CPC in its free form state.[19] Therefore, in addition to having a high CPC concentration, CPC mouthwash formulations should limit the incorporation of constituents known to cause mycelial formation. The CPC formulations discussed within this special issue have been designed using surfactant species at levels that allow CPC to remain in its fCPC form, thus preserving CPC antimicrobial activity and clinical efficacy.

Incorporating Zinc Lactate

The incorporation of zinc lactate into high-concentration CPC mouthwashes brought several improvements in the original CPC formulations. Zinc is an essential antioxidant and antimicrobial agent, often added as zinc lactate, due to its high and stable solubility in aqueous solution, thus avoiding the need for ethanol in the formulation. Direct comparison studies have demonstrated that mouthwashes containing 0.075% CPC and 0.28% zinc lactate have more pronounced effects on supragingival biofilm and gingivitis control, as compared to rinses containing only CPC or alcohol-free essential oil (EO) rinses.[20-22]

Notably, the addition of zinc lactate into CPC-containing mouthwash has enhanced the formulation's antiplaque and antigingivitis effects to levels equivalent to those achieved with the daily use of EO rinses containing 21.6% ethanol.[23] This finding is highly significant as EO rinses with ethanol have long been considered the most efficient daily use formulation for the control of supragingival plaque and gingivitis, with clinical results often superior to those obtained by the use of rinses containing CPC only. Thus, there is now an enhanced CPC formula containing zinc lactate that performs like alcohol-based EO rinses, but without causing the typical burning sensation experienced with the use of alcohol-based EO rinses, which may compromise patient adherence. Unlike alcohol-based EO rinses that are contraindicated for children, individuals with alcohol dependency, and those with soft-tissue lesions, CPC-zinc lactate rinses are considered a safer option for routine use. They are associated with low rates of adverse events[15] and promote shifts in the oral microbiome that favor the restoration of a microbial balance without inducing microbial resistance.[24,25] Also, in the context of infection control, both CPC-zinc lactate and chlorhexidine pre-procedural rinses reduce contaminated aerosols by approximately 70% during aerosol-generating procedures.[26]

CPC-zinc lactate rinses are also a valuable option for patients with halitosis.[27] In addition to its antimicrobial effects, zinc's organoleptic properties allow it to oxidize thiol groups in precursors

of volatile sulfur compounds converting them into non-volatile odorless compounds. Considering that halitosis has a high prevalence, affecting approximately 30% of the adult population,[28] and its negative impact on oral-health-related quality of life,[29] dental providers should adopt comprehensive treatment approaches that both address the intraoral underlying causes of halitosis and support patients' overall well-being in their daily life. In this scenario, CPC rinses containing zinc lactate can serve as a valuable adjunctive therapy.

Effectiveness of CPC and Zinc Lactate

This special issue highlights the latest findings on the effectiveness of CPC and zinc lactate mouthwash in controlling supragingival biofilm, gingival inflammation, and oral malodor, while also discussing its impact on the oral microbiome. The evidence presented further supports the recommendation of CPC-zinc lactate rinses for adults with halitosis or periodontal diseases, based on the clinical and microbiological benefits associated with daily use.

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Impact of Cetylpyridinium Chloride and Zinc Mouthwash on Oral Health and the Microbiome

Meghan A. Berryman, PhD

Abstract: The widespread use of antimicrobial mouthwashes highlights the importance of understanding their impact on both clinical outcomes and the oral microbiome. This literature review seeks to critically evaluate the current academic knowledge regarding the clinical efficacy of mouthwash containing cetylpyridinium chloride (CPC) and zinc lactate in reducing plaque, gingivitis, and oral malodor, with a particular focus on its interactions with the oral microbiome. Clinical trials have validated the efficacy of CPC and zinc lactate in enhancing oral health metrics, although the long-term impact of their combined use on the oral microbiome warrants further exploration. CPC and zinc lactate in a mouthwash is particularly effective against oral biofilms. While bacteria has the potential to develop resistance against antiseptics, there is no evidence at this time to suggest that CPC and zinc lactate influences resistance in the oral cavity. However, there is evidence that CPC and zinc lactate in combination may be superior to other antibacterial mouthwashes at controlling periodontal pathogens while promoting a healthy and balanced oral microbiome. Future research should prioritize longitudinal, multi-omics investigations to elucidate the nature and extent of these interactions across diverse bacterial communities. The capacity of CPC and zinc lactate to support a healthy oral microbiome, without promoting antimicrobial resistance, underscores their combined potential as a safe and effective oral hygiene solution.

Maintaining optimal oral hygiene is a critical aspect of overall health, serving as the primary defense against an array of oral diseases, including dental caries, gingivitis, and periodontitis.[1] The significance of controlling plaque—the bacterial biofilm that forms on teeth—is supported across the literature as having positive impacts on oral health outcomes.[2] While mechanical plaque disruption through regular toothbrushing and flossing is considered to be the foundation of oral hygiene, its effectiveness can be limited by the topography of the oral cavity, individual dexterity, and personal practice.[3] To overcome these limitations and to access hard-to-reach areas within the oral cavity, such as interproximal spaces, mouthwashes serve as adjunctive therapies.[4] Their usage has seen an increase worldwide, reflecting a growing awareness of their potential benefits.[5] Mouthwashes offer such advantages as dental plaque reduction, control of gingival inflammation, and mitigation of oral malodor.[4]

The oral microbiome, a diverse ecosystem of microorganisms that reside within the oral cavity, plays a vital role in the maintenance of oral health and overall systemic health.[6] A balanced microbiome acts as a natural defense against the colonization of pathogenic microorganisms, and imbalances can lead to localized oral diseases like dental caries and gingivitis as well as systemic diseases such as cardiovascular diseases, gastrointestinal diseases, and neurological disorders.[6] Consequently, understanding how the ingredients in mouthwash formulations interact with microbial balance is important when evaluating the overall impact of oral hygiene products.

Widely used in mouthwashes for its broad-spectrum antiseptic properties, cetylpyridinium chloride (CPC) is a monocationic

quaternary ammonium compound capable of disrupting bacterial cell membranes and interfering with essential bacterial metabolic processes.[7,8] The amphiphilic nature of CPC's structure allows for an electrostatic interaction with negatively charged bacterial surfaces.[9] The positive pyridine head displaces cations on the membrane and the hexadecane tail inserts into the lipid bilayer, disorganizing the bacterial membrane and causing leakage of cellular components.[8] CPC's surfactant properties allow for even distribution of the liquid within the oral cavity regardless of surface irregularities.[8] Clinical studies show CPC's activity against the oral pathogens linked to periodontal disease and its bactericidal effects on oral biofilms.[10]

Zinc salts, including zinc lactate, are commonly added to oral hygiene products because of, most notably, their involvement in the reduction of oral malodor.[11] Zinc ions inhibit the formation of volatile sulfur compounds (VSCs) due to their strong affinity for the thiol groups within VSCs.[12] When zinc ions interact with these sulfur-containing molecules, they form insoluble odorless sulfides.[12] Additionally, the inclusion of zinc in mouthwash formulas has shown to enhance long-term antibacterial activity and inhibit bacterial metabolism.[13]

Therefore, the rationale for combining CPC and zinc lactate in a single mouthwash formulation (CPC + Zn) stems from the concept of harnessing their individual strengths with the intention of achieving a comprehensive oral hygiene product that effectively targets plaque, gingivitis, and halitosis. This review seeks to synthesize the current academic knowledge regarding the clinical efficacy of mouthwash containing both CPC and zinc lactate, with a particular focus on its interactions with the oral microbiome.

Clinical Efficacy of CPC and Zinc Lactate Mouthwash

Several clinical trials have provided evidence for the superior antiplaque and antigingivitis efficacy of mouthwash formulas containing CPC + Zn in comparison to fluoride-based mouthwash, CPC mouthwash, alcohol-free essential oil mouthwash, and essential oil mouthwash in an alcohol base. Two 3-month, parallel-group clinical studies evaluating the efficacy of mouthwash containing 0.075% CPC and 0.28% zinc lactate compared to mouthwash containing 0.02% sodium fluoride found that the CPC + Zn mouthwash provided significantly greater plaque control and reduction in gingival inflammation.[14] The two clinical trials were conducted at Loma Linda University in California and an Oral Health Clinical Services site in New Jersey and reported very similar results. The groups that were treated with CPC + Zn mouthwash had a 20.6% and 21.5% greater reduction in whole-mouth gingivitis and a 27.4% and 25.3% greater reduction in whole-mouth plaque scores compared to the fluoride mouthwash groups in the California and New Jersey sites, respectively. Greater reductions in gingival severity, which is categorized as inflammation and bleeding, were also observed at both sites for the CPC + Zn group compared to the fluoride groups; however, the California site reported a 62.5% greater reduction and the New Jersey site reported a 38.6% greater reduction in gingival severity.

Additionally, two 6-week studies were conducted on the efficacy of 0.075% CPC and 0.28% zinc lactate compared to a 0.07% CPC mouthwash with no zinc lactate—one at the Federal University of Rio Grande do Sul in Brazil and one at the Universidad Católica Santo Domingo in Dominican Republic.[15,16] After 6 weeks of treatment, the CPC + Zn groups showed a 16.8% and 13.2% greater reduction in whole-mouth gingivitis and a 16.8% and 16.1% greater reduction in whole-mouth plaque index scores than the group treated with CPC alone at the Brazil and Dominican Republic sites, respectively. Clinicians at the two sites also reported greater reductions in gingival severity for the group treated with CPC + Zn compared to those treated with CPC alone: the Brazil site reported a 54.5% greater reduction and the Dominican Republic site reported a 28.6% greater reduction in gingival severity. The results of these studies suggest that the addition of zinc lactate to a CPC-based mouthwash formula enhances the antiplaque and antigingivitis effects of the product.

Essential oil–based mouthwashes are marketed with or without an alcohol base, and claims have been made that report equivalent control of plaque and gingivitis regardless of alcohol content.[17-19] However, this evidence is ambiguous when evaluating the efficacy of these mouthwashes in comparison to mouthwashes containing CPC + Zn. A 6-week study comparing mouthwash with 0.075% CPC and 0.28% zinc lactate and alcohol-free essential oil mouthwash found that the CPC + Zn treatment significantly outperformed the essential oil mouthwash across all antiplaque and antigingivitis indices.[20] CPC + Zn had a 26.7% greater reduction in whole-mouth plaque and a 10.6% greater reduction in whole-mouth gingivitis scores than the alcohol-free essential oils. However, in a 6-week clinical trial comparing mouthwash with 0.075% CPC and 0.28% zinc lactate and essential oil mouthwash with 21.6% ethanol, both mouthwash formulas significantly improved plaque and gingivitis scores for all timepoints compared to baseline.[21] After 6 weeks, the CPC + Zn group exhibited a 37.2% reduction in plaque severity and 47.7% reduction in gingivitis severity, and the essential oils with alcohol group showed a 35.9% reduction in plaque severity and 38.6% reduction in gingivitis severity compared to baseline, resulting in no statistically significant difference measured between the two mouthwash treatments. Given that alcohol-based mouthwash has been associated with increased abundance of oral opportunistic bacteria and significantly impacts the oral microbiome,[22] a CPC + Zn mouthwash is an effective alcohol-free alternative for plaque and gingivitis control.

The CPC and zinc combination has also demonstrated efficacy in reducing aerosolized bacterial load, a promising finding. A randomized clinical trial evaluated the effect of a pre-procedural mouthwash containing 0.075% CPC and 0.28% zinc lactate on reducing bacteria in dental aerosols after ultrasonic scaling compared to chlorhexidine, water, and no rinsing.[23] Notably, the results of this study indicated that the colony-forming units detected in the aerosols from the CPC + Zn group and the chlorhexidine group were statistically similar. Both mouthwashes resulted in significantly less aerosolized bacteria than when patients were treated with water or no rinsing. Chlorhexidine has known bactericidal efficacy and has been shown to reduce gingivitis and plaque across many clinical

trials.[24] While chlorhexidine is effective at targeting multispecies biofilms that include pathogenic bacteria like *Streptococcus mitis* and *Porphrymonas gingivalis*, it also decreases bacterial diversity, which one study found led to more acidic oral conditions in healthy individuals.[25,26] This pre-procedural rinse study indicates that CPC + Zn is as effective as chlorhexidine in reducing the risk of infection during dental procedures and may be a better alternative for a balanced oral microbiome.

Intriguingly, a recent study evaluating the efficacy of gargling with mouthwash in preventing the development of respiratory symptoms suggests that adding a 0.075% CPC and 0.28% zinc lactate mouthwash to oral care regimens is beneficial in lowering the incidence of upper respiratory symptoms associated with cold and flu.[27] Specifically, the study found that adding regular gargling with the CPC + Zn mouthwash to an oral care regimen resulted in a 21.5% decrease in respiratory symptoms and a 11% decrease in severity of symptoms compared to brushing alone.

This body of evidence underscores the effectiveness of mouthwash formulations containing CPC and zinc lactate as superior agents for managing plaque and gingivitis compared to other commonly used mouthwash formulas, including those based on fluoride, CPC alone, and essential oils, with or without alcohol. The studies consistently demonstrate the enhanced capability of CPC + Zn combinations in reducing plaque, gingival inflammation, bacterial load, and respiratory symptoms associated with cold and flu. Furthermore, given the potential adverse effects of alcohol-based mouthwashes on the oral microbiome, an alcohol-free option containing CPC and zinc lactate represents an effective and safer alternative for individuals seeking robust oral health benefits. These findings support the consideration of CPC and zinc lactate mouthwash as a preferred option in oral hygiene regimens aimed at reducing plaque and gingivitis.

CPC and Zinc Lactate Mouthwash and a Healthy Oral Microbiome

The combined action of CPC and zinc lactate in a mouthwash is particularly effective against oral biofilms. CPC has been shown to effectively inhibit the activity of bacterial glucosyltransferase, which is the enzyme responsible for synthesizing glucan, a key biofilm component.[28] This biofilm-specific mechanism may be why CPC was also seen to have a 20% biofilm kill depth with static immersion into the mouthwash compared to the 5% kill depth seen with chlorhexidine immersion.[8,29] Simultaneously, zinc's ability to inhibit bacterial adhesion and weaken biofilm matrix could further enhance mouthwash efficacy against oral biofilms.[30] The dual approach to targeting both microbial components and biofilm suggests a complementary action that is reflected in antimicrobial studies.

An in vitro biofilm study comparing mouthwash containing 0.075% CPC and 0.28% zinc lactate to a negative control with no active ingredients, mouthwash containing only 0.075% CPC, essential oil mouthwash, and mouthwash with essential oils in an alcohol base found that CPC + Zn continued to significantly reduce bacterial biofilm viability 2 and 5 hours post-treatment by 42.8% and 62.1% compared to negative control, respectively.[31] It was the only mouthwash in the study that significantly reduced biofilm viability over time.

In conjunction with the antibacterial properties of CPC and zinc lactate, it is important to consider the impact of this mouthwash on a healthy oral microbiome. Disrupting the natural balance of the oral microbiome has the potential to lead to dysbiosis and, concerningly, antimicrobial resistance.[32,33] It is possible for bacteria to develop resistance to quaternary ammonium compounds like CPC through upregulated efflux pumps, outer membrane alterations targeting binding sites, and biodegradation; however, there is no evidence to suggest that CPC is influencing resistance in the oral cavity.[32] When comparing resistance to chlorhexidine in *Enterococcus faecalis* and *Streptococcus mutans*, it was observed that repeated exposure to chlorhexidine resulted in resistance in *E faecalis*, but no increased resistance to CPC was observed in either *E faecalis* or *S mutans*.[34]

Notably, a recent study evaluating the effects of mouthwash containing 0.075% CPC and 0.28% zinc lactate, mouthwash with 0.12% chlorhexidine, and 0.075% CPC mouthwash in a multispecies biofilm model found that all mouthwashes reduced metabolic activity, biofilm viability, and several species counts, including *P gingivalis*, *Fusobacterium nucleatum*, *Parvimonas micra*, *Campylobacter gracilis*, and *S mutans*.[35] However, only the CPC + Zn combination reduced the pathogen *Prevotella intermedia*. The authors specifically highlight that *P intermedia* is associated with oral biofilm dysbiosis and reducing the species is integral to maintaining homeostasis.[35] Perhaps most importantly, however, is the evidence that the CPC + Zn mouthwash did not disrupt the balance of health-associated bacterial species, while treatment with either chlorhexidine or CPC without zinc lactate reduced these species. These results suggest that CPC and zinc lactate in combination may be superior at controlling periodontal pathogens, while promoting a healthy and balanced oral microbiome.

These preliminary findings indicate that CPC and zinc lactate may act synergistically or additively to enhance antimicrobial activity against a range of oral pathogens and potentially modulate the biofilm environment in a way that is more favorable for oral health. However, future research should prioritize longitudinal, multi-omics investigations to elucidate the nature and extent of these interactions across the diverse bacterial species and communities within the oral microbiome. Understanding the specific impact on a wider range of bacterial species and their functional activities will be crucial for a comprehensive assessment of the combined effect of CPC and zinc lactate on oral health.

Conclusions

This comprehensive evaluation of the current literature on the impact of mouthwash containing CPC and zinc lactate on oral heath underscores its ability to effectively reduce plaque accumulation, gingival inflammation, and oral malodor. The additive antimicrobial properties of CPC + Zn allow for sustained antibacterial action and effective control of oral malodor, while minimizing the disruption of the oral microbiome. Clinical trials show that the addition of zinc lactate to a CPC-based mouthwash formula may enhance the antiplaque and antigingivitis effects of the product, outperforming essential oil mouthwashes and showing parity with alcohol-based mouthwash. Given that alcohol-based mouthwash significantly

impacts the oral microbiome, CPC + Zn mouthwash may be an effective alcohol-free alternative for plaque and gingivitis control. It was also observed that CPC + Zn is as effective as chlorhexidine in reducing the risk of infection during dental procedures. Additionally, the capacity of CPC + Zn to support a healthy oral microbiome, without promoting bacterial resistance, underscores the combination as a safe and effective oral hygiene solution. Overall, these findings advocate for the adoption of CPC and zinc lactate mouthwash as an effective adjunctive to oral care strategies.

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Antibacterial and Anti-Malodor Efficacy of a Cetylpyridinium Chloride and Zinc Lactate Mouthwash

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Abstract: *Background:* Oral malodor represents a common health concern affecting a substantial portion of the global population, the prevalence of which can range from 15% to 60%, highlighting its widespread occurrence. Bad breath, originating from pathogens in the oral cavity, can be mediated through treatment with antibacterial mouthwashes. This clinical trial explores the antibacterial effects and anti-malodor properties of a mouthwash containing both cetylpyridinium chloride and zinc lactate (CPC + Zn). *Methods:* In vitro antibacterial efficacy studies were run in the form of single-species short-interval kill tests on *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* treated with CPC + Zn mouthwash, a placebo, and a negative control; a whole saliva bacterial kill test; and a biofilm viability test treated with CPC + Zn mouthwash, CPC alone mouthwash, essential oils with alcohol mouthwash, and essential oils alone mouthwash. A 3-week, double-blind, parallel clinical trial was also conducted in Chengdu, Sichuan, China, to evaluate the clinical efficacy of CPC + Zn mouthwash compared to a fluoride mouthwash for overnight oral malodor (12 hours post rinsing). *Results:* When CPC + Zn was tested against an *A actinomycetemcomitans* strain and *S mutans* strain, it gave a 7.11 (±0.549) and 8.83 (±0.405) log reduction in colony forming units (CFUs) relative to the phosphate buffered saline control, respectively, resulting in 99.9% reduction in bacterial load. Compared to a negative control, CPC + Zn mouthwash treatment significantly reduced bacterial biofilm viability 2 and 5 hours post treatment by 42.83% ($P = .018$) and 62.07% ($P = .001$), respectively. After 3 weeks of product use, the CPC + Zn test group exhibited a 33.5% decrease in oral malodor with a final baseline-adjusted mean score of 4.89 (±0.06; confidence interval 95% [4.76, 5.02]; $P < .001$). *Conclusions:* CPC + Zn mouthwash delivered superior antibacterial effects in both planktonic and biofilm cultures when compared to negative control in vitro and superior malodor reduction compared to control in vivo. The substantial reduction observed in both bacterial load and oral malodor suggests that the CPC + Zn mouthwash could serve as a highly effective oral hygiene product. The ability to maintain a "pleasant breath" status further enhances its applicability in daily oral care, improving users' social interactions and overall quality of life. *Practical Implications:* Alcohol-free CPC + Zn mouthwash may be an effective treatment for prolonged oral malodor suppression through antibacterial properties.

Oral malodor, sometimes referred to as halitosis, represents a common oral condition affecting a substantial portion of the global population, the prevalence of which can range from 15% to 60%, highlighting its widespread occurrence.[1] Bad breath, originating from pathogens in the oral cavity, can significantly influence an individual's social interactions, as emotional well-being is negatively correlated with levels of total volatile sulfur compounds (VSCs) among other malodorous compounds.[2] Bacterial degradation of amino acids from within the oral cavity results in the production of malodorous VSCs like hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, the major contributors to bad breath.[3] Multiple interventions have been assessed over the years for the control of oral malodor.[4] Targeting the bacteria that cause bad breath is an important portion of those efforts because the bacteria that cause oral malodor are also implicated in periodontal disease.[1]

Cetylpyridinium chloride (CPC) has been a widely used antiseptic in mouthwashes and dentifrices since 1939.[5] As a monocationic surfactant, its structure includes a positively charged hydrophilic pyridine head and a hydrophobic hexadecane tail. This amphiphilic nature allows it to interact with the oral environment and bacterial cell membranes. CPC's antibacterial mechanism starts with an electrostatic interaction with negatively charged bacterial surfaces.[6] The positive pyridine head displaces essential divalent cations on the membrane and the hydrophobic tail inserts into the lipid bilayer, disrupting membrane integrity and exhibiting broad-spectrum antibacterial activity.[5] CPC's surfactant properties ensure even distribution in the oral cavity.[5] Clinical studies show CPC's activity against oral pathogens linked to gingivitis and periodontal disease and its demonstrated bactericidal effects on biofilms.[7] These multifaceted antibacterial mechanisms provide a strong rationale for the inclusion of CPC in mouthwash to combat VSC-producing bacteria.

Zinc ions, often in the form of zinc salts like zinc lactate, are common in anti-malodor mouthwashes[8] and other oral hygiene products for benefits such as malodor reduction, plaque and calculus control, and antibacterial action.[9] Zinc ions exhibit antibacterial activity against various oral bacteria,[10] interfering with metabolic processes and reducing acid production by *Streptococcus* species.[11] The addition of zinc lactate to mouthwash specifically shows long-term antibacterial effects.[12] A key anti-malodor mechanism of zinc ions is their strong affinity for thiol groups within VSCs.[8] Zinc ions interact with hydrogen sulfide to form insoluble, odorless zinc sulfide.[8] In vitro studies show zinc salts can almost completely inhibit hydrogen sulfide volatilization.[8] Clinical trials show zinc lactate mouthwashes reduce VSC concentrations and improve breath odor.[9] The dual action of zinc lactate—antibacterial and VSC-neutralizing—makes it a valuable component in anti-malodor mouthwashes, potentially acting additively with CPC.

This clinical trial explores the antibacterial effects and anti-malodor properties of a mouthwash containing both CPC and zinc lactate. Reducing the oral bacterial load, especially anaerobic gram-negative bacteria on the tongue and in periodontal pockets, is key to managing oral malodor.[13] This research provides an understanding of the potential additive nature between CPC and zinc lactate in combating oral malodor. A more effective anti-malodor mouthwash formulation could improve oral health and quality of life.

Materials and Methods

In vitro Antibacterial Efficacy Analysis

Single-Species Bacterial Kill Test

Treatments were as follows:

1. A test mouthwash containing 0.075% CPC and 0.28% zinc lactate in an alcohol-free base (CPC + Zn).
2. A matching placebo mouthwash.
3. A negative control containing phosphate buffered saline (PBS).

Single-species short-interval kill tests are a generally accepted measure of the antibacterial efficacy of liquid oral care formulations. Fine et al established a method using representative single species cultures of bacteria to enumerate the population of bacteria killed by a mouthwash formulation in a 30-second exposure, the recommended use time for most oral rinse formulas.[14] In the present study, the authors employed a method similar to that of Fine et al using modifications previously reported in Schaeffer et al.[15]

Aggregatibacter actinomycetemcomitans (ATCC #43178) was grown from a single colony on a plate of brain heart infusion (BHI) agar with 10% sheep's blood (Hardy Diagnostics, hardydiagnostics.com) by seeding a 30 mL culture of BHI broth supplemented with 1% sodium bicarbonate. Cultures were grown overnight at 37°C.

Streptococcus mutans (ATCC #25175) was grown from a single colony on a trypticase soy agar plate containing 5% sheep's blood (Becton Dickinson, bd.com) by seeding a 30 ml culture of trypticase soy broth. Cultures were grown overnight at 37°C in a 5% carbon dioxide (CO_2) atmosphere.

Briefly, 1 mL aliquots of an OD_{600}~0.8 culture were harvested and treated for 30 seconds with the indicated mouthwash or control. Samples were pelleted and washed three times with sterile PBS to remove the treatment. Washed pellets were resuspended in 1 ml of sterile PBS, serially diluted and plated on agar plates for colony enumeration. *A actinomycetemcomitans* samples were plated on BHI agar containing 5% defibrinated sheep's blood. Plates were incubated for 48 to 72 hours in a semi-anaerobic atmosphere prior to counting colonies. *S mutans* samples were plated on tryptic soy broth agar plates containing 5% defibrinated sheep's blood and incubated in a 5% CO_2 atmosphere for 24 to 48 hours prior to counting.

Colony counts were used to determine the numbers of viable bacteria per mL sample (CFU/mL), and this value was used to determine the log reduction in CFUs relative to the PBS-treated samples.

Whole Saliva Bacterial Kill Test

In order to validate the efficacy of formulas against a more realistic, robust bacterial population, the authors harvested whole saliva from a single, healthy adult volunteer via expectoration. The volunteer provided unstimulated saliva after having abstained from

eating, drinking, and all oral hygiene for at least 8 hours.

Whole saliva was aliquoted into 200 µL samples in individual sterile microfuge tubes. Aliquots of saliva were treated 1:1 with the indicated mouthwash and allowed to incubate for 30 seconds. Following the 30-second treatment, exposure was disrupted by adding 1 mL of sterile Dey-Engley neutralization broth (D/E broth) to each sample. This medium contains a mix of thiols and detergents capable of neutralizing a wide range of antimicrobial agents, such as quaternary ammonium compounds, metals, and surfactants.

Samples were pelleted by centrifugation and then washed one time by resuspending in 1 mL of sterile PBS and centrifuging again. Washed samples were resuspended in a fresh 1 mL aliquot of sterile PBS. Two hundred µL of each sample was transferred in duplicate to a sterile 96-well plate, and serial 10-fold dilutions were performed in sterile PBS. One hundred µL of relevant dilutions was plated on trypticase soy agar plates supplemented with 5% sheep's blood. Plates were incubated for 24 hours at 37°C in a 5% CO_2 atmosphere. Colony counts were obtained from relevant plates. Data are reported as a reduction in CFUs/mL relative to a negative control sample treated with sterile PBS.

Biofilm Viability Assay

Treatments were as follows:

1. A test mouthwash containing 0.075% CPC, 0.28% zinc lactate, and 0.05% sodium fluoride in an alcohol-free base (CPC + Zn).
2. A commercially available mouthwash containing 0.075% CPC in an alcohol-free base (CPC) (Colgate-Palmolive Co., colgatepalmolive.com).
3. A commercially available mouthwash containing essential oils and 21.6% ethanol (EO + EtOH) (Johnson & Johnson, jnj.com).
4. A commercially available mouthwash containing essential oils and no alcohol (EO) (Johnson & Johnson).
5. A negative control containing PBS.

Laboratory biofilms used in this study were cultured from whole saliva collected from unbrushed donors following Institutional Review Board approval. The donors were asked to refrain from eating, drinking, and oral hygiene 12 hours prior to saliva donation. Before collection, the donors were provided with an unused toothbrush and instructed to brush their teeth without toothpaste for 1 minute in order to dislodge plaque from tooth surfaces. They were instructed to proceed to spit in a sterile 50 mL conical tube until a total volume of 10 mL was reached. The whole saliva was then vortexed to homogenize the samples prior to the addition of 40 mL McBain medium supplemented with 5 µg/mL hemin (final concentration; ThermoFisher, thermofisher.com) and 1 µg/mL menadione (final concentration; ThermoFisher). The resulting bacterial suspension was distributed in 1.5 mL aliquots into a sterile 24-well polystyrene plate. The biofilms were cultured on hydroxyapatite disks for 24 hours at 37°C under an environment containing 5% CO_2. Media was replaced twice daily thereafter and biofilms were cultured as described for an additional 48 hours. Biofilms were treated with undiluted mouthwashes for 30 seconds at room temperature on an elliptical shaker at the rate of 90 revolutions per minute. After treatment, the hydroxyapatite disks with the cultured biofilms were rinsed by dipping five times in sterile deionized water for a total of two rounds. This wash step was carried out in sterile 24-well polystyrene plates containing 1.7 mL of sterile deionized water. After washing, the biofilms were allowed to recover in sterile deionized water at 37°C under 5% CO_2 for 2 and 5 hours. To collect the biofilms for viability measurement, the treated biofilms were sonicated for a total of 2 minutes at 30-second intervals per side. Biofilm viability was measured through the quantification of total ATP (adenosine 5'-triphosphate) using BacTiter-Glo™ Microbial Cell Viability kit (Promega, promega.com). The test reagents were prepared as described by the manufacturer with 100 µL of the ATP reagent added to 50 µL of bacterial suspension. The reaction was incubated at room temperature in the dark for no more than 5 minutes. Total ATP was measured through luminometry with values expressed at relative light units (RLUs). Additionally, RLUs were normalized versus total bacterial mass as determined through Syto 9 staining (ThermoFisher) with the control group set to "1." Percent reduction in biofilm viability was calculated relative to the control. The experiment was performed a total of four times. Statistical significance was determined using Tukey's multiple comparison test.

Clinical Malodor Analysis

Interventions

1. A test mouthwash containing 0.075% CPC, 0.28% zinc lactate, and 0.05% sodium fluoride in an alcohol-free base (CPC + Zn).
2. A commercially available mouthwash with 100 ppm fluoride (F) (Colgate-Palmolive Co.).

Study Design

This 3-week, double-blind, parallel clinical trial was conducted in Chengdu, Sichuan, China, to evaluate the clinical efficacy of CPC + Zn mouthwash compared to F mouthwash for overnight oral malodor (12 hours post rinsing). Qualifying participants were randomly assigned via a computer-generated random number list to either the CPC + Zn test group or the F control group in such a way that examiner, subjects, and statistician were blind to allocation. All products were concealed and coded by the sponsor to preserve blinding.

Participants and Inclusion/Exclusion Criteria

Eighty healthy adults, male and female, 18 through 70 years old were recruited and divided into two groups of 40 participants per group. For inclusion in the study, participants must have aligned with the following inclusion criteria: availability for the 3-week duration, initial mean organoleptic oral malodor score ≥6.0 and ≤8.4, and willingness to sign informed consent form. Individuals were excluded from this study if they had orthodontic bands, ≥1 tumor of the soft or hard tissue within the oral cavity, advanced periodontal disease, ≥5 carious lesions requiring restorative treatment, use of antibiotics or participation in another clinical study within 1 month of this study, received dental prophylaxis within 2 weeks of baseline examination, taken prescription medications that may interfere with the study outcome, existing medical

TABLE 1

Reductions in CFUs Following Treatment of *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans* With a Placebo or the Test CPC + Zn Mouthwash

TREATMENT	*A ACTINOMYCETEMCOMITANS*			*S MUTANS*		
	Log CFU/ml Reduction (vs. PBS)	% Reduction (vs. PBS)	Log CFU/ml Reduction vs. Placebo	Log CFU/ml Reduction (vs. PBS)	% Reduction (vs. PBS)	Log CFU/ml Reduction vs. Placebo
Placebo	1.04 ± 0.326			0.80 ± 0.166		
CPC + Zn	7.11 ± 0.549	>99.9%	6.11	8.83 ± 0.405	>99.9%	8.03

Data are reported as a log reduction in CFUs relative to the negative control sample treated with sterile PBS.
CFU = colony forming unit, CPC = cetylpyridinium chloride, PBS = phosphate buffered saline, Zn = zinc lactate

TABLE 2

Summary of Clinical Malodor Trial Participants

TREATMENT GROUP	NUMBER OF PARTICIPANTS (FEMALE)	MEAN AGE (SD)	AGE RANGE
CPC + Zn	39 (18)	53.28 (9.03)	30–69
Control	39 (21)	51.64 (9.72)	24–68

No statistically significant difference was indicated between the two groups with respect to gender and age.
SD = standard deviation

condition prohibiting the individual from eating or drinking for up to 4 hours, history of allergies to oral care products, history of drug or alcohol abuse, or self-reported pregnancy or lactation.

Study Protocol

All qualifying participants were provided with their group-specific mouthwash, a regular fluoride toothpaste containing 0.76% sodium monofluorophosphate, and a manual toothbrush. Participants were asked to brush their teeth for 2 minutes with the provided toothpaste and toothbrush once in the morning and once in the evening. All rinsing followed product-specific per label instructions. Following each instance of brushing, the CPC + Zn test group members were asked to rinse for 30 seconds with 20 mL of assigned mouthwash without rinsing with water afterwards, and the F control group members were asked to rinse for 1 minute with 10 mL of assigned mouthwash without rinsing with water afterwards as per label mouthwash instructions.

Oral malodor evaluations were conducted by four trained judges using a nine-point hedonic scale from most unpleasant (1) to most pleasant (9). Baseline evaluations were conducted in the morning after participants refrained from eating, drinking, and all oral hygiene, including brushing, rinsing, and flossing for at least 6 hours. Participants were then evaluated after following the oral care regimen for 3 weeks. This evaluation was conducted 12 hours post rinsing (overnight).

Statistical Analysis

Participant group size was determined as about 40 for a significance with 80% power. Subject-wise baseline oral malodor scores were determined by taking the mean of the scores provided by all four judges for each subject. Baseline and week 3 group scores were determined by calculating the mean of all scores within each group. Paired t-test was used to compare the within-treatment, week 3 scores to baseline scores. Between-treatment comparison was performed with analysis of covariance baseline-adjusted week 3 means and baseline as the covariate. All tests were two-sided with a 0.05 significance level.

Results

In vitro Antibacterial Efficacy

Single-Species Bacterial Kill Test

Simple in vitro bacterial kill studies are a useful tool for enumerating the ability of a formula to kill target bacteria. The treatment of bacterial samples with the placebo formula without CPC or other active ingredients resulted in a small (≤1 log) but consistent reduction in CFUs for both strains due to the presence of excipients such as surfactants. The placebo treatment reduced *S mutans* counts by 0.80 (±0.166) log CFUs/mL and *A actinomycetemcomitans* by 1.04 (±0.326) log CFUs/mL. Due to these clear differences in the performance of the placebo formula, the performance of the test mouthwashes with CPC + Zn was reported relative to the colony counts obtained following treatment with both placebo and the negative control mouthwash to help distinguish formula effects from active ingredient effects.

When CPC + Zn was tested against an *A actinomycetemcomitans* strain, it gave a 7.11 (±0.549) log reduction in CFUs relative to the PBS control. This represented a >6 log increase beyond the placebo formula. CPC + Zn treatment resulted in statistically

significant greater reduction ($P < .0001$) in planktonic *A actinomycetemcomitans* than the negative control. CPC + Zn reduced planktonic *A actinomycetemcomitans* by >99.9% over a negative control formula (Table 1).

CPC + Zn was also tested against planktonic *S mutans*. CPC + Zn gave a reduction of 8.83 (±0.405) log CFUs compared to the PBS-treated negative control. This was >8 logs greater reduction in CFUs than the matched placebo formula. The test formula was statistically significantly ($P < .0001$) different from the negative control. Both formulas gave reductions in planktonic *S mutans* that were >99.9% greater than the negative control formulas (Table 1).

Whole Saliva Bacterial Kill Test

While single-species kill assays are a valuable tool for quantifying the impact of oral care products on individual species of bacteria found in the oral cavity, these assays are not reflective of the way in which bacteria exist in patients' mouths. Therefore, a whole saliva short exposure test was used to validate the in vitro performance of formulations.

This method is used to measure the immediate impact of typical use time treatments with the indicated formula on the viability of planktonic bacteria, represented by whole saliva. The CPC + Zn mouthwash gave a 1.69 log reduction (>90%) in total

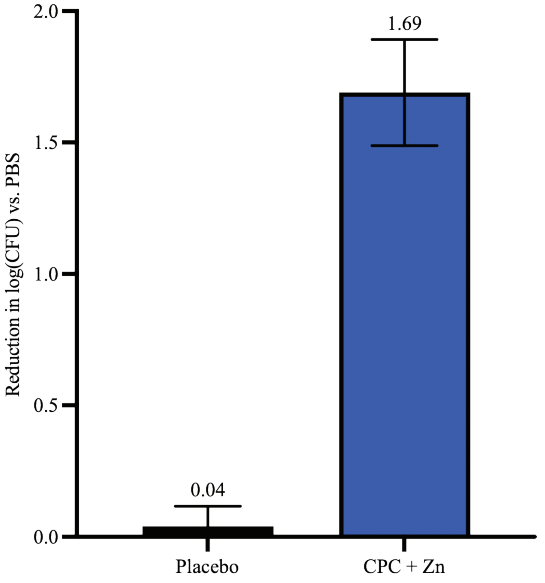

Fig 1. CPC + Zn = cetylpyridinium chloride + zinc lactate

Fig 1. Short exposure killing of total salivary bacteria by test mouthwashes. Formulas are incubated 1:1 with whole saliva for 30 seconds and then diluted and plated. Results are presented as the log reduction in CFUs/mL relative to a PBS-treated sample.

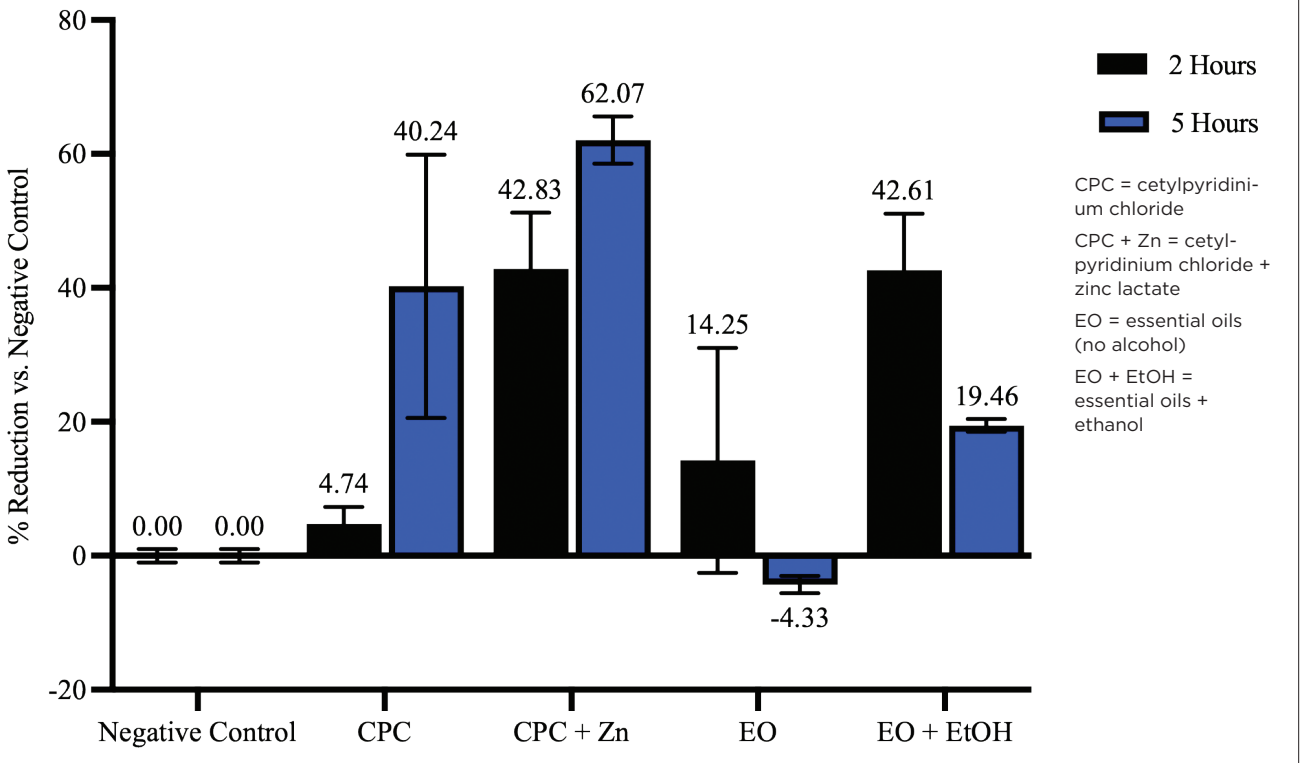

Fig 2.

Fig 2. In vitro biofilm viability after mouthwash treatment. In vitro biofilms were treated with undiluted oral rinses for 30 seconds with remaining biofilm viability measured 2 and 5 hours post treatment through ATP quantification. Percent viability of remaining bacteria in the biofilms was quantified relative to the negative control biofilm group.

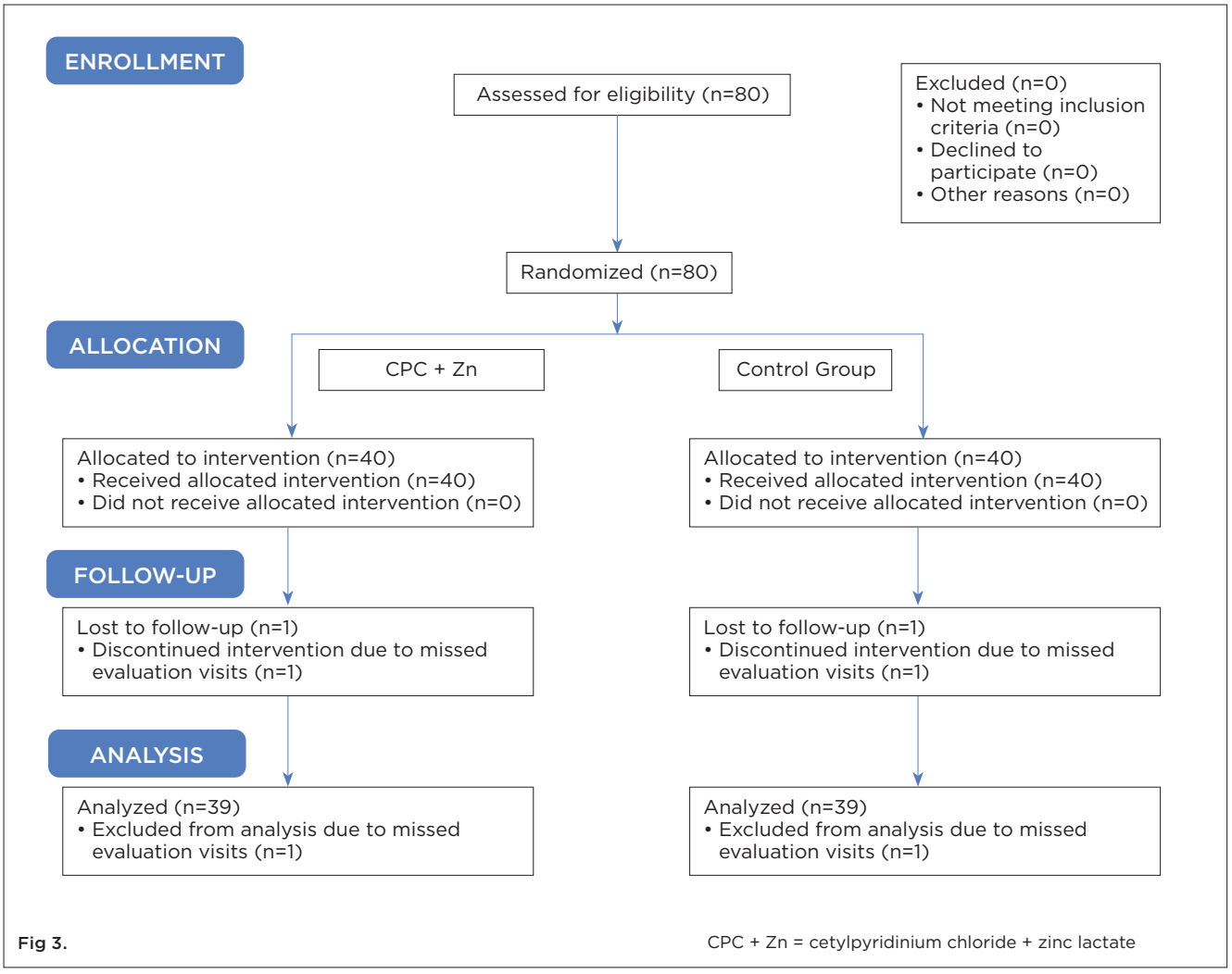

Fig 3. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

salivary bacterial counts (Figure 1). For comparison purposes, a matched placebo mouthwash with no CPC or Zn gave only a 0.04 log reduction in CFUs/ml with the same treatment. This difference in killing was statistically significantly (P = .0002) greater than the placebo.

Biofilm Viability

Compared to the negative control, treatment with the CPC + Zn mouthwash showed statistically significant reduction in biofilm viability 2 and 5 hours post treatment by 42.83% (P = .018) and 62.07% (P = .001), respectively. CPC mouthwash reduced 4.74% (P > .999) and 40.24% (P = .079) biofilm viability 2 and 5 hours post treatment, respectively, but the reduction was not significant compared to the negative control. Mouthwash containing EO + EtOH reduced 42.61% (P = .103) and 19.46% (P = .861) biofilm viability 2 and 5 hours post treatment, respectively, but it was not statistically significant compared to the negative control. Mouthwash containing essential oils and no alcohol reduced biofilm viability by 14.25% (P = .987) after 2 hours and showed similar viability to the negative control 5 hours after treatment (P > .999). CPC-containing mouthwashes were the only treatments that continued to suppress bacterial biofilm viability over time; however, CPC + Zn was the only treatment that showed significant reduction at both timepoints compared to the negative control (Figure 2).

Clinical Malodor Reduction

Eighty individuals were recruited based on inclusion criteria to participate in this study. A total of 78 participants completed the study. One person per group was dropped from analysis because they failed to make the final appointment (Figure 3). There were no statistically significant differences between the two treatment groups with respect to gender (P = .497) and age (P = .442) (Table 2). No adverse events were recorded, including hard and soft tissue examination results.

There was no statistically significant (P = .244) difference between baseline organoleptic scores for each treatment group. The CPC + Zn test group had a mean baseline score of 7.32 (±0.28; confidence interval [CI] 95% [7.09, 7.68]). The control group had a mean baseline score of 7.39 (±0.25; CI 95% [7.19, 7.71]). Both mean scores

represent breath considered to be between moderately unpleasant (7) and very unpleasant (8). After 3 weeks of product use, both treatments showed statistically significant ($P < .05$) reduction in malodor compared to baseline. The CPC + Zn test group exhibited a 33.5% decrease in oral malodor with a final baseline-adjusted mean score of 4.89 (±0.06; CI 95% [4.76, 5.02]; $P < .001$) (Figure 4). This mean score is considered to represent breath quality between neutral (5) and slightly pleasant breath (4). The control group had a 12.0% reduction in oral malodor with a baseline-adjusted mean organoleptic score of 6.49 (±0.06; CI 95% [6.36, 6.62]; $P < .001$), which is considered to be breath quality between slightly unpleasant (6) and moderately unpleasant (7). The CPC + Zn group had a 24.7% greater reduction in oral malodor than the control group.

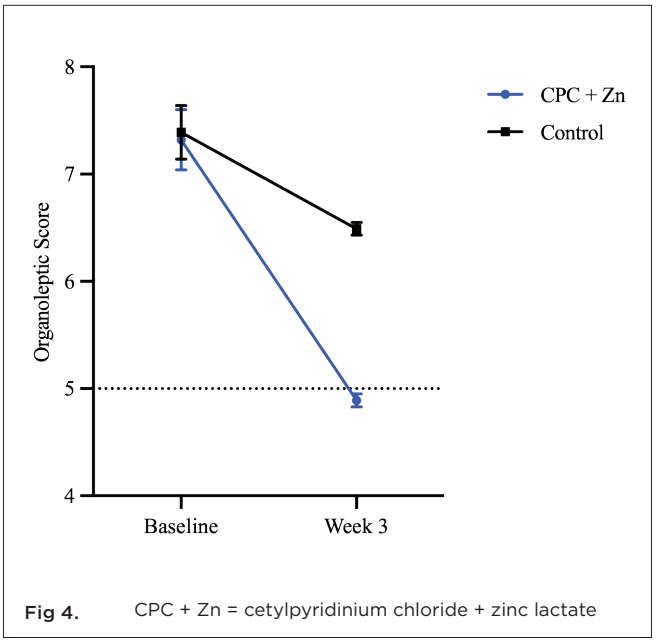

Fig 4. CPC + Zn = cetylpyridinium chloride + zinc lactate

Fig 4. Participant mean organoleptic scores at baseline and after 3 weeks of product use. The dotted line represents the score for neutral breath.

Discussion

These series of in vitro and in vivo studies demonstrated the potent antibacterial and anti-malodor effects of a CPC + Zn mouthwash, achieving significant reductions in bacterial presence across various environments. The >99.9% reduction in planktonic single-species bacteria and >90% reduction of salivary bacteria showcased the mouthwash's formidable antibacterial capacity. In biofilm, CPC + Zn mouthwash was the only treatment that showed significant and compounding reduction in bacterial biofilm as time increased compared to the negative control. While the EO + EtOH mouthwash was observed to have a similar 43% reduction in biofilm viability as the CPC + Zn mouthwash at 2 hours, the antibacterial efficacy of EO + EtOH declined after 5 hours, whereas the antibacterial efficacy of CPC + Zn increased over time. Notably, the continued significant suppression of bacterial biofilm viability over time corroborates the mouthwash's efficacy in improving breath quality in clinical trials. The transition from the "unpleasant" to the "pleasant" breath range in organoleptic scores after 3 weeks of use illustrated CPC + Zn's tangible benefits in a daily-use scenario.

Previous studies with CPC-based mouthwashes reflect the ingredient's antibacterial properties. A similar in vitro single-species kill study on *A actinomycetemcomitans* and *S mutans* performed with two different mouthwashes containing 0.075% CPC and 0.05% sodium fluoride also showed a >99.9% reduction in each species.[15] A biofilm study performed with confocal laser scanning microscopy and fluorometric analyses observed that treatment with an alcohol-free 0.075% CPC-containing mouthwash showed a significantly increased number of damaged biofilm cells compared to placebo mouthwash.[15,16] Additionally, oral care formulations have been enhanced with the addition of zinc salt in previous studies, showing that zinc acts in a compounding manner to antimicrobial properties.[12,17,18] A clinical study featuring a mouthwash formula containing 0.075% CPC and 0.28% zinc lactate versus mouthwash with 0.075% CPC found that the addition of zinc lactate to the formula significantly enhanced the antiplaque and antigingivitis efficacy of the mouthwash.[19] These results combined suggest that the antibacterial properties seen in the CPC + Zn mouthwash here may be driven by the 0.075% CPC and further strengthened by the zinc lactate.

In addition to its broad spectrum antibacterial properties, CPC has also been shown to specifically suppress the expression of genes related to VSC production in anaerobic pathogens in single-species studies,[20] indicating that CPC acts in conjunction with zinc lactate's well-known VSC neutralization[8] to provide the malodor protection seen in the clinical trial performed here. Supporting this, a previous clinical trial where participants brushed with a regular fluoride toothpaste and rinsed with a 0.075% CPC mouthwash showed significant reduction in VSC concentrations in addition to significant improvement in oral malodor compared to brushing alone.[21]

Conclusion

The substantial reduction observed in both bacterial load and oral malodor suggests that the CPC + Zn mouthwash serves as a highly effective oral hygiene product, combatting oral biofilms and targeting malodor. The ability to provide "pleasant breath" further enhances its applicability in daily oral care, improving users' social interactions and overall quality of life. These findings underscore the potential for this mouthwash formulation to become a preferred option in oral health regimens, particularly for individuals seeking enhanced antibacterial and breath-freshening benefits beyond conventional products.

ACKNOWLEDGMENTS

Technical writing and data visualization were provided by Meghan A. Berryman, PhD. The author contributions were as follows: LS and CD: investigation, formal analysis; RA: methodology; LM: formal analysis, validation; NL and YZ: conceptualization, funding acquisition, supervision; DH: project administration, investigation. All authors contributed to writing, review, and editing.

DISCLOSURES

This clinical trial was supported by funding from Colgate-Palmolive Company. The study was reviewed and approved by the Institutional Review Board of China Oral Health Foundation, 18-A South Avenue, Zhongguancun, Haidian District Beijing, 100081.

DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Mouthwash Containing Cetylpyridinium Chloride and Zinc Lactate Shows Enhanced Antiplaque and Antigingivitis Efficacy

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Abstract: *Background:* While toothbrushing remains the primary technique recommended for mechanically removing plaque above the gumline, it often leaves interproximal plaque unaddressed. Cetylpyridinium chloride (CPC) is a well-known antibacterial for use in mouthwash formulas, demonstrated to successfully reduce plaque and gingivitis throughout the mouth. There is evidence, however, that the addition of zinc lactate to the formula increases its efficacy in vivo. *Methods:* A randomized, single-center, three-arm, examiner-blind, parallel-group clinical trial was conducted over 6 weeks in the Dominican Republic to assess the efficacy of two alcohol-free mouthwashes containing (1) 0.075% CPC, 0.28% zinc lactate, and 0.05% sodium fluoride (CPC + Zn) and (2) 0.075% CPC and 0.05% sodium fluoride (CPC) compared to a fluoride-free, alcohol-free placebo mouthwash on established dental plaque and gingivitis. One hundred and sixteen participants took part in the phase III clinical study. The Stewart Quantification Plaque Index was used to measure whole mouth, interproximal, gumline, and severity for plaque, and the Löe-Silness Gingival Index was used to measure whole mouth, interproximal, and bleeding for the gingiva. *Results:* All plaque and gingivitis scores improved statistically significantly ($P < .05$) for both the CPC + Zn and CPC treatment groups for all timepoints compared to the placebo group. After 6 weeks, the CPC + Zn treatment group showed statistically significantly ($P < .05$) greater reductions of 16.1% in whole-mouth plaque index, 16.4% in interproximal plaque, 9.4% in gumline plaque, 27.0% in plaque severity, 13.2% in whole-mouth gingival index, 14.8% in interproximal gingivitis, and 28.6% in gingival bleeding compared to the CPC treatment group. *Conclusions:* These results demonstrated that both the CPC + Zn and CPC-only mouthwashes significantly reduced established dental plaque and gingivitis compared to a fluoride-free, alcohol-free placebo mouthwash after 4 and 6 weeks. Importantly, the CPC + Zn mouthwash provided significantly greater reductions in all measured indices after 4 and 6 weeks compared to the CPC-only mouthwash. *Practical Implications:* The alcohol-free CPC + Zn mouthwash studied here is a superior option when choosing an alcohol-free mouthrinse for control of plaque and gingivitis.

While toothbrushing remains the primary technique recommended for mechanically removing plaque above the gumline, it often leaves interproximal plaque unaddressed.[1] The addition of a mouthwash to the oral care regimen can greatly impact the management of oral health by reducing plaque biofilms.[2] Using antibacterial mouthwash specifically in conjunction with toothbrushing has been demonstrated to successfully reduce interproximal plaque and gingivitis throughout the mouth.[3-5] Cetylpyridinium chloride (CPC) is a well-known antibacterial ingredient in many mouthwashes and is recognized as safe and effective for use against plaque and gingivitis in the US Food and Drug Administration's 2003 Advance Notice of Proposed Rulemaking for Over-the-Counter Antigingivitis/Antiplaque Drug products, based on the recommendation of the Dental Plaque Subcommittee of the Nonprescription Drugs Advisory Committee.[6,7]

The present study focuses on CPC, a quaternary ammonium compound whose amphiphilic properties disrupt the bacterial cell membrane, resulting in antimicrobial efficacy against plaque.[8] A recent review highlighting the efficacy of CPC mouthwash against interproximal plaque and gingival inflammation included studies with CPC levels ranging from 0.05% to 0.075% and all eight studies found that CPC demonstrated significantly better results than the control tested.[3] However, most of the studies tested mouthwash featuring CPC alone compared to a mouthwash with or without fluoride.

This clinical trial evaluated the antiplaque and antigingivitis efficacy of a mouthwash formula containing 0.075% CPC and 0.28% zinc lactate in an alcohol-free base (CPC + Zn) versus a mouthwash with 0.075% CPC in an alcohol-free base (CPC) and a fluoride-free and alcohol-free placebo mouthwash. The addition of zinc salt to enhance oral care formulations has been seen previously with evidence that zinc salt acts in a compounding manner to antimicrobial properties.[9-11] Previous research in vitro supports the evidence that CPC + Zn combats pathogens associated with periodontal disease without destroying the healthy balance of the oral microbiome.[12] The addition of zinc lactate to the formula also increased its efficacy in vivo, as evidenced by a 54.5% greater reduction in gingivitis severity after 6 weeks of use compared to a mouthwash formula containing 0.07% CPC alone.[13] Of note, zinc has been shown to combat halitosis-associated bacteria as a clinically effective additive against oral malodor.[14,15]

Therefore, the authors hypothesize that CPC + Zn mouthwash will be significantly better at reducing plaque and gingivitis compared to both the CPC mouthwash and the placebo due to the addition of zinc lactate. These results suggest that the alcohol-free CPC + Zn mouthwash studied here may be a superior option when choosing an alcohol-free mouthrinse for control of plaque and gingivitis.

Materials and Methods

Interventions

1. A 0.075% CPC, 0.28% zinc lactate, and 0.05% sodium fluoride mouthwash in an alcohol-free base (CPC + Zn) (Colgate-Palmolive Co., colgatepalmolive.com).
2. A 0.075% CPC and 0.05% sodium fluoride mouthwash in an alcohol-free base (CPC) (Colgate-Palmolive Co.).
3. A placebo mouthwash in a fluoride-free, alcohol-free base.

Study Design

To assess the clinical antiplaque and antigingivitis efficacy of a mouthwash containing CPC + Zn compared to a mouthwash with just CPC and a placebo mouthwash, 120 adult male and female individuals with established dental plaque and gingivitis were recruited for a 6-week, three-cell, parallel-group, randomized clinical trial conducted at a single site in the Santo Domingo, Dominican Republic, area. Adverse events were noted by the study coordinator through participant interview and dental examination. The study was reviewed and approved by Consejo Nacional de Bioética en Salud Av. Bolívar No. 902, Santo Domingo, República Dominicana, Dentro de la Universidad Católica Santo Domingo.

Inclusion Criteria: For inclusion in the study, participants had to: (1) be between the ages of 18 and 70; (2) be available for the full duration of the study; (3) have ≥20 uncrowned permanent teeth (excluding third molars); (4) have an average whole-mouth plaque score of ≥1.5 on the Stewart Quantification Plaque Index when enrolled; (5) have an average whole-mouth gingivitis score of ≥1.0 on the Löe-Silness Gingival Index when enrolled.

Exclusion Criteria: Individuals were excluded from this study if they: (1) had periodontal disease, ≥5 decayed dental sites, tumors of the soft or hard oral tissue; (2) were taking antimicrobial medication and/or medication that affected salivary flow within 1 month of study start date; (3) were pregnant or lactating; (4) were enrolled in another clinical study within 1 week of study start date; (5) had a history of allergies to oral care products or ingredients or a medical condition that prohibited eating and drinking for periods up to 4 hours.

Randomization and Blinding: Random assignment of qualified participants was performed by first providing a chronological identification number then assigning a treatment group with a computer-generated randomization list. Neither examiner, study site personnel, statistician, nor participant was informed of product allocation. To further ensure blinding, white paper concealed all products, and label information was limited to a code number corresponding to mouthwash, instructions, and safety information.

Study Protocol

Participants were asked to refrain from all oral hygiene practices for 12 hours and eating, drinking, or smoking for 4 hours prior to baseline assessment. A soft-bristled manual toothbrush and a 6-oz tube of a commercially available fluoride toothpaste were provided to the patient to use in addition to the mouthwash treatment. Instructions stated that participants were required to brush for 1 minute in the morning and in the evening, followed by rinsing for 30 seconds with 20 mL of assigned mouthwash each time. Participants were asked to refrain from flossing, using interdental stimulators, or eating/drinking for 30 minutes after rinsing.

Dental plaque scores were assigned according to the Stewart Quantification Plaque Index.[16] Dental plaque was dyed with a red/blue disclosing solution and scored at the maxillary and mandibular surfaces on each tooth using a dental light and mirror.

Whole-mouth plaque score was calculated by adding all the scores from each scoreable surface and dividing by the total number of scoreable surfaces. Interproximal, gumline, and severity scores were calculated by dividing the tooth into nine zones and quantifying each zone as: 0 = no plaque; 1 = separate flecks of plaque covering less than one third of the surface; 2 = plaque covering one third but less than two thirds of the surface; 3 = plaque covering two thirds or more of the surface. The gumline was referred to as zones A, B, and C, which were added and divided by the number of surfaces. Interproximal regions were regarded as D and F. Severity was determined by adding all zones scored as 2 or 3 and dividing by the number of surfaces.

Gingival inflammation scores were assigned according to the Löe-Silness Gingival Index.[17,18] Scoring was performed at six sites: distobuccal, midbuccal, mesiobuccal, distolingual, midlingual, and mesiolingual. Whole-mouth gingivitis score was calculated by adding all scores from each scoreable surface and dividing by the total number of scoreable surfaces. Interproximal scores were the addition of the mesial and distal scores divided by the number of mesial and distal surfaces. Bleeding scores were the addition of the 2 and 3 scores divided by the total number of surfaces scored.

Statistical Analysis

Statistical analysis was performed as stated in Stewart et al.[19] In brief, sample size was determined for an attrition rate of 10% and power of 80%, a significance of $\alpha = 0.05$, and response measure of 0.58. The per protocol population was analyzed.

Results

Trial Participants

Of the 120 individuals accepted into the study based on inclusion criteria, 116 participants completed all 6 weeks (Figure 1). One-hundred-sixteen participants were randomized into the CPC + Zn treatment group (n = 40; female = 20), the CPC treatment group (n = 39; female = 20), and the placebo group (n = 37; female = 18). The age range for the CPC + Zn group was 21 to 63 (mean: 35.3). The age range for the CPC group was 20 to 56 (35.3). The age range for the placebo group was 21 to 56 (33.1). There was no statistically significant difference between gender ($P > .05$) or age ($P > .05$) across the three treatment groups (Table 1). No adverse events were reported.

TABLE 1

Demographic Summary of Participants

TREATMENT GROUP	NUMBER OF PARTICIPANTS (FEMALE)	MEAN AGE	AGE RANGE
CPC + Zn	40 (20)	35.3	21–63
CPC	39 (20)	35.3	20–56
Placebo	37 (18)	33.1	21–56

No statistically significant ($P > .05$) difference was indicated across the three treatment groups respective to age and gender.
CPC = cetylpyridinium chloride, Zn = zinc lactate

Plaque Index Analysis

Within-Treatment Comparison to Baseline

Mean whole-mouth plaque index scores statistically significantly decreased by 26.5% ($P < .05$) after 4 weeks and by 33.3% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 2 A). Mean whole-mouth plaque index scores significantly decreased by 16.9% ($P < .05$) after 4 weeks and by 24.7% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean whole-mouth plaque index scores did not statistically significantly decrease after 4 weeks or 6 weeks of treatment with placebo mouthwash compared to baseline.

Mean interproximal plaque index scores statistically significantly decreased by 26.2% ($P < .05$) after 4 weeks and by 36.2% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 2 B). Mean interproximal plaque index scores statistically significantly decreased by 15.6% ($P < .05$) after 4 weeks and by 24.3% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean interproximal plaque index scores statistically significantly decreased by 4.4% ($P < .05$) after 4 weeks and by 4.1% ($P < .05$) after 6 weeks of treatment with placebo mouthwash compared to baseline.

Mean gumline plaque index scores statistically significantly decreased by 9.7% ($P < .05$) after 4 weeks and by 12.5% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 2 C). Mean gumline plaque index scores statistically significantly decreased by 3.7% ($P < .05$) after 4 weeks and by 7.6% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean gumline plaque index scores statistically significantly increased by 8.2% ($P < .05$) after 4 weeks and by 10.5% ($P < .05$) after 6 weeks of treatment with placebo mouthwash compared to baseline.

Mean plaque severity scores statistically significantly decreased by 35.6% ($P < .05$) after 4 weeks and by 44.4% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 2 D). Mean plaque severity scores statistically significantly decreased by 20.7% ($P < .05$) after 4 weeks and by 29.9% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean plaque severity scores statistically significantly increased by 4.3% ($P < .05$) after 4 weeks and by 9.8% ($P < .05$) after 6 weeks of treatment with placebo mouthwash compared to baseline.

Between-Treatment Comparison

The CPC + Zn treatment group showed a statistically significant 16.1% greater reduction ($P < .05$) in whole-mouth plaque index scores after 4 weeks and 6 weeks of treatment compared to the CPC treatment group (Figure 2 A). Compared to the placebo treatment group, the CPC + Zn group had a 29.1% ($P < .05$) and a 36.5% ($P < .05$) statistically significantly greater reduction in whole-mouth plaque index after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 15.4% ($P < .05$) and

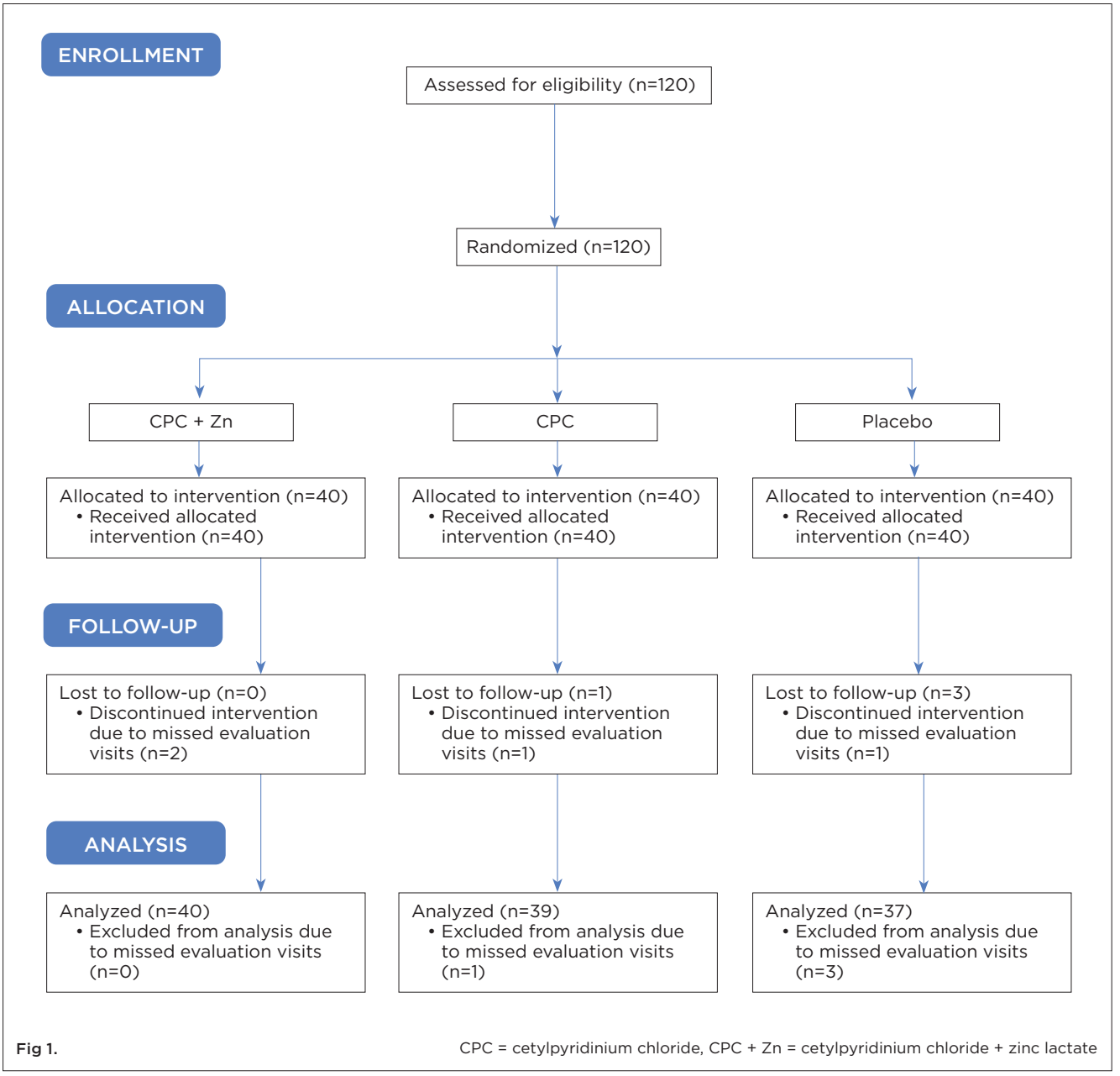

Fig 1. CPC = cetylpyridinium chloride, CPC + Zn = cetylpyridinium chloride + zinc lactate

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

a 24.3% ($P < .05$) statistically significantly greater reduction in whole-mouth plaque index compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

The CPC + Zn treatment group showed a statistically significant 13.4% greater reduction ($P < .05$) in interproximal plaque scores after 4 weeks and a 16.4% greater reduction ($P < .05$) after 6 weeks of treatment compared to the CPC treatment group (Figure 2 B). Compared to the placebo treatment group, the CPC + Zn group had a 22.4% ($P < .05$) and a 33.0% ($P < .05$) statistically significantly greater reduction in interproximal plaque after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 10.4% ($P < .05$) and a 19.9% ($P < .05$) statistically significantly greater reduction in interproximal plaque compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

The CPC + Zn treatment group showed a statistically significant 10.3% greater reduction ($P < .05$) in gumline plaque scores after 4 weeks and a 9.4% greater reduction ($P < .05$) after 6 weeks of treatment compared to the CPC treatment group (Figure 2 C). Compared to the placebo treatment group, the CPC + Zn group had a 18.2% ($P < .05$) and a 22.5% ($P < .05$) statistically significantly greater reduction in gumline plaque after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 8.8% ($P < .05$) and a 14.5% ($P < .05$) statistically significantly greater reduction in gumline plaque compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

The CPC + Zn treatment group showed a statistically significant

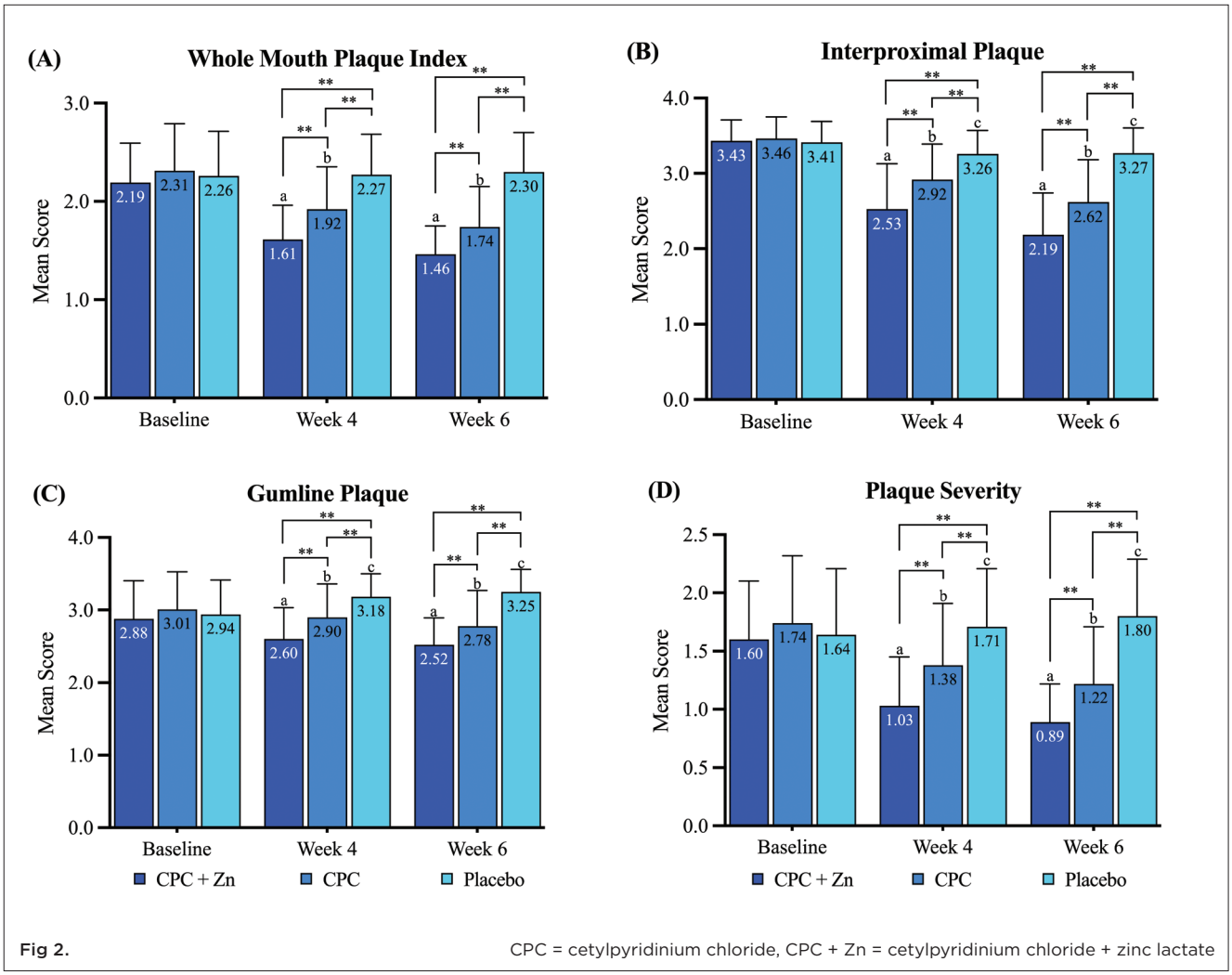

Fig 2. CPC = cetylpyridinium chloride, CPC + Zn = cetylpyridinium chloride + zinc lactate

Fig 2. Mean Plaque Index Scores Over Time. (A) Mean whole-mouth plaque index scores over time. (B) Mean interproximal plaque index scores over time. (C) Mean gumline plaque index scores over time. (D) Mean plaque severity scores over time. Statistical analysis was performed via paired t-test for within-treatment comparisons to baseline: a = $P < .05$ compared to CPC + Zn treatment group baseline; b = $P < .05$ compared to CPC treatment group baseline; c = $P < .05$ compared to placebo treatment group baseline. Statistical analysis was performed via analysis of covariance (ANCOVA) for between-treatment comparisons of baseline-adjusted means: ** $P < .05$.

25.4% greater reduction ($P < .05$) in plaque severity scores after 4 weeks and a 27.0% statistically significantly greater reduction ($P < .05$) after 6 weeks of treatment compared to the CPC treatment group (Figure 2 D). Compared to the placebo treatment group, the CPC + Zn group had a 39.8% ($P < .05$) and a 50.6% ($P < .05$) statistically significantly greater reduction in plaque severity after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 19.3% ($P < .05$) and a 32.2% ($P < .05$) statistically significantly greater reduction in plaque severity compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

Gingival Index Analysis

Within-Treatment Comparison to Baseline

Mean whole-mouth gingival index scores statistically significantly decreased by 18.1% ($P < .05$) after 4 weeks and by 30.0% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 3 A). Mean whole-mouth gingival index scores statistically significantly decreased by 9.9% ($P < .05$) after 4 weeks and by 19.9% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean whole-mouth gingival index scores statistically significantly increased by 2.0% ($P < .05$) after 4 weeks and by 4.1% ($P < .05$) after 6 weeks of treatment with placebo mouthwash compared to baseline.

Mean interproximal gingivitis scores statistically significantly decreased by 19.9% ($P < .05$) after 4 weeks and by 32.7% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 3 B). Mean interproximal gingivitis scores statistically significantly decreased by 10.5% ($P < .05$) after 4 weeks and by 21.1% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean interproximal gingivitis scores statistically significantly increased by 2.5% ($P < .05$) after 4 weeks but did not significantly increase or decrease after 6 weeks of treatment with placebo mouthwash compared to baseline.

Mean gingival bleeding scores statistically significantly

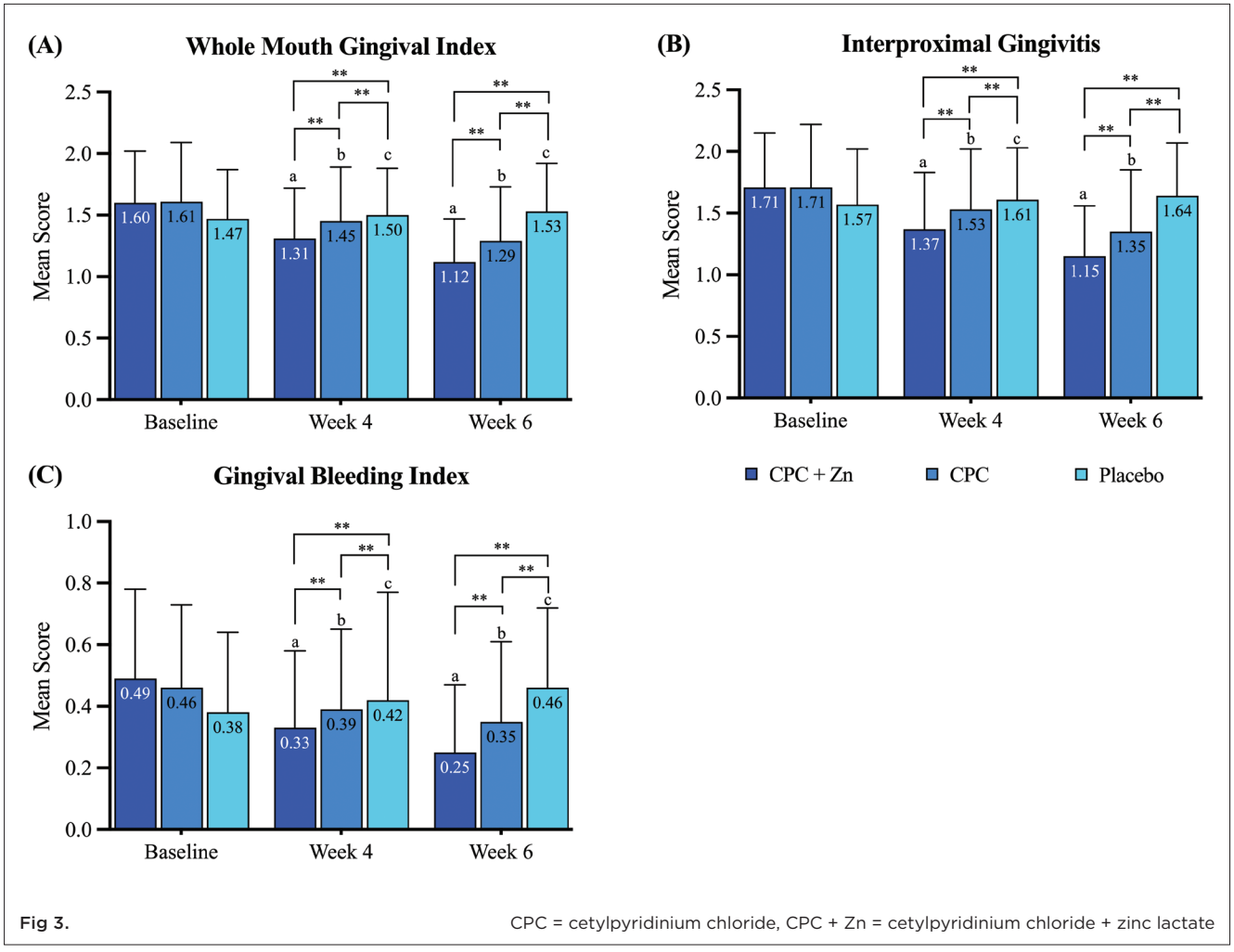

Fig 3. CPC = cetylpyridinium chloride, CPC + Zn = cetylpyridinium chloride + zinc lactate

Fig 3. Mean Gingival Index Scores Over Time. (A) Mean whole-mouth gingival index scores over time. (B) Mean interproximal gingivitis scores over time. (C) Mean gingival bleeding scores over time. Statistical analysis was performed via paired t-test for within-treatment comparisons to baseline: a = $P < .05$ compared to CPC + Zn treatment group baseline; b = $P < .05$ compared to CPC treatment group baseline; c = $P < .05$ compared to placebo treatment group baseline. Statistical analysis was performed via ANCOVA for between-treatment comparisons of baseline-adjusted means: ** $P < .05$.

decreased by 32.7% ($P < .05$) after 4 weeks and by 49.0% ($P < .05$) after 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Figure 3 C). Mean gingival bleeding scores statistically significantly decreased by 15.2% ($P < .05$) after 4 weeks and by 23.9% ($P < .05$) after 6 weeks of treatment with CPC mouthwash compared to baseline. Mean gingival bleeding scores statistically significantly increased by 10.5% ($P < .05$) after 4 weeks and by 21.1% ($P < .05$) after 6 weeks of treatment with placebo mouthwash compared to baseline.

Between-Treatment Comparison

The CPC + Zn treatment group showed a statistically significant 9.7% greater reduction ($P < .05$) in whole-mouth gingival index scores after 4 weeks and a 13.2% greater reduction ($P < .05$) after 6 weeks of treatment compared to the CPC treatment group (Figure 3 A). Compared to the placebo treatment group, the CPC + Zn group had a 12.7% ($P < .05$) and a 26.8% ($P < .05$) statistically significantly greater reduction in whole-mouth gingival index after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 3.3% ($P < .05$) and a 15.7% ($P < .05$) greater reduction in whole-mouth gingival index compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

The CPC + Zn treatment group showed a statistically significant 10.5% greater reduction ($P < .05$) in interproximal gingivitis scores after 4 weeks and a 14.8% greater reduction ($P < .05$) after 6 weeks of treatment compared to the CPC treatment group (Figure 3 B). Compared to the placebo treatment group, the CPC + Zn group had a 14.9% ($P < .05$) and a 29.9% ($P < .05$) statistically significantly greater reduction in interproximal gingivitis after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 5.0% ($P < .05$) and a 17.7% ($P < .05$) greater reduction in interproximal gingivitis compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

The CPC + Zn treatment group showed a statistically significant 15.4% greater reduction ($P < .05$) in gingival bleeding scores after 4 weeks and a 28.6% greater reduction ($P < .05$) after 6 weeks of

treatment compared to the CPC treatment group (Figure 3 C). Compared to the placebo treatment group, the CPC + Zn group had a 21.4% ($P < .05$) and a 45.7% ($P < .05$) statistically significantly greater reduction in gingival bleeding after 4 weeks and 6 weeks of treatment, respectively. The CPC treatment group had a 7.1% ($P < .05$) and a 23.9% ($P < .05$) greater reduction in gingival bleeding compared to the placebo group after 4 weeks and 6 weeks of treatment, respectively.

Discussion

This 6-week study demonstrated the superiority of CPC + Zn mouthwash in treating individuals with established dental plaque and gingivitis compared to mouthwash containing CPC alone or rinsing with a fluoride-free, alcohol-free placebo mouthwash. Both CPC + Zn and CPC treatments significantly reduced plaque and gingivitis across all indices compared to baseline and the placebo treatment. However, the CPC + Zn treatment provided significantly greater reductions at 4 weeks than the CPC treatment was able to achieve even after 6 weeks.

The results of this study are similar to a previously published 6-week plaque and gingivitis clinical trial comparing the same CPC + Zn mouthwash with a 0.07% CPC and 0.05% sodium fluoride mouthwash.[13] After 6 weeks, Rösing et al observed a 16.8% greater reduction in whole-mouth plaque index and a 14.3% greater reduction in whole-mouth gingival index for CPC + Zn compared to the 0.07% CPC mouthwash, which is comparable to the 16.1% and 13.2% seen in this study, respectively. However, mean gingival bleeding scores, which were referred to as gingival severity in Rösing et al, were reduced 28.6% by the CPC + Zn mouthwash in this study and 54.5% in the Rösing et al study compared to the CPC-alone group. This discrepancy could be due to the CPC-alone mouthwash in this study containing 0.075% CPC as opposed to 0.07% CPC as in the Rösing et al study. A lower percentage of the active ingredient could result in a greater difference between the two test groups, but more likely the difference may be due to formulation differences or difference in study populations.

The inclusion of zinc lactate in this CPC mouthwash formulation enhanced the antiplaque and antigingivitis efficacy of CPC. The addition of a zinc salt to enhance oral care formulations has been seen previously. Brading et al in 2003 showed that a 0.3% triclosan toothpaste with added zinc citrate had superior antimicrobial efficacy compared to a 0.3% triclosan toothpaste alone.[9] A small SARS-CoV-2 study found that 0.075% CPC plus 0.28% zinc lactate mouthwash treatment showed a greater reduction in viral load in saliva after immediate use and 30 minutes compared to a 0.075% CPC-alone mouthwash.[10] In addition, a recent study found that the addition of zinc lactate to an amine/fluoride mouthwash increased the long-term antibacterial activity.[11]

Conclusion

This clinical trial was designed to evaluate the antiplaque and antigingivitis efficacy of a CPC + Zn mouthwash compared to a CPC-only mouthwash and a placebo mouthwash with no active ingredients. The authors hypothesized that the CPC + Zn would be superior in reducing plaque and gingivitis significantly compared to both the CPC-only mouthwash and the placebo due to the addition of zinc lactate. The results of the trial fully supported this hypothesis. Therefore, the alcohol-free CPC + Zn mouthwash studied here is a viable option when choosing an alcohol-free mouthwash for control of plaque and gingivitis.

ACKNOWLEDGMENTS

Technical writing was provided by Meghan A. Berryman, PhD. The author contributions were as follows: BS and BG: conceptualization, funding acquisition, supervision; LM: formal analysis, validation; JN and AE: project administration, investigation. All authors contributed to writing, review, and editing.

DISCLOSURES

This clinical trial was supported by funding from the Colgate-Palmolive Company. Institutional Review Board Approval: The study was reviewed and approved by Consejo Nacional de Bioética en Salud (CONABIOS) Av. Bolívar No. 902, Santo Domingo, República Dominicana, Dentro de la Universidad Católica Santo Domingo.

DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Antiplaque and Antigingivitis Efficacy of Mouthwash Containing Cetylpyridinium Chloride and Zinc Lactate Compared to Essential Oils With Alcohol

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Abstract: *Background:* Antibacterial mouthwashes are an effective method for reducing plaque and gingivitis when used regularly as part of an oral hygiene regimen that includes brushing and flossing. However, mouthwashes formulated with high ethanol content can be associated with a burning sensation that typically leads to lack of compliance. Alcohol-free, antibacterial mouthwash may be an effective alternative for antiplaque and antigingivitis treatment without the burn. *Methods:* A 118-participant, phase III, randomized, single-center, three-arm, examiner-blind, parallel-group clinical trial was conducted over 6 weeks in the Dominican Republic to assess the efficacy of a 0.075% cetylpyridinium chloride (CPC), 0.28% zinc lactate, and 0.05% sodium fluoride mouthwash in an alcohol-free base (CPC + Zn), an essential oils and 21.6% ethanol mouthwash (EO + EtOH), and a placebo mouthwash on established dental plaque and gingivitis. Scoring indices were used to measure whole mouth, interproximal, and severity for both plaque and gingivitis. *Results:* All plaque and gingivitis scores improved statistically significantly for both the CPC + Zn (all: $P < .001$) and EO + EtOH (all: $P < .001$) treatment groups for all timepoints compared to baseline. After 6 weeks, the CPC + Zn group exhibited a 37.2% reduction in plaque severity and a 47.7% reduction in gingivitis severity ($P < .001$), and the EO + EtOH group had a 35.9% reduction in plaque severity and a 38.6% reduction in gingivitis severity compared to baseline ($P < .001$). There was no statistically significant ($P > .05$) difference between the impact that CPC + Zn and EO + EtOH had on plaque and gingivitis reduction for all scores measured. *Conclusions:* These results demonstrated parity between CPC + Zn and EO + EtOH mouthwash formulas in the reduction of dental plaque and gingivitis. Both treatments significantly reduced whole-mouth, interproximal, and severity scores compared to the placebo. *Practical Implications:* Alcohol-free CPC + Zn mouthwash may be an effective alternative to control plaque and gingivitis for patients who struggle to comply with a regimen with alcohol-containing mouthwashes.

Mouthwash plays an important role in the reduction of plaque biofilm and the management of oral disease.[1] While toothbrushing continues to be the predominant recommendation for mechanical removal of supragingival plaque, interproximal plaque remains intact on the tooth surface after brushing alone with regular toothbrushes.[2] Antibacterial mouthwashes have proven to be an effective method for reducing interproximal plaque and whole-mouth gingivitis in adjunct use with toothbrushing.[3-5] However, many commercially available mouthwashes are formulated with a high ethanol content, which is associated with intense oral pain and lower user compliance.[6-8] Alcohol-free, antibacterial mouthwash may be an effective alternative for antiplaque and antigingivitis reduction without the burn.

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound widely used for its antibacterial properties across several types of dental treatments. It is recognized as safe and effective for use against plaque and gingivitis in the US Food and Drug Administration's 2003 Advance Notice of Proposed Rulemaking for Over-the-Counter Antigingivitis/Antiplaque Drug products, based on the recommendation of the Dental Plaque Subcommittee of the Nonprescription Drugs Advisory Committee.[9,10] The amphiphilic nature of the CPC compound disrupts and destroys bacterial cell membranes, allowing for the cellular remnants to be rinsed off the oral surface.[11] A recent review investigating eight randomized clinical trials featuring CPC-containing mouthwashes found that CPC demonstrated significant and superior antiplaque efficacy across all studies compared to the controls.[3]

The mouthwash formula presented in this study contained 0.075% CPC and 0.28% zinc lactate (CPC + Zn). In vitro studies found that CPC + Zn reduced periodontal pathogens while allowing for the colonization of oral health–associated bacterial species in biofilm.[12] The addition of zinc lactate to the formula also increases its efficacy in vivo, as evidenced by a 54.5% greater reduction in gingivitis severity after 6 weeks of use compared to a mouthwash formula containing 0.07% CPC alone.[13] Zinc has been shown to have antimicrobial properties in the context of halitosis-associated bacteria and can be clinically effective against halitosis in a mouthwash.[14,15] CPC + Zn has also been shown to have a 44.8% greater reduction in plaque compared to an alcohol-free essential oil mouthwash.[16]

Mouthwashes with essential oils in an ethanol base (EO + EtOH) are among the most widely studied mouthwashes due to their antimicrobial properties and effective plaque control.[17] A systematic review found that EO + EtOH significantly reduced plaque and gingivitis compared to a CPC mouthwash without zinc lactate.[18] A recent clinical trial even indicates that EO + EtOH achieved a more thorough reduction in interproximal plaque than flossing as an adjunct to brushing.[19]

The present clinical trial was designed to evaluate the antiplaque and antigingivitis efficacy of a CPC + Zn mouthwash compared to an EO + EtOH mouthwash and a placebo mouthwash with no active ingredients. The authors hypothesized that both CPC + Zn and EO + EtOH will equally reduce plaque and gingivitis significantly compared to the placebo.

Materials and Methods

Interventions

1. A test mouthwash containing 0.075% CPC, 0.28% zinc lactate, and 0.05% sodium fluoride in an alcohol-free base (CPC + Zn) (Colgate-Palmolive Co., colgatepalmolive.com).
2. A commercially available mouthwash containing essential oils and 21.6% ethanol (EO + EtOH) (Johnson & Johnson, jnj.com).
3. An alcohol-free placebo mouthwash with 0.05% sodium fluoride.

Study Design

This phase III, randomized, single-center, three-arm, examiner-blind, parallel-group clinical trial was conducted in the Santo Domingo, Dominican Republic, area to assess the clinical efficacy of an alcohol-free CPC + Zn mouthwash compared to an EO + EtOH mouthwash and a negative control mouthwash without CPC in a population with established dental plaque and gingivitis over a 6-week period.

Antibacterial mouthwashes are effective for reducing interproximal plaque and whole-mouth gingivitis in adjunct use with toothbrushing. However, many commercially available mouthwashes are formulated with a high ethanol content, which is associated with intense oral pain and lower user compliance. Alcohol-free, antibacterial mouthwash may be an effective alternative for antiplaque and antigingivitis reduction without the burn.

Qualifying participants were randomly assigned to one of three treatment groups in such a way that neither the examiner nor the statistician was aware of the identity of the product allocation. Participants were assigned an identification number in chronological order from 001 to 120. They were then randomized to a study group by a computer-generated randomization list.

All products were concealed with white overwrapped paper. Label information was limited to a mouthwash code, instructions for at-home use, and safety information. The examiner, study site personnel, and statistician were blinded to product assignment.

Participants and Inclusion Criteria: A total of 120 healthy female and male adults between the ages of 18 and 70 were recruited from the Santo

TABLE 1

Demographic Summary of Participants

TREATMENT GROUP	NUMBER OF PARTICIPANTS (FEMALE)	MEAN AGE (SD)	AGE RANGE
CPC + Zn	39 (21)	39.67 (10.86)	23–65
EO + EtOH	40 (20)	41.90 (11.81)	22–65
Placebo	39 (21)	40.56 (12.24)	24–69

CPC + Zn = cetylpyridinium chloride + zinc lactate, EO + EtOH = essential oils and ethanol

TABLE 2

Mean Index Scores by Treatment Group at Baseline, Week 4, and Week 6

		GINGIVAL INDEX			PLAQUE INDEX		
Treatment	Timepoint	Total	Interproximal	Severity	Total	Interproximal	Severity
CPC + Zn	Baseline	1.43 ± 0.39	1.50 ± 0.43	0.40 ± 0.28	3.54 ± 0.55	3.93 ± 0.61	0.74 ± 0.17
	Week 4	1.16 ± 0.32	1.22 ± 0.39	0.26 ± 0.20	2.82 ± 0.57	3.23 ± 0.62	0.54 ± 0.17
	Week 6	1.01 ± 0.32	1.07 ± 0.39	0.20 ± 0.17	2.50 ± 0.56	2.90 ± 0.62	0.47 ± 0.16
EO + EtOH	Baseline	1.64 ± 0.50	1.73 ± 0.54	0.50 ± 0.30	3.82 ± 0.52	4.21 ± 0.53	0.82 ± 0.12
	Week 4	1.36 ± 0.46	1.42 ± 0.51	0.35 ± 0.28	3.07 ± 0.52	3.51 ± 0.57	0.62 ± 0.14
	Week 6	1.22 ± 0.44	1.28 ± 0.49	0.30 ± 0.26	2.70 ± 0.51	3.14 ± 0.58	0.52 ± 0.15
Placebo	Baseline	1.48 ± 0.41	1.56 ± 0.45	0.43 ± 0.29	3.68 ± 0.62	4.04 ± 0.68	0.78 ± 0.18
	Week 4	1.49 ± 0.43	1.57 ± 0.47	0.43 ± 0.30	3.68 ± 0.65	4.05 ± 0.70	0.77 ± 0.19
	Week 6	1.48 ± 0.43	1.57 ± 0.47	0.43 ± 0.30	3.67 ± 0.64	4.05 ± 0.70	0.76 ± 0.19

Scores reported as mean ± standard deviation. Non-baseline scores reported as unadjusted scores. There was no statistically significant difference between mean index scores of the three treatment groups at baseline. Total gingivitis, $P = .095$; interproximal gingivitis, $P = .086$; gingivitis severity, $P = .302$; total plaque, $P = .096$; interproximal plaque, $P = .136$; plaque severity, $P = .122$

Domingo, Dominican Republic, area and randomized equally into three groups of 40 participants. To be included in the study, participants were required to be available for the 6-week duration, had to be considered in good health by the practitioner, and had to have ≥20 uncrowned permanent natural teeth, excluding the third molars. Upon baseline inspection, participants were required to have a Löe-Silness Gingival Index score of ≥1.0 and a Turesky modification of the Quigley-Hein Plaque Index score of ≥1.5.

Exclusion criteria included the presence of orthodontic bands, partial removable dentures, tumors of the soft or hard tissue in the oral cavity, advanced periodontal disease, or ≥5 decayed carious lesions requiring restorative treatment. Individuals were also excluded if they were pregnant or lactating women, had received dental prophylaxis 2 weeks prior to entry into the study, had participated in a clinical study within 1 month of this study, had a history of allergies to oral care products or their ingredients, had an existing medical condition that prevented eating or drinking for periods up to 4 hours, had a history of alcohol or drug use, or were prescribed any medication that may interfere with the study outcome.

Study Protocol

In addition to study mouthwash, all qualifying participants were provided with a regular fluoride toothpaste containing 0.76% sodium monofluorophosphate and a manual soft-bristled toothbrush. Participants were instructed to brush in the morning and in the evening for 1 minute with approximately 1.5 g of the provided toothpaste on the provided toothbrush. Following each instance of brushing, participants were instructed to rinse for 30 seconds with 20 mL of their assigned mouthwash for 6 weeks.

Participants were assessed for dental plaque according to the Turesky modification of the Quigley-Hein Plaque Index.[20,21] Dentition of each tooth was disclosed and plaque was scored at the distofacial (DF), midfacial (MidF), mesiofacial (MF), distolingual (DL), midlingual (MidL), and mesiolingual (ML) surfaces. The scoring system criteria were: 0 = no plaque; 1 = separate flecks of plaque at the cervical margin; 2 = thin, continuous ≤1 mm plaque band at the cervical margin; 3 = plaque band with >1 mm width covering < one third of the crown of the tooth; 4 = ≥ one third and < two thirds plaque coverage on the crown of the tooth, 5 = ≥ two thirds plaque coverage on the crown of the tooth.

TABLE 3

Within-Treatment Analysis of Plaque Index Scores Compared to Baseline

		WEEK 4		WEEK 6	
Treatment	Plaque Index	% Reduction	*P* Value	% Reduction	*P* Value
CPC + Zn	Total	21.2	< .001	30.2	< .001
	Interproximal	18.7	< .001	26.8	< .001
	Severity	26.9	< .001	37.2	< .001
EO + EtOH	Total	18.8	< .001	28.5	< .001
	Interproximal	15.5	< .001	24.6	< .001
	Severity	24.4	< .001	35.9	< .001
Placebo	Total	0.0	.979	0.3	.906
	Interproximal	0.0	.898	0.0	.943
	Severity	1.3	.598	2.6	.435

Positive percent reduction represents decrease in baseline-adjusted mean index score compared to baseline means. Significance determined via paired t-test compared to baseline.

TABLE 4

Within-Treatment Analysis of Gingival Index Scores Compared to Baseline

		WEEK 4		WEEK 6	
Treatment	Gingival Index	% Reduction	*P* Value	% Reduction	*P* Value
CPC + Zn	Total	19.7	< .001	30.3	< .001
	Interproximal	19.4	< .001	29.4	< .001
	Severity	36.4	< .001	47.7	< .001
EO + EtOH	Total	15.8	< .001	25.0	< .001
	Interproximal	16.9	< .001	25.6	< .001
	Severity	27.3	< .001	38.6	< .001
Placebo	Total	0.0	.811	0.7	.949
	Interproximal	0.0	.818	0.6	.899
	Severity	0.0	.941	0.0	.952

Positive percent reduction represents decrease in baseline-adjusted mean index score compared to baseline means. Significance determined via paired t-test compared to baseline.

Whole-mouth plaque score = [(DF + MF + MidF) + (DL + ML + MidL)] / 6
Interproximal plaque score = [(DF + MF) + (DL + ML)] / 4
Plaque severity score = total number of 3–5 scores / 6

Participants were assessed for gingival inflammation and scored at the DF, MidF, MF, DL, MidL, and ML sites of each tooth according to the Löe-Silness Gingival Index.[22,23] The criteria for scoring were: 0 = no inflammation; 1 = slight change in color and texture, indicating mild inflammation; 2 = moderate glazing, redness, edema, and hypertrophy, indicating moderate inflammation; 3 = marked redness and spontaneous bleeding, indicating severe inflammation.

Whole-mouth gingivitis score = [(DF + MF + MidF) + (DL + ML + MidL)] / 6
Interproximal gingivitis score = [(DF + MF) + (DL + ML)] / 4
Gingivitis severity score = total number of 2–3 scores / 6

Participants were also assessed by the dental examiner visually with a dental light and mirror to evaluate the soft and hard palate, gingival mucosa, buccal mucosa, mucogingival fold areas, tongue,

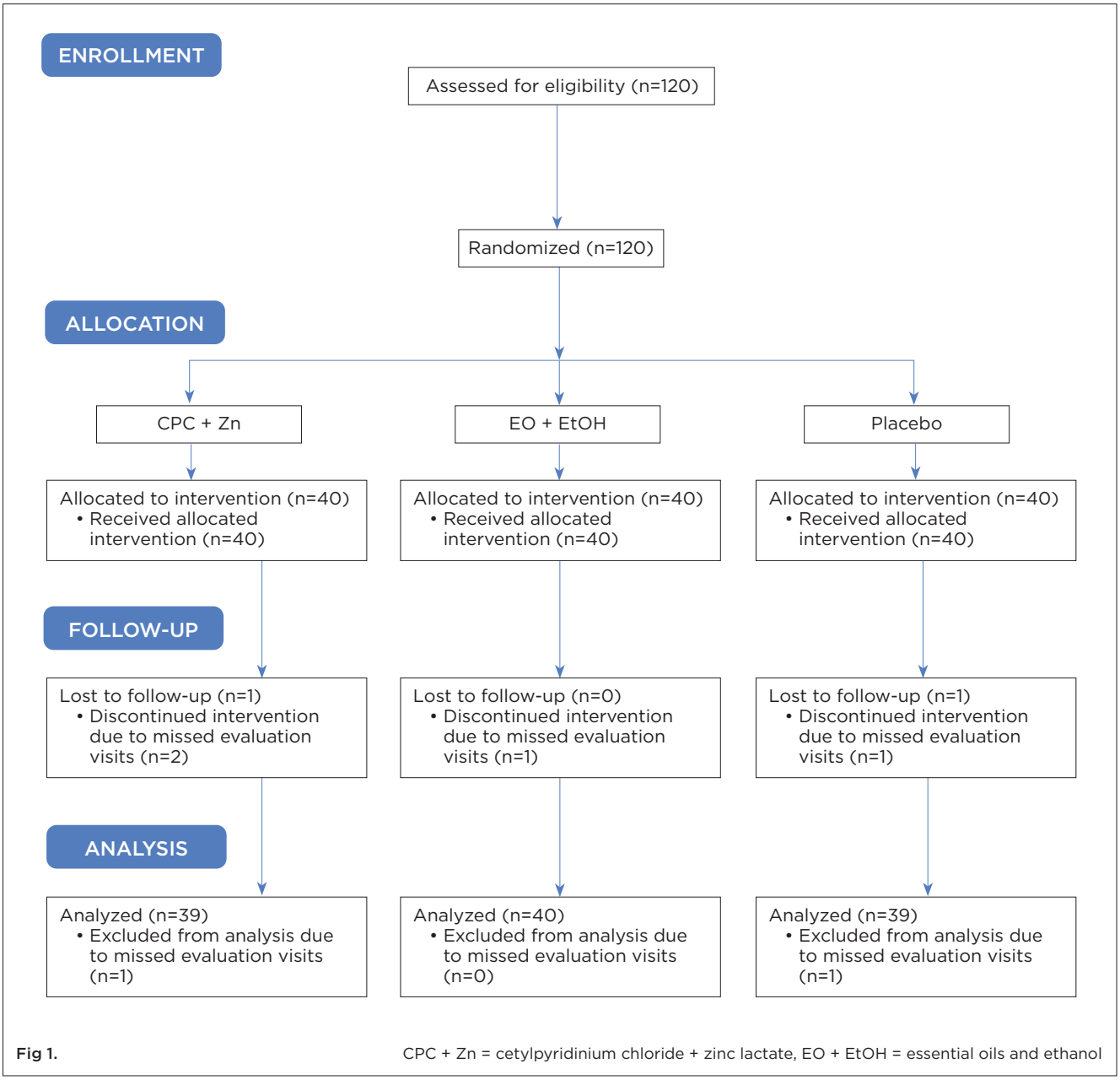

Fig 1. CPC + Zn = cetylpyridinium chloride + zinc lactate, EO + EtOH = essential oils and ethanol

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

sublingual and submandibular areas, salivary glands, tonsillar and pharyngeal areas, and teeth.

Adverse events were monitored via participant interview and dental examination.

Statistical Analysis

A sample size of 120 participants divided into three groups of 40 participants was based on a response measure of 0.58, a significance level of $\alpha = 0.05$, a 10% attrition rate, and an 80% power level as previously described in the literature.[24,25] The per protocol population was analyzed. Chi-square analysis was used to determine significant differences for gender between groups. An independent t-test was used to determine significant differences for age between groups.

Statistical analyses were performed separately for scores from Löe-Silness Gingival Indices and Quigley-Hein Plaque Indices assessments. Analysis of variance (ANOVA) was performed for between-treatment baseline comparisons. Paired t-tests were used for within-treatment baseline versus follow-up score comparisons. Analysis of covariance (ANCOVA) was performed for within-treatment baseline-adjusted versus follow-up score comparisons. Tukey's test for multiple comparisons was used to conduct post-ANCOVA pair-wise comparisons of treatment groups. All statistical tests conducted used a significance of $\alpha = 0.05$ and were two-sided.

TABLE 5

Between-Treatment Analysis of Plaque Index Scores

Treatment Comparison	Plaque Index	WEEK 4		WEEK 6	
		% Difference	*P* Value	% Difference	*P* Value
CPC + Zn vs. EO + EtOH	Total	3.0	.678	2.3	.837
	Interproximal	3.8	.552	2.9	.760
	Severity	3.4	.628	2.0	.946
CPC + Zn vs. Placebo	Total	21.2	< .001	30.0	< .001
	Interproximal	18.7	< .001	26.8	< .001
	Severity	26.0	< .001	35.5	< .001
EO + EtOH vs. Placebo	Total	18.8	< .001	28.3	< .001
	Interproximal	15.5	< .001	24.6	< .001
	Severity	23.4	< .001	34.2	< .001

A positive percent difference indicates a greater reduction in baseline-adjusted mean index score for the first treatment group listed compared to the second treatment group. Significance determined by ANCOVA.

TABLE 6

Between-Treatment Analysis of Gingival Index Scores

Treatment Comparison	Gingival Index	WEEK 4		WEEK 6	
		% Difference	*P* Value	% Difference	*P* Value
CPC + Zn vs. EO + EtOH	Total	4.7	.665	7.0	.500
	Interproximal	3.0	.822	5.0	.712
	Severity	12.5	.697	14.8	.577
CPC + Zn vs. Placebo	Total	19.7	< .001	29.8	< .001
	Interproximal	19.4	< .001	28.9	< .001
	Severity	36.4	.001	47.7	< .001
EO + EtOH vs. Placebo	Total	15.8	.001	24.5	< .001
	Interproximal	16.9	.002	25.2	< .001
	Severity	27.3	.015	38.6	< .001

A positive percent difference indicates a greater reduction in baseline-adjusted mean index score for the first treatment group listed compared to the second treatment group. Significance determined by ANCOVA.

Results

Trial Participants

One-hundred-twenty participants were accepted into the study based on the inclusion and exclusion criteria (Figure 1). Two participants were dismissed for not complying with protocol requirements. One-hundred-eighteen participants were randomized into the CPC + Zn treatment group (n = 39; female = 21), the EO + EtOH treatment group (n = 40; female = 20), and the placebo group (n = 39; female = 21). The age range for the CPC + Zn group was 23 to 65 (mean ± standard deviation [SD]: 39.67 ± 10.86). The age range for the EO + EtOH group was 22 to 65 (41.90 ± 11.81). The age range for the placebo group was 24 to 69 (40.56 ± 12.24). There were no statistically significant differences between gender ($P = .925$) or age ($P = .693$) across the three treatment groups (Table 1).

Neither examiner nor participants reported any adverse effects on the oral hard or soft tissue. The two participants who did not complete the study reported reasons unrelated to the treatments.

Within-Treatment Analysis

At baseline, there were no statistically significant ($P > .05$) differences between mean scores for any of the plaque or gingival

indices between the three treatment groups (Table 2). For the placebo treatment group, there was no significant difference between mean plaque index or gingival index scores compared to baseline at either week 4 or week 6 (Table 3).

CPC + Zn Treatment Over Time Compared to Baseline

All plaque index scores decreased significantly over time for the CPC + Zn treatment group (Table 3). Total plaque scores were reduced by 30.2% from an average of 3.54 (±0.55) at baseline to 2.57 (±0.08) baseline-adjusted mean score by week 6 ($P < .001$). Interproximal plaque scores were reduced by 26.8% from an average of 3.93 (±0.61) at baseline to 2.97 (±0.09) baseline-adjusted mean score by week 6 ($P < .001$). Plaque severity was reduced by 37.2% from an average of 0.74 (±0.17) at baseline to 0.49 (±0.02) baseline-adjusted mean score by week 6 ($P < .001$). All mean plaque index scores showed a continuous reduction after 4 and 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Table 2).

All gingival index scores decreased significantly over time for the CPC + Zn treatment group (Table 4). Total gingivitis was reduced by 30.3% from an average of 1.43 (±0.39) at baseline to 1.06 (±0.05) baseline-adjusted mean score by week 6 ($P < .001$). Interproximal gingivitis was reduced by 29.4% from an average of 1.50 (±0.43) at baseline to 1.13 (±0.06) baseline-adjusted mean score by week 6 ($P < .001$). Gingivitis severity was reduced by 47.7% from an average of 0.40 (±0.28) at baseline to 0.23 (±0.03) baseline-adjusted mean score by week 6 ($P < .001$). All mean gingival index scores showed a continuous reduction after 4 and 6 weeks of treatment with CPC + Zn mouthwash compared to baseline (Table 2).

This phase III, randomized clinical trial was conducted to assess the clinical efficacy of an alcohol-free CPC + Zn mouthwash compared to an EO + EtOH mouthwash and a negative control mouthwash without CPC in a population with established dental plaque and gingivitis over a 6-week period.

EO + EtOH Treatment Over Time Compared to Baseline

All plaque index scores decreased significantly over time for the EO + EtOH treatment group (Table 3). Total plaque scores were reduced by 28.5% from an average of 3.82 (±0.52) at baseline to 2.63 (±0.08) baseline-adjusted mean score by week 6 ($P < .001$). Interproximal plaque scores were reduced by 24.6% from an average of 4.21 (±0.53) at baseline to 3.06 (±0.09) baseline-adjusted mean score by week 6 ($P < .001$). Plaque severity was reduced by 35.9% from an average of 0.82 (±0.12) at baseline to 0.50 (±0.02) baseline-adjusted mean score by week 6 ($P < .001$). All mean plaque index scores showed a continuous reduction after 4 and 6 weeks of treatment with EO + EtOH mouthwash compared to baseline (Table 2).

All gingival index scores decreased significantly over time for the EO + EtOH treatment group (Table 4). Total gingivitis was reduced by 25.0% from an average of 1.64 (±0.50) at baseline to 1.14 (±0.05) baseline-adjusted mean score by week 6 ($P < .001$). Interproximal gingivitis was reduced by 25.6% from an average of 1.73 (±0.54) at baseline to 1.19 (±0.06) baseline-adjusted mean score by week 6 ($P < .001$). Gingivitis severity was reduced by 38.6% from an average of 0.50 (±0.30) at baseline to 0.27 (±0.03) baseline-adjusted mean score by week 6 ($P < .001$). All mean gingival index scores showed a continuous reduction after 4 and 6 weeks of treatment with EO + EtOH mouthwash compared to baseline (Table 2).

Between-Treatment Analysis

CPC + Zn Treatment Compared to EO + EtOH Treatment

While both CPC + Zn and EO + EtOH treatment groups significantly decreased plaque over time, there was no significant difference between the groups' percentage plaque reductions observed across any of the three plaque indices at either week 4 or week 6 (all: $P > .05$) (Table 5). Gingivitis was also significantly reduced in both the CPC + Zn and EO + EtOH treatment groups, but there was no significant difference between the two groups in percentage reduction observed across any of the gingival indices at either week 4 or week 6 (all: $P \geq .05$) (Table 6).

CPC + Zn Treatment Compared to Placebo Treatment

The CPC + Zn treatment group had a 30.0% greater reduction in total plaque, a 26.8% greater reduction in interproximal plaque, and a 35.5% reduction in plaque severity after 6 weeks compared to the group that rinsed with a placebo mouthwash (all: $P < .001$) (Table 5).

This group also exhibited a significantly greater reduction in gingivitis across all indices. Clinicians observed a 29.8% greater reduction in total gingivitis, a 28.9% greater reduction in interproximal gingivitis, and a 47.7% reduction in gingivitis severity after 6 weeks of rinsing with CPC + Zn mouthwash compared to the group that rinsed with a placebo mouthwash (all: $P < .001$) (Table 6).

EO + EtOH Treatment Compared to Placebo Treatment

The EO + EtOH treatment group had a 28.3% greater reduction in total plaque, a 24.6% greater reduction in interproximal plaque, and a 34.2% reduction in plaque severity after 6 weeks of rinsing with EO + EtOH mouthwash compared to the group that rinsed with a placebo mouthwash (all: $P < .001$) (Table 5).

This group also exhibited a significantly greater reduction in gingivitis across all indices. Clinicians observed a 24.5% greater reduction in total gingivitis, a 25.2% greater reduction in interproximal gingivitis, and a 38.6% reduction in gingivitis severity after 6 weeks of rinsing with EO + EtOH mouthwash compared to the group that rinsed with a placebo mouthwash (all: $P < .001$) (Table 6).

Discussion

This 6-week clinical trial demonstrated parity between CPC + Zn and EO + EtOH mouthwash formulas in the reduction of dental plaque and gingivitis. Both treatment groups significantly reduced whole-mouth, interproximal, and severity plaque and gingivitis scores compared to baseline and the placebo. While there was no significant difference between the efficacy of these two mouthwashes compared to each other, CPC + Zn consistently outperformed EO + EtOH compared to placebo, as evidenced by a 47.7% greater reduction in gingivitis severity by CPC + Zn after 6 weeks of treatment compliance compared to a 38.6% greater reduction after EO + EtOH treatment.

A previous clinical trial featuring a CPC mouthwash without zinc lactate compared to EO + EtOH mouthwash also demonstrated no statistical difference between the treatments for all plaque and gingivitis indices measured after 6 weeks of use.[26] Given that CPC + Zn has been reported to have a 54.5% greater reduction in gingivitis severity after 6 weeks of use compared to a mouthwash formula containing 0.07% CPC alone,[13] there was reason to believe that the CPC + Zn formula would have a greater statistically significant reduction in plaque and gingivitis indices compared to EO + EtOH. Additionally, Schaeffer et al has shown in an in vitro biofilm study that CPC + Zn and EO + EtOH had a similar percent reduction in biofilm viability after 2 hours of mouthwash treatment compared to the negative control at 42.8% and 42.6% reduction, respectively.[27] However, after 5 hours of mouthwash treatment the CPC + Zn formula resulted in a 62.1% reduction and the EO + EtOH declined to a 19.46% reduction, showing that the bacteria in biofilm increased in viability after prolonged exposure to EO + EtOH but decreased in viability after prolonged exposure to CPC + Zn. The reason that the clinical study reported here did not find a clinically significant difference between CPC + Zn and EO + EtOH despite CPC + Zn's proven anti-biofilm properties could reflect that standard plaque indices do not distinguish between living and dead bacteria and may not correlate with the proportion of bacterial viability within the plaque biofilm.

Despite suggestions that the act of mouth rinsing alone can reduce plaque and gum inflammation,[28] the placebo mouthwash in this trial demonstrated no reduction in total plaque and total gingivitis after 4 weeks and 6 weeks of use. The 30% greater reduction than placebo in whole-mouth plaque and gingivitis after 6 weeks of CPC + Zn treatment observed in this study is supported by two clinical studies comparing CPC + Zn mouthwash compared to mouthwash containing 0.02% sodium fluoride, which found that rinsing with CPC + Zn significantly decreased whole-mouth plaque and gingivitis by an average of 26.4% and 21.1% more than the control, respectively.[29]

Conclusion

This clinical trial evaluated the antiplaque and antigingivitis efficacy of a CPC + Zn mouthwash compared to an EO + EtOH mouthwash and a placebo mouthwash. It was hypothesized that both CPC + Zn and EO + EtOH would equally reduce plaque and gingivitis significantly compared to the placebo. The results of the trial fully supported this hypothesis. There was, however, no significant difference between the two test mouthwashes at the measured timepoints. Given that mouthwashes formulated with ethanol can be associated with intense oral pain and lower user compliance, an alcohol-free CPC + Zn mouthwash may be an effective alternative for reducing plaque and treating gingivitis in patients who prefer to avoid the oral pain associated with alcohol-containing mouthwashes.

ACKNOWLEDGMENTS

Technical writing was provided by Meghan A. Berryman, PhD. The author contributions were as follows: BS and BG: conceptualization, funding acquisition, supervision; RD: methodology; LM: formal analysis, validation; JN and AE: project administration, investigation. All authors contributed to writing, review, and editing.

DISCLOSURES

This clinical trial was supported by funding from Colgate-Palmolive Company. Institutional Review Board Approval: The study was reviewed and approved by Consejo Nacional de Bioética en Salud (CONABIOS) Av. Bolívar No. 902, Santo Domingo, República Dominicana, Dentro de la Universidad Católica Santo Domingo.

DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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