

Investigative Study



# EFFECT OF TOOTHPASTE BASED ON SODIUM FLUORIDE AND ZINC LACTATE ON BACTERIAL CONTROL

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## ABSTRACT

To assess the anti-plaque effects of a toothpaste containing sorbitol, zinc-lactate, and sodium fluoride (TCSF) in subjects with moderate plaque-induced gingivitis, a total of 10 patients with gingivitis were enrolled. None of these patients had previously been treated for periodontal disease and demonstrated radiographic evidence of bone loss. Inclusion characteristics included good general health, with both male and female subjects aged 18–70. Informed consent was obtained from all individual participants included in the study. Patients underwent professional oral hygiene (POH) and were instructed to use toothpaste TCSF at home twice a day for 2 weeks. Microbial analyses were performed prior to POH and at the end of the second week, and the results were statistically compared to the initial findings. The Student t-test was used to identify statistically significant results. All subjects completed the study. The results showed statistically significant reductions in total bacterial loading. The overall conclusion was that TCSF toothpaste was highly effective in significantly reducing bacterial load, demonstrating its comprehensive benefits as a dentifrice.

KEYWORDS: toothpaste, bacterial, oral microbiota, plaque

## INTRODUCTION

Home-based oral hygiene is a crucial element in preventing oral diseases, such as dental caries and periodontal disease (1-2). These pathologies are strongly influenced by the presence and proliferation of pathogenic bacteria within the oral cavity. Poor dental hygiene can lead to cavities, gingivitis, periodontitis, tooth loss, halitosis, fungal infections, and gum disease. Adequate daily oral hygiene helps reduce the bacterial load, thereby preventing the onset of these diseases. The use of a toothbrush is the most important measure for oral hygiene, with evidence suggesting that electric toothbrushes provide a significant benefit compared to manual toothbrushes in terms of plaque reduction, both in the short and long term. However, it is also relevant that using toothpaste improves oral hygiene.

The oral microbiota is a complex ecosystem composed of billions of microorganisms that coexist in a balanced state. This ecosystem includes both commensal bacteria, which perform beneficial roles, and potential pathogens, which can cause diseases when the balance is disturbed. Understanding the general concepts of the oral microbiota is essential for developing effective strategies to prevent and treat oral infections.

Previous studies have evaluated the effectiveness of different oral hygiene practices and products in reducing the bacterial load present in the oral cavity (3-5). These works have provided data for the use of specific oral hygiene products as an integral part of strategies to prevent oral diseases.

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The most effective way to prevent the development of dental diseases is to control the production of dental plaque, a thin, soft layer that deposits on teeth, gums, and all appliances present in the mouth through microbial action. Dietary sugars, particularly sucrose, contribute to plaque formation, and their presence increases the rate and thickness of plaque formation.

Removing plaque from teeth and surrounding areas is essential for maintaining a healthy mouth (6-10). There are various tools that patients can use for oral hygiene, including manual toothbrushes, electric toothbrushes, interdental brushes, dental floss, and mouthwash. The literature has attempted to demonstrate which method is most effective, but this does not alter the fact that the crucial point is to perform home hygiene procedures.

In this context, we conducted a preliminary study on a commercially available toothpaste to evaluate its effectiveness in reducing oral bacterial load.

## MATERIAL AND METHODS

From September to October 2023, 10 healthy patients were randomly selected. Patients were in the 18-70 age group. Subjects have not received any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they met any of the following criteria: (1) pregnancy; (2) a history of taking antibiotics or using antibacterial mouth rinses for the past 6 months; (3) smoking, drug, or alcohol abuse. Subjects participating in the study followed a detailed verbal description of the procedure and signed consent forms.

Patients underwent professional oral hygiene (POH) and were instructed to use Meridol (TCSF) toothpaste at home twice daily for 2 weeks. A total of 10 patients were selected. All patients underwent POH at the baseline measurement. Prior to POH, microbial analysis was performed. Then, POH was completed after two weeks; microbiological samples were collected again from the sites in each patient, using sterile paper tips.

For bacteria analysis, sites were isolated using cotton rolls. Sterile, absorbable paper points (size 60) were used for collecting subgingival samples, which were then immediately transferred to the microbiological laboratory for processing. Aggregatibacter actinomycetemcomitans (AA), Porphyromonas gingivalis (PG), Tannerella forsythia (TF), Treponema denticola (TD), Fusobacterium Nucleatum (FN), Campylobacter rectus (CR), and Total Bacterial Loading (CBT) were evaluated.

#### Real-time polymerase chain reaction

Oligonucleotide probes were designed based on 16S rRNA gene sequences from the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1), comprising 845 entries. All the sequences were aligned to find either a consensus sequence or less conserved spots. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified all selected bacteria in multiplex PCR assays. This reaction included a total of twelve primers and six probes that were highly specific for each species. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity, and the absence of inhibition in cases where target amounts were unbalanced. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10-minute incubation period at 95°C to activate polymerase, followed by a two-step amplification consisting of 15 seconds at 95°C and 60 seconds at 57°C for 40 cycles. All these experiments were performed, including non-template controls to exclude reagent contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg, Germany) were used as a standard for the quantitative analysis. Standard curves for each target were constructed in two triplex reactions, using a mix of the same number of plasmids in serial dilutions ranging from 101 to 107 copies. A linear relationship was observed between the threshold cycle values and the log of the copy number across the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations were estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of the relative amount of red complex species. To prevent contamination of samples and the polymerase chain reaction, plasmid purification and handling were performed in a separate laboratory using dedicated pipettes.

### Statistical analysis

The SPSS program and a paired simple t-test were used to detect statistically significant differences.

## RESULTS

Both clinical and microbiological parameters showed improvements. After 15 days of TCSF toothpaste, microbiological analysis showed a significant reduction of total bacterial loading (Table I).

Table I. Mean amounts of specific bacterial species before (1) and after (2) treatment.

Paired sample test										
Pairwise differences						t	Df	Sig. (2-		
								code)		
		Mean	Standard deviation	Mean Error	95% confidence difference	interval for the				
					inferior	superior				
Couple 1	AA1-AA2	51 50000	105.9	33.5	-24.3	127.3	1.536	9	159	
Couple 2	PG1-PG2	-10000	31623	10000	-32622	12622	-1.000	9	.343	
Couple 3	TF1-TF2	59.3	155.7	49.2	-52.1	170.7	1.204	9	.259	
Couple 4	TD1-TD2	-10000	4.2	1.3	-3.1	2.9	-075	9	.942	
Couple 5	FN1-FN2	-327.2	1585.6	501.4	-1461.5	807.1	-653	9	.530	
Couple 6	CR1-Cr2	70000	18.8	5.9	-12.7	14.1	118	9	.909	
Couple 2	TBL1-TBL2	315099.9	252639.2	79891.5	134372.6	495827.1	3.944	9	.003	

AA: Aggregatibacter actinomycetemcomitans; PG: Porphyromonas gingivalis; TF: Tannerella forsythia; TD: Treponema denticola; FN: Fusobacterium nucleatum; CR: Campylobacter rectus; TBL: total bacteria loading. Total bacterial loading was significantly reduced after treatment.

## DISCUSSION

Dental plaque is recognized as a significant etiological factor in the development of dental caries and plaqueinduced gingival diseases. Mechanical removal of plaque through toothbrushing, toothpaste, and mouth rinses helps counteract the accumulation of pathogenic plaque, thereby contributing to the prevention of these conditions. Effective and therapeutic plaque control is a crucial aspect of personal hygiene, and the appropriate use of TCSF toothpaste is documented as an effective tool among plaque control measures.

Preliminary results indicate that the use of TCSF as an adjunctive treatment is associated with a reduction in the plaque index post-treatment. Studies have shown that TCSF toothpaste helps reduce plaque and gingivitis. Most research on TCSF toothpaste focuses on periodontitis, a prevalent, chronic, nonspecific, and immunological disease of the periodontal tissues caused by microbial infections. TCSF toothpaste reduces plaque, gingivitis, and bleeding without showing a significant effect on clinical attachment loss.

The investigated toothpaste is composed of the following main components: Sodium Fluoride (NaF) and Zinc. Sodium Fluoride (NaF) is primarily used to reduce the prevalence of caries and to improve enamel remineralization (11) The antibacterial and cariostatic effects of fluorides are widely accepted, and the widespread use of fluorides has been attributed to the decline of dental caries in Western countries in recent years (12). Fluorides primarily act by forming fluorapatite crystals, which have greater resistance to organic acids than the hydroxyapatite crystals of tooth enamel. It has also been shown to reduce the production of organic acids in cariogenic bacteria such as Streptococcus mutans (13). Zinc, present in this toothpaste as Zinc Lactate, is a non-toxic, non-cumulative essential trace element. Zinc inhibits the pathway of glucose uptake by Streptococcus mutans, Streptococcus Sanguis, and Actinomyces Naeslundii, and the metabolism of glucose to lactic acid. This helps reduce plaque formation and maintain a healthy microbial balance within the oral cavity (14-18).

It can be hypothesized that the ability of TCSF toothpaste to promote antibacterial activity likely contributes to an overall improvement in oral hygiene for the patient. Further investigation may be necessary to fully understand the mechanism of action of sodium fluoride and confirm its long-term benefits in managing dental diseases associated with bacterial plaque.

## CONCLUSIONS

The results of this clinical study are very promising regarding the benefits of using TCSF toothpaste as a complement in the standard treatment of gingivitis. It helps reduce the plaque index and bacterial load, leading to an overall improvement in the patient's oral health. It is therefore essential to motivate and raise awareness among patients about oral health, explaining the importance of proper oral hygiene at home.

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