



Abstract # 0389

In vitro Antibacterial Comparison of Dentifrices Claiming Antigingivitis and Gum-Healing Properties

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Abstract

Antibacterial activity is a key part of the efficacy of toothpastes currently marketed with gum-healing and antigingivitis claims. Prior *in vitro* testing has shown zinc to be efficacious against organisms associated with caries, supragingival plaque, and gingivitis.

Objective: To compare the antibacterial efficacy of prototype antigingivitis toothpastes to currently marketed toothpaste products making gum-related claims, when tested vs. oral microorganisms of significance in an agar dilution MIC test.

Methods: All toothpaste samples were placed in identical tubes and submitted blind for testing. The toothpastes were diluted in sterile, deionized water and tested in a twofold dilution series ranging from 0.05 g/mL to 0.0008 g/mL. A toothpaste without antibacterial claims (Crest Regular) was employed as the control. Test organisms were: *S. mutans*, *S. sanguinis*, *F. nucleatum*, *A. naeslundii* and *A. viscosus*. Each agar dilution was inoculated with spots of approximately 1×10^5 cfu of each test organism. Plates were incubated at 37°C, aerobically or anaerobically as appropriate. All toothpastes were tested in triplicate vs. each organism.

Results: Antibacterial activity was defined by concentration variance from the control. MICs equal to control values were considered non-active. Colgate Total displayed the best activity against the three organisms associated with gum disease. Natural Care Tom's of Maine Antigingivitis Toothpaste (with and without fluoride) with Zinc Citrate, Viadent Advanced Care, and Listerine Essential Care were superior to control and equivalent to each other. Jason Healthy Mouth was equivalent to control (no activity), while The Natural Dentist Herbal Toothpaste and Gum Therapy was inferior to control.

Conclusion: An all-natural antigingivitis toothpaste with zinc citrate displayed superior antibacterial properties to all other natural brands tested, equivalency to another zinc citrate product, and equivalency to a four essential oil-based dentifrice.

Introduction

There is a plethora of currently marketed toothpaste products making gum claims. Among products included in this study, one lists several botanical extracts as "gum stimulants" (although none are listed as active ingredients), another lists four essential oils as active ingredients for antiplaque/antigingivitis, while another lists a chlorophenol as the active ingredient for antigingivitis. Still another toothpaste marketed with gum claims lists a botanical extract as a "gum soother" (although, again, it is not listed as an active ingredient), while the last product in the study lists a metal ion as the active ingredient for fighting plaque and gingivitis.

Objective

The objective of this study was to compare the *in vitro* antibacterial activity of several toothpastes marketed with gum-related benefits against that of two prototype antigingivitis toothpastes (one with fluoride and one without). Note: Since this investigation the non-fluoride product has been launched (please see Figures 1 & 2); however, due to Tom's of Maine's non-animal testing policy, the fluoridated version will not be ready for market until 2004.

Materials & Methods

Materials

The commercially available toothpastes listed previously were all purchased at a local Kennebunk market, while the Tom's of Maine prototype toothpastes were manufactured in the R&PD Laboratory. Cultures of the following bacteria were obtained from the American Type Culture Collection (Manassas, VA): *Streptococcus mutans* ATCC



Figure 1.

Table 1. Minimum inhibitory concentration to yeast.

Toothpaste	Streptococcus mutans ATCC 10419	Actinomyces naeslundii ATCC 10418	Actinomyces viscosus ATCC 10417	Streptococcus sanguinis ATCC 10556	Streptococcus faecalis ATCC 10454
Control	0.00116	0.00125	0.00625	0.00625	0.00625
Colgate Total	0.00116	0.00125	0.00625	0.00625	0.00625
Jason Healthy Mouth	0.00116	0.00125	0.00625	0.00125	0.00625
Listerine	0.00116	0.00125	0.00625	0.00125	0.00625
Colgate Total	0.0008	0.00125	0.00116	0.00125	0.00116
Crest Regular	0.00116	0.00625	0.0125	0.00625	0.00625
Natural Dentist	0.00112	0.00625	0.0125	0.00625	0.00625
GNONS	0.00625	0.00125	0.00625	0.00125	0.00112

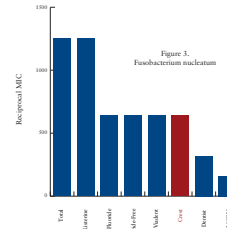


Figure 3. *Fusobacterium nucleatum*

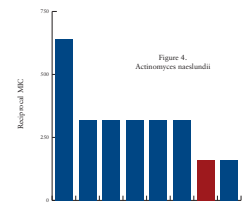


Figure 4. *Actinomyces naeslundii*

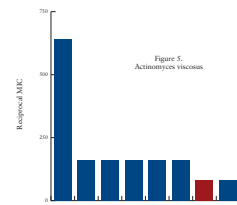


Figure 5. *Actinomyces viscosus*

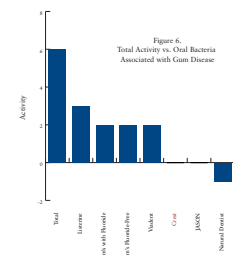


Figure 6. Total Activity vs. Oral Bacteria Associated with Gum Disease



Figure 2.

25175, *Streptococcus sanguinis* ATCC 10556, *Fusobacterium nucleatum* ATCC 10953, *Actinomyces naeslundii* ATCC 19039, and *Actinomyces viscosus* ATCC 15987. BHI, Wilkins-Chalgren, and Cooked Meat media were obtained from Becton Dickinson Laboratories (Sparks, MD), and CDC anaerobic blood agar plates were obtained from Remel (Lenexa, KS). The Gas Pak jar and gas generating envelopes were obtained from Becton Dickinson Laboratories (Sparks, MD).

Methods

In order to reduce waste, the test method was based on the limited agar dilution procedure described by NCCLS (Document M11-A3, December 1993). Each toothpaste was diluted to 0.5 g/mL by delivering 2.5 g to 5 mL tubed, sterile deionized water and vortexing thoroughly. Doubling dilutions were further prepared in sterile deionized water, giving a series of 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156, and 0.008 g/mL for each paste. Aliquots of 0.5 mL from each dilution of toothpaste were delivered to tubes containing 4.5 mL sterile agar held at about 50°C (BHI agar was the test medium for *S. mutans* and *S. sanguinis*; WC agar was used for *A. naeslundii*, *A. viscosus*, and *F. nucleatum*). Tubes were vortexed thoroughly and contents delivered to appropriately labeled quad plates. Final test concentrations of toothpastes, therefore, were 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156, and 0.0008 g/mL.

S. mutans and *S. sanguinis* were grown on BHI agar under 5% CO₂ at 37°C in a Gas Pak jar, while *A. viscosus*, *A. naeslundii* and *F. nucleatum* were grown on CDC blood agar, anaerobically, at 37°C in a Gas Pak jar. Suspensions of the test organisms were prepared by picking colonies from appropriately incubated agar cultures to sterile broth, to match a McFarland 0.5 turbidity standard (approximately 1.5×10^8 cfu mL⁻¹). Inocula were applied to agar surfaces in 1 uL spots, giving approximately 1.5×10^5 cfu per spot. Plates prepared without test product were inoculated as viability controls, and uninoculated media were also included to confirm sterility. All plates were inverted and incubated appropriately for 48 hours in Gas Pak jars. The MIC was considered as the lowest concentration of toothpaste which caused a marked inhibition in growth as compared to growth control. Each toothpaste was tested in triplicate vs. each organism (three separate inoculum preparations on three different days).

Results

Table I displays the minimum inhibitory concentrations (MIC) results for all bacteria in this investigation. Figures 3-5 show reciprocal MIC of toothpastes vs. the three oral bacteria associated with gum disease, *Fusobacterium nucleatum*, *Actinomyces naeslundii*, and *Actinomyces viscosus*, respectively. For this investigation, antibacterial activity was further defined by concentration variance from the control (Crest Regular). MICs equal to control values were considered non-active. As an example, if the MIC of the control was 0.00156 g/mL for a given bacterium, an MIC of 0.0008 g/mL would warrant a score of +1. Figure 6 displays the total activity of the samples investigated against the three gum-related bacteria. Colgate Total displayed the best overall activity vs. organisms associated with gum disease. Natural Care Tom's of Maine Antigingivitis Toothpaste (with and without fluoride), Viadent Advanced Care, and Listerine Essential Care were superior to control and equivalent to each other. Jason Healthy Mouth was equivalent to control (no activity), while The Natural Dentist Herbal Toothpaste and Gum Therapy was inferior to control.

Conclusions

Natural Care Tom's of Maine Natural Antigingivitis Toothpaste with zinc citrate displayed superior *in vitro* antibacterial properties to all other all-natural brands tested, equivalency to another zinc citrate product, and equivalency to a four essential oil-based dentifrice, when tested vs. oral bacteria of significance in an agar dilution MIC test.