Clin Oral Invest (2009) 13:195–202
DOI 10.1007/s00784-008-0227-3

ORIGINAL ARTICLE

Clinical anti-microbial efficacy of a new zinc citrate dentifrice

P. K. Sreenivasan · D. Furgang · K. Markowitz · M. Mckiernan · D. Tischio-Bereski · W. Devizio · D. Fine

Received: 2 June 2008 / Accepted: 10 September 2008 / Published online: 11 October 2008

Abstract This crossover design clinical study compared the anti-microbial effects of a new 1% zinc citrate dentifrice with a control formulation. Thirty adults completed a washout phase and baseline samples of dental plaque, buccal mucosa, tongue, saliva, and plaque collected to enumerate anaerobes and streptococci. Subjects were randomly assigned a test dentifrice to use for the next 13 days. Oral samples similar to the baseline were collected on day 14 prior to oral hygiene for microbial analysis. The subject then placed a custom intra-oral stent with hydroxyapatite (HA) squares and brushed their teeth with their assigned dentifrice. Oral samples and HA squares were collected 5 h later for microbial analyses. This completed the study with one test dentifrice. The entire study was repeated with the alternate dentifrice after a second washout phase. Whereas baseline samples demonstrated no significant differences in microbial parameters between the two treatment groups ($p>0.05$), subjects provided the zinc citrate dentifrice demonstrated 24–52% reductions in anaerobic bacteria and streptococci on day 14 versus the control paste ($p<0.05$). In the 5-h post-brushing samples, subjects provided the zinc citrate toothpaste demonstrated 27–49% reductions for anaerobic bacteria and streptococci ($p<0.05$). Additionally, *in situ* microbial biofilm formation on HA disks was significantly inhibited amongst the zinc citrate group ($p<0.05$). Significant reductions in anaerobic bacteria and streptococci were observed amongst all intra-oral locations along with *in situ* biofilm formation after use of the zinc citrate dentifrice.

Keywords Anaerobic · Bacteria · Dentifrice · Fluoride · Saliva · Streptococci · Tongue · Zinc citrate

P. K. Sreenivasan · W. Devizio
Colgate–Palmolive Company,
909 River Road,
Piscataway, NJ 08855, USA

D. Furgang · K. Markowitz · M. Mckiernan · D. Tischio-Bereski · D. Fine (✉)
Oral Biology Department,
University of Medicine and Dentistry of New Jersey,
MSB, 185 South Orange Avenue,
Newark, NJ 07103-2714, USA
e-mail: finedh@umdnj.edu

Introduction

The human mouth is home to a large and diverse group of endogenous bacteria [23]. A range of factors promote the growth and proliferation of these organisms and include diet, a moist and warm environment, and several unique anatomical features including both shedding and non-shedding surfaces. Complex naturally formed multilayered biofilms can be routinely isolated from oral surfaces such as the plaque from the surfaces of the exposed teeth and the surface of the tongue [14, 21]. Dental plaque represents the most extensively examined biofilm and may comprise more than 300 layers of oral bacteria. Estimates indicate that approximately 10^{10} organisms can be recovered per gram of dental plaque [22, 23]. Saliva represents another oral environment that has been the focus of many clinical investigations for microbiological analysis. However, few clinical investigations provide microbiological assessments of the tongue and buccal mucosa. Haraszthy et al. [10] utilized molecular methods and report the isolation of a unique group of organisms from the tongue surface of subjects with clinical symptoms of halitosis. Similarly, evidence suggests that the buccal microflora maybe more diverse and associated with oral conditions [20].

A central focus of oral microbiological investigations has sought to analyze the number and diversity of oral microorganisms in health and disease. The influences of these organisms on dental plaque formation and their relationship to oral disease progression have been demonstrated [15]. Based on these studies, it is now well accepted that accumulations of oral bacteria play a role in the progression of common diseases such as gingivitis, caries, and periodontal disease. The unrestricted accumulation of dental plaque leads to gingivitis, an inflammation of gingival tissue [1, 3, 14]. Epidemiological surveys indicate that gingivitis may afflict large portions of populations worldwide [19, 21].

Brushing is an effective means to remove the accumulated dental plaque [2, 4]. However, despite education and other preventative programs, poor brushing habits and inadequate oral hygiene are common and are reflected in the high worldwide prevalence of gingivitis and other oral conditions. Oral hygiene formulations with anti-microbial agents represent an important advance to mitigate the effects of dental plaque [1, 3, 4, 7, 16, 17]. A primary rationale for the inclusion of anti-microbial agents is their ability to control dental plaque and related gingivitis [9, 16, 24]. These agents include chlorhexidine, triclosan, essential oils, metal salts, and other ingredients with a significant history of safe and effective use [1, 7, 9, 17, 24]. Their use in over-the-counter oral hygiene formulations is supported by results from a large number of clinical studies which demonstrate significant reductions in dental plaque and gingivitis [9, 12, 24].

One ingredient used widely in dentifrices is zinc citrate, a salt with a long history of safe and efficacious use in dentifrices to control dental plaque. The US FDA has classified zinc citrate as class I for safety and class III for efficacy [24]. A variety of human clinical studies with these formulations are readily available in the literature [9, 12, 16, 24]. Studies have examined the effect of brushing for up to 6 months with these dentifrices [2, 8, 11], on accumulation of dental plaque. Whereas previous clinical studies have examined the ability of dentifrices formulated with zinc citrate to reduce dental plaque, the effects on levels of specific oral organisms remain unexplored.

This clinical study investigated the effects of brushing for 13 days with a newly formulated dentifrice with 1% zinc citrate with a control formulation. Microbiological analyses on samples of dental plaque, scrapings from the surface of the cheeks, tongue, and saliva compared the anti-microbial effect of the two formulations on bacterial populations found in several distinct oral niches. An additional objective examined the accumulation of plaque bacteria on hydroxyapatite surfaces (HA) worn by the subjects in custom stents. This assessment compared the effects of these dentifrices on *in situ* post-brushing microbial colonization and provides a measure of the residual anti-microbial effects of the tested dentifrices resulting in delayed plaque formation.

Materials and methods

Study design

This controlled and double-blind clinical study utilized a 2×2 crossover design with randomized assignment of test dentifrices. An independent institutional review board reviewed and approved the clinical study protocol. Adult subjects from the Newark, NJ area expressing an interest in the study completed an informed consent and prospective subjects were scheduled for a screening visit that included a complete oral examination. Subjects who could comply with study schedules and met the inclusion and exclusion criteria were enrolled.

Inclusion criteria included adults of either gender (age range 18–72 years) with a minimum of 20 natural teeth with buccal and lingual scoreable surfaces (not including abutment and third molars), no significant oral soft tissue pathology, and periodontal pockets less than 6 mm. Subjects with gingival index scores greater than 1.0 by the Turesky Modification of the Loe–Silness gingival index [a four-point scale that ranges from no inflammation (0) to 3 (severe inflammation)] and dental plaque by the Silness–Loe plaque index (plaque index scores—0=no plaque/debris; 1=separate flecks of plaque at cervical margin of tooth; 3=a thin band of continuous plaque up to 1 mm at the cervical margin of tooth; 4=plaque covering at least 1/3 but less than 2/3 of the crown of the tooth; 5=plaque covering 2/3 or more of the tooth crown) greater than 1.5 were enrolled. Exclusion criteria included the presence of grossly carious teeth, extensive crowns or restorations, orthodontically banded teeth, systemic diseases, pregnancy, and lactation. Subjects using prescription medications in the past 30 days or those participating in any clinical trial in the past 30 days were excluded. Thirty adults who met the inclusion and inclusion criteria were enrolled.

(a) Clinical Procedures for intra-oral stent preparation:

An alginate impression of the subject's mandibular teeth was used to prepared a custom-made intra-oral stent with hydroxyapatite (HA) squares for all subjects enrolled (Fig. 1) as described previously [5]. Each subject was provided with two stents of dental acrylic (Ortho-Jet, Lang Dental Manufacturing, Wheeling, IL, USA) that were worn over the mandibular right and left posterior teeth. Each stent held two 3×3 mm HA squares prepared with sintered-food grade hydroxyapatite (NEI Industries, Sesser, IL, USA).

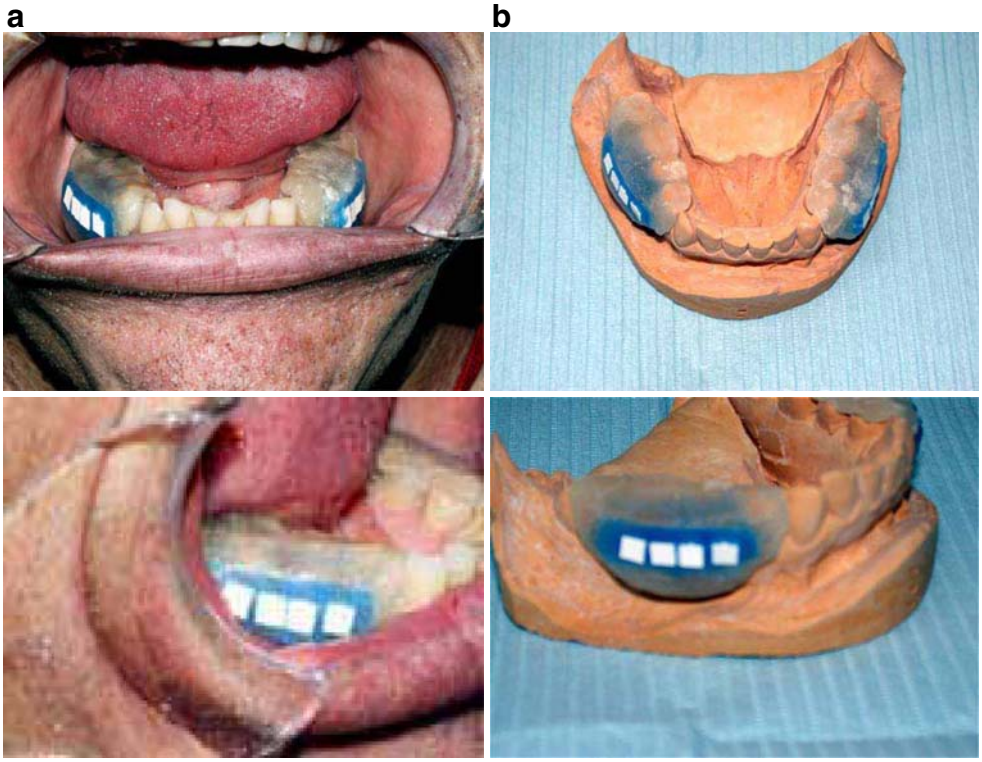

Fig. 1 Photographs of intra-oral stent with hydroxyapatite (HA) squares. Each stent was worn over the mandibular posterior teeth and had a wax-filled groove on the facial aspect into which 3×3-mm HA squares can be placed. Following periods of time, squares can be removed for microbiological analysis. **a** Intra-oral *photograph* with stents in patient. **b** *Photograph* with stents in a model

Following stent fabrication, the dentist adjusted the fit and supervised their use during the study.

(b) Toothpastes, instructions to subjects, and clinical design:

Enrolled subjects were provided a commercially available fluoride dentifrice [Colgate Great Regular Flavor (Colgate–Palmolive Co., NY, NY, USA)] and a soft-bristled adult toothbrush (Colgate–Palmolive) prior to the start of the study. They used these articles for 7 days during the washout (break-in) period prior to the baseline sampling. Following study enrollment, all subjects were instructed to discontinue the use of all other oral hygiene formulations such as chewing gums, mints, mouthwashes, and dentifrices for the duration of the study period. Subjects were instructed to brush their teeth with the washout dentifrice for 1 min using at least a 1 in. strip of the assigned tooth paste. Identical brushing instructions were provided to subjects for all phases of the study. All subjects also completed a 1-week washout phase between the two treatments.

Test dentifrices included a formulation with 1% zinc citrate and a control dentifrice without zinc citrate. Both test dentifrices were formulated with fluoride and prepared by Colgate–Palmolive Co. Test dentifrices were overwrapped and a unique code assigned to each group. Codes were not identified until the conclusion of the study.

Subjects completing the 1-week washout phase arrived in the morning at the dental clinic having refrained from oral hygiene procedures. Oral samples (dental plaque from the buccal surfaces of two teeth, saliva, and samples of tongue and cheek scrapings) were collected for baseline microbiological assessments (described in section below). Each subject was randomly assigned a test dentifrice, a soft-bristled toothbrush, and instructed to brush twice daily. Subjects were recalled on day 14 and arrived at the dental clinic prior to oral hygiene. Post-treatment oral samples were collected as described during the baseline visit and the subjects were provided their custom stent with HA squares. Subjects brushed with the test dentifrice assigned for the previous 13 days and returned to the dental clinic after 5 h. Oral samples (dental plaque, saliva, and samples of tongue and cheek scrapings) and two HA squares (from right and left stent) were collected during this visit. The entire procedure was repeated for the alternate test dentifrice after a 1-week washout phase.

Procedures for collection of oral samples

Identical procedures for sample collection were utilized for both test dentifrices at baseline and post-brushing samples on day 14 that included a pre-brushing sample and another collected at 5 h post-brushing. These procedures are described below:

(a) *Supragingival plaque*: Each plaque sample was removed from the buccal surface of a molar and a non-adjacent bicuspid in the same quadrant. Baseline and

pre-brushing day 14 samples were taken from pairs of mandibular teeth. The 5-h post-brushing day 14 sample was taken from a pair of maxillary teeth. Care was taken to ensure that no set of teeth was sampled more than once. A sterile Columbia 13/14 scaler was used to collect the plaque. Plaque obtained from the two teeth designated to be sampled at each time period (pre-brushing or 5 h) was pooled and placed into a tube containing 1 ml of sterile phosphate-buffered saline (PBS) for processing.

(b) *Saliva*: Before plaque collection, subjects provided unstimulated saliva obtained by expectorating a minimum of 1 ml into a test tube for microbial analysis.

(c) *Buccal and tongue scrapings*: These samples were taken using the edge of a wooden tongue blade for each of two randomly chosen sites on the cheek and the tongue (for baseline and 5 h post-brushing on day 14). Each site entailed five scrapes per sample. Tongue blades were placed in a tube with 3 ml PBS and vortexed for 30 s to shake loose collected oral sample.

(d) *Stent samples*: Two hydroxyapatite (HA) squares (one each from the right and left stent) were collected from each subject. For each test dentifrice, these samples were collect on day 14 at the 5-h post-brushing time points.

Microbiological procedures

All samples were subjected to brief periods of sonic dispersion using a Branson 200 sonicator with a Cup Horn for 30 s, pulsed (settings are output=1, duty cycle=50%), and serial 10-fold dilutions were prepared in PBS. Dilutions from 10^0 to 10^{-4} were plated using a Spiral Systems Autoplate 4000 Spiral plater according to manufacturer's directions. Samples were plated in duplicate on 5% sheep blood agar and Mitis–Salivarius Agar obtained from Beckton-Dickinson, Franklin Lakes, NJ and incubated at 37°C for 5–7 days under anaerobic conditions. Colony forming units (CFU) were calculated from dilutions yielding at least 20 colonies per plate as described previously [6].

Statistical analysis

Duplicate microbial counts of anaerobic bacteria and streptococci from each subject and oral site sampled were recorded as CFU/ml and averaged. The number of viable bacteria for each dentifrice, bacterial type, and oral site was averaged for the entire population. Statistical analyses utilized the Student *t* test and were completed by the JMP software (Cary, NC, USA). Analyses compared the effects of the two dentifrices on each of the two types of oral bacteria recovered from each oral site sampled (saliva, dental plaque, tongue surface, and cheek) and the hydroxyapatite squares on the stent. These analyses were conducted for the baseline and each of the post-treatment assessments. Statistical significance is reported at $p<0.05$.

Results

The demographic of the subjects enrolled in the study is presented in Table 1. All subjects completed the study and their age ranged from 23 to 54 years, with a mean age of 39 years. Microbiological assessments compared the control dentifrice with the 1% zinc citrate toothpaste. Statistical analyses comparing the baseline samples from each site for the two treatment groups indicate no statistical differences ($p>0.05$) at baseline.

The effects of the test and control dentifrices on total anaerobic bacteria are shown in Fig. 2 and indicate effects on dental plaque, saliva, and the scrapings obtained from the tongue and buccal mucosa. The use of the zinc citrate dentifrice resulted in significant reductions of anaerobic bacteria in all oral samples versus the control ($p<0.05$). Samples collected on the morning of day 14 prior to oral hygiene indicate 50%, 24%, 32.8%, and 35.6% inhibition of anaerobic bacteria in the dental plaque, buccal, tongue, and saliva, respectively for the zinc citrate formulation versus the control. The 5-h post-brushing samples collected on day 14 indicate significant effects by the zinc citrate dentifrice versus the control ($p<0.05$). Percent inhibition of

Table 1 Demographic characteristics of enrolled subjects

Characteristics	
N=30	
Age (years)	
Mean	39.0
SD	8.8
Range	23–54
Gender	
Male	11
Female	19
Race	
White	6
Black	11
Hispanic	2
Asian	7
Other	4
Smoker	
Yes	5
No	25

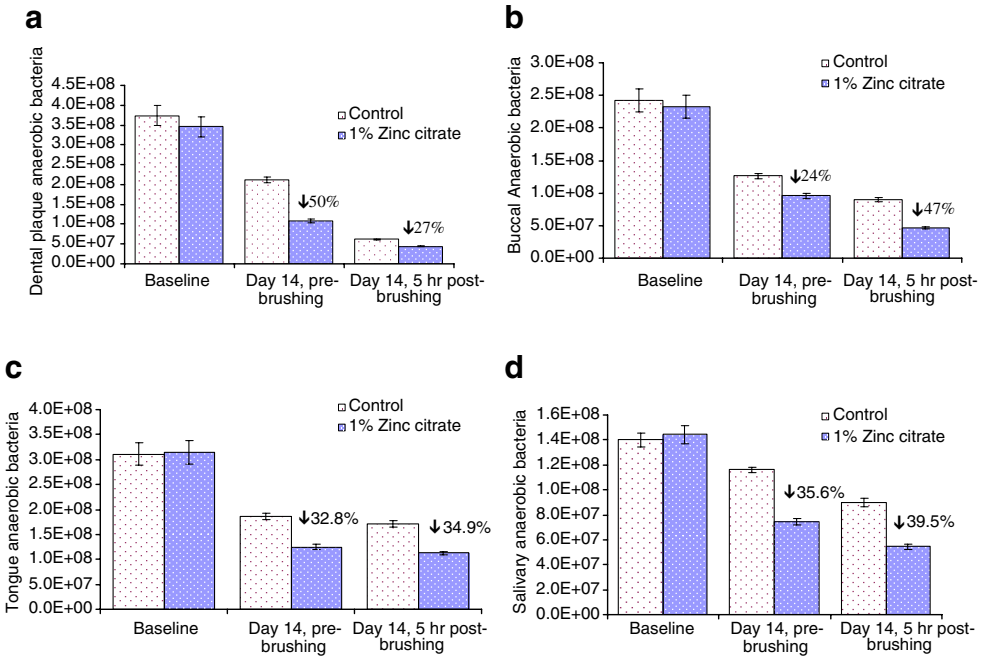

Fig. 2 The effects of brushing with the control and 1% zinc citrate dentifrices on anaerobic bacteria recovered from oral sites. Shown in figure are results from dental plaque (**a**), buccal samples (**b**), tongue samples (**c**), and saliva (**d**). *Bars* represent mean viable anaerobic bacteria (CFU/ml)±standard error of the mean recovered at each sampling point. Statistically significant ($p<0.05$) percentage reductions for the 1% zinc citrate group compared with the control group are shown

anaerobic bacteria by zinc citrate were 27%, 47%, 34.9%, and 39.5% for samples of dental plaque, buccal, tongue, and saliva samples, respectively.

The samples, i.e., dental plaque, saliva, and the scrapings from tongue and buccal mucosa collected following a period of product use and used to enumerate anaerobic organisms were also used to assess treatment effects on oral streptococci. These results (Fig. 3) indicate the mean and SEM of viable streptococci isolated from each sample. Statistical analyses indicate no differences in the baseline samples obtained from each of these intra-oral sites ($p>0.05$). All samples collected after the use of the zinc citrate dentifrice demonstrate significant reductions in oral streptococci versus the control ($p<0.05$). Analysis of the samples collected on the morning of day 14 prior to oral hygiene indicates 52%, 26%, 32%, and 36% inhibition of the dental plaque, buccal, tongue, and saliva, respectively for the zinc citrate formulation versus the control. The 5-h post-brushing samples collected on day 14 indicate significant effects by the zinc citrate dentifrice versus the control ($p<0.05$). Subjects using zinc citrate demonstrated a 31%, 49%, 35%, and 42% inhibition of the streptococci in the dental plaque, buccal, tongue, and saliva samples, respectively.

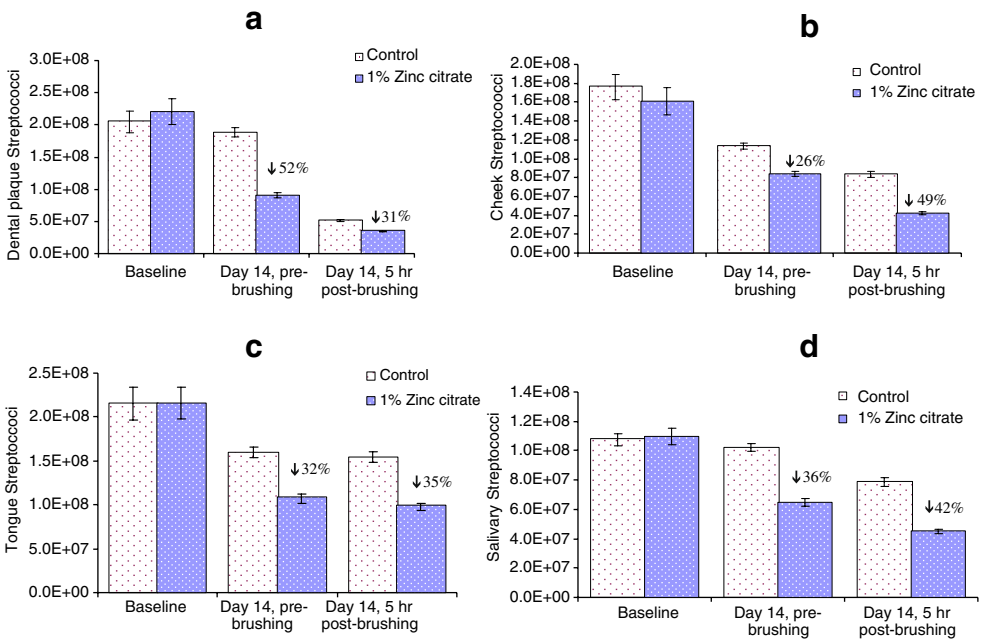

Fig. 3 The effects of brushing with the control and 1% zinc citrate dentifrices on oral streptococci recovered from oral sites. Shown in figure are results from dental plaque (**a**), buccal samples (**b**), tongue samples (**c**), and saliva (**d**). *Bars* represent mean viable streptococci (CFU/ml)±standard error of the mean recovered at each sampling point. Statistically significant ($p<0.05$) percentage reductions by *t* test for the 1% zinc citrate group compared with the control group are shown

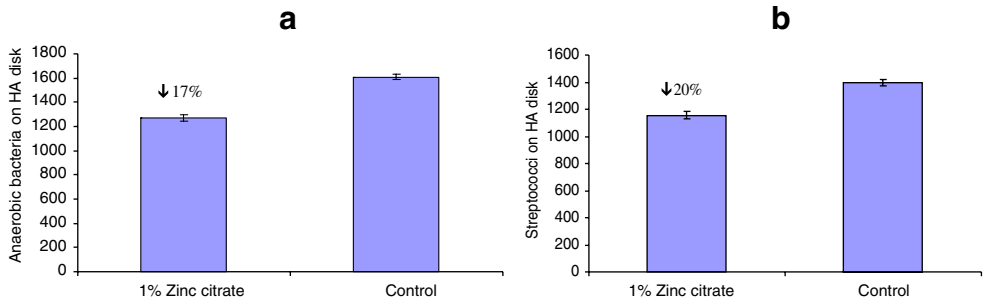

Fig. 4 The effects of brushing with the control and 1% zinc citrate dentifrices on *in situ* biofilm formation by anaerobic bacteria (**a**) and streptococci (**b**). *Bars* represent mean viable bacteria (CFU/ml)± standard error of the mean recovered from the hydroxyapatite disks. Statistically significant ($p<0.05$) percentage reductions by *t* test for the 1% zinc citrate group compared with the control group are shown

Effects of these treatments on *in situ* plaque formation are presented in Fig. 4 and indicate average numbers of viable bacteria recovered. HA squares collected 5 h after the use of the zinc citrate dentifrice indicate significant inhibitions in anaerobic organisms (Fig. 4a) and streptococci (Fig. 4b) versus the control formulation ($p<0.05$). Versus the control toothpaste, anaerobic bacteria and streptococci colonizing the HA squares were inhibited by 17% and 20%, respectively after the use of the zinc citrate dentifrice.

Discussion

This clinical study demonstrated significant reductions in anaerobic bacteria and streptococci of the dental plaque, saliva, and on the surface of the tongue and buccal mucosa following 13-day use of a newly formulated dentifrice with 1% zinc citrate. Oral organisms examined for the study were selected based on their numerical dominance and ability to readily grow in the laboratory. Whereas previous clinical studies with zinc citrate have examined effects on clinical measures of dental plaque and gingivitis and the effects of long-term use on the oral microflora, there are no studies that determine the short-term microbiological efficacy of zinc citrate on oral bacteria [4, 11, 24]. Several features of this study remain novel. The study concurrently sampled several oral sites and determined the numbers of anaerobic bacteria and streptococci in these samples. In addition, the study determined the effect of the dentifrices on intra-oral biofilm formation on HA surfaces *in situ*.

Concurrent assessment of the microflora of four oral sites, i.e., dental plaque, tongue surface, buccal mucosa, and saliva highlight a methodological difference of this study. Clinical studies commonly assess the effects of oral hygiene regimens on the microflora of the dental plaque and saliva [7]. These samples reflect the clinical observations relating salivary and dental plaque microflora with progression of clinical disease [23]. On the other hand, advances in the microbiology of the human mouth indicate distinct microflora on the surface of the tongue and buccal mucosa. Clinical studies indicate the role of the tongue microflora in halitosis [10] and evidence suggests that buccal mucosa may serve as a reservoir for periodontal pathogens [13]. Therefore, it is rational to assess the efficacy of oral hygiene regimens on the microflora of all these oral sites. However, studies that simultaneously assess effects on different oral sites are not available. We have explored some parts of this concept in a previous study [6]. This design offers clear patient-directed advantages to determine anti-microbial effects on oral regions that are not subjected to routine oral hygiene. Whereas it is widely known that oral organisms are found on the surface of the tongue and buccal mucosa, routine oral hygiene is frequently restricted to the teeth. Therefore, this clinical study was based on this common oral hygiene practice of brushing the teeth without instructing subjects to cleanse other regions of the mouth. With this design, the results indicate the additional effects of the zinc citrate dentifrice at significantly reducing the numbers of organisms on the tongue, buccal mucosa, saliva, and on HA surfaces *in situ.*

The clinical design for this study incorporated several steps to reduce the variability in the oral microflora and control for factors which could confound statistical analyses [18]. One approach to reduce microbial variation was to sample two teeth instead of one as described previously [6]. The second approach utilized a crossover design for the study with randomized allocation of test formulations during the two test phases of the study and two washout phases. The first washout phase provides a period where all enrolled subjects practice a standard regimen for their oral hygiene. The second washout phase is after the completion of the first test phase and provides a period to wash out the effects of the first test formulation. No statistical differences were observed in the baseline samples in any of the oral samples for total anaerobic bacteria and streptococci between the subjects receiving the two dentifrices. This indicates a sufficient duration of the washout phases for the oral microflora of the subjects and allows further statistical analyses of the effects of the two treatments.

Microbial analyses during each phase of the study examined total anaerobic bacteria levels which reflect plaque development in the human mouth. The organisms selected readily grow on enriched microbiological media and comprise the large majority of cultivable gram-positive and gram-negative microflora. Also included in the studies was a concurrent assessment of the oral streptococci. Streptococci constitute between 50% and 70% of the oral organisms and comprise the most commonly isolated oral bacteria [23]. The anti-microbial effects of zinc citrate were observed in the overnight samples collected approximately 12 h after the last use of the formulation. Significant anti-microbial effects were observed for the zinc citrate in samples from each of the four oral sites as well as from the HA surface. Further, the percentage reductions observed for the anaerobic bacteria were similar to those seen for the streptococci. Additional analyses reveal that the anti-microbial effects of zinc citrate were observed in the 5-h post-brushing samples with similar percentage reduction in the anaerobic bacteria and streptococci. Together, these observations indicate both the short-term and the longer-term effects of zinc citrate that can help provide the therapeutic benefits of reductions in dental plaque observed in longer-term clinical studies.

Recent investigations demonstrate extensive interest in exploring the growth and maturation of biofilms in their natural environment. This comprises an important area designed to explore and develop intervention strategies. The study examined intra-oral formation of the microbial biofilm following each treatment based on an *in situ* approach developed previously [5]. Advantages of this approach include biofilm formation within the mouth to help explain the anti-microbial results observed with the zinc citrate in the samples from the four oral sites. Samples collected 5 h post-use of the zinc citrate indicate significantly lower microbial colonization of the HA squares in the custom stents provided to the subjects. The ability of the oral bacteria to colonize the HA surface reflects the critical initial steps for biofilm formation. From an analytical standpoint, the HA squares are of uniform surface area and analysis of the microflora on these squares allows a direct comparison between the two treatments on inhibiting *in situ* microbial biofilm formation. Additionally, the study collected duplicate samples of HA squares from bilateral locations and assessed the numbers of both anaerobic organisms and streptococci. Results from HA squares demonstrate similar inhibitions of the anaerobic bacteria and streptococci after the use of zinc citrate and indicate the effects at preventing biofilm formation. The inclusion of the HA squares in this study helps explain the results of the comprehensive microbiological analyses conducted at the four different sites of the human mouth and provide corroborating data on the effects of the zinc citrate.

In conclusion, results from this study provide microbiological evidence that demonstrates the effects of the formulation at reducing oral bacteria. The effects observed on the oral streptococci from several oral sites observed at several time points and support the results observed with the anaerobic microflora. These results corroborate previous studies on the effects of the zinc citrate dentifrice at reducing supragingival plaque and gingivitis. The significant reductions in the oral bacteria found in the different oral sites were corroborated by the results from the *in situ* microbial biofilm studies. These studies reveal lower rates of intra-oral microbial biofilm formation after the use of the zinc citrate formulation.

Conflict of interest P. K. Sreenivasan is an employee of the Colgate–Palmolive Co. No other author has any financial relationship with the sponsor (Colgate–Palmolive) of this research.

References

1. Ciancio SG (1992) Agents for the management of plaque and gingivitis. J Dent Res 71:1450–1454
2. Davies RM, Davies GM, Ellwood RP (2003) Prevention. Part 4: Toothbrushing: what advice should be given to patients. Br Dent J 195:135–1413
3. Eley BM (1999) Antibacterial agents in the control of supragingival plaque—a review. Br Dent J 186:286–2964
4. Fine DH (1995) Chemical agents to prevent and regulate plaque development. Periodontol 8:87–1075
5. Fine DH, Furgang D, Steinberg LM, Olshan AM, Kohut RE, Coelho JF, Harper DS (2000) A model for clinical evaluation of anti-microbial effects of agents on plaque colonization. Am J Dent 13:153–158
6. Fine DH, Furgang D, Markowitz K, Sreenivasan PK, Klimpel K, De Vizio W (2006) The antimicrobial effect of a triclosan/copolymer dentifrice on oral microorganisms in vivo. J Am Dent Assoc 137:1406–1413
7. Forward GC, James AH, Barnett P, Jackson RJ (1997) Gum health product formulations: what is in them and why. Periodontol 15:32–39
8. Gjermo P, Saxton CA (1991) Antibacterial dentifrices. Clinical data and relevance with emphasis on zinc/triclosan. J Clin Periodontol 18:468–473
9. Gunsolley JC (2006) A meta-analysis of six-month studies of antiplaque and antigingivitis agents. J Am Dent Assoc 137:1649–1657
10. Haraszthy VI, Zambon JJ, Sreenivasan PK, Zambon MM, Gerber D, Rego R, Parker C (2007) Identification of oral bacterial species associated with halitosis. J Am Dent Assoc 138:1113–1120
11. Hall PJ, Green AK, Horay CP, de Brabander S, Beasley TJ, Cromwell VJ, Holt JS, Savage DJ (2005) Plaque antibacterial levels following controlled food intake and use of a toothpaste containing 2% zinc citrate and 0.3% triclosan. Int Dent J 53(6 Suppl 1):379–384
12. Hioe KP, van der Weijden GA (2005) The effectiveness of self-performed mechanical plaque control with triclosan containing dentifrices. Int J Dent Hyg 3:192–204
13. Leung NM, Chen R, Rudney JD (2006) Oral bacteria in plaque and invading buccal cells of young orthodontic patients. Am J Orthod Dentofacial Orthop 130:698.e11–698.e18
14. Listgarten MA (1988) The role of dental plaque in gingivitis and periodontitis. J Clin Periodontol 15:485–487

15. Löe H (1988) Research on oral diseases and its impact on dental education and practice. Adv Dent Res 2:199–203
16. Mandel ID (1988) Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontol 15:488–498
17. Marsh PD (1991) Dentifrices containing new agents for the control of plaque and gingivitis: microbiological aspects. J Clin Periodontol 18:462–467
18. Moore WE, Holdeman LV, Cato EP, Good IJ, Smith EP, Ranney RR, Palcanis KG (1984) Variation in periodontal floras. Infect Immun 46:720–726
19. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C (2005) The global burden of oral diseases and risks to oral health. Bull World Health Organ 83:661–669
20. Rudney JD, Chen R, Sedgewick GJ (2005) *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* are components of a polymicrobial intracellular flora within human buccal cells. J Dent Res 84: 59–63
21. Pihlstrom BL, Michalowicz BS, Johnson NW (2005) Periodontal diseases. Lancet 366:1809–1820
22. Socransky SS, Haffajee AD (2002) Dental biofilms: difficult therapeutic targets. Periodontol 2000 28:12–55
23. Socransky SS, Haffajee AD (2005) Periodontal microbial ecology. Periodontol 2000 38:135–18726
24. Wu CD, Savitt ED (2002) Evaluation of the safety and efficacy of over-the-counter oral hygiene products for the reduction and control of plaque and gingivitis. Periodontol 2000 28:91–105