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ORIGINAL ARTICLE

Effect of a triclosan/PVM/MA copolymer/fluoride dentifrice on volatile sulfur compounds *in vitro*

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OBJECTIVE: The objective of the investigation was to document the *in vitro* efficacy of a triclosan/PVM/MA copolymer/fluoride (TCF) dentifrice against the formation of volatile sulfur compounds (VSC) as well as the growth of H_2S-producing bacteria. Clinical studies using organoleptic judges, gas chromatography, or a portable sulfide monitor have generally been employed in the assessment of treatments for the control of oral malodor. However, these studies are not appropriate for screening purposes because of the expense and time required.

METHODS: An *in vitro* method was developed for the purpose of screening new compounds, agents or formulations for their ability to control VSC formation and for determining bio-equivalence of efficacy when implementing changes in existing formulations. The method combines basic microbiological methods, dynamic flow cell techniques and head space analysis. The *in vitro* VSC method was validated by comparing the efficacy of two dentifrices containing TCF with a control fluoride dentifrice as the TCF products have been clinically proven to control oral malodor.

RESULTS: In the validation studies, the TCF-containing dentifrices were significantly better ($P < 0.05$) than the control dentifrice in inhibiting VSC formation and reducing H_2S-producing bacteria. For example, when compared with baseline, the TCF dentifrices reduced VSC formation between 42 and 49% compared with the control dentifrice which reduced VSC formation 3%. There was no significant difference ($P > 0.05$) between the two TCF dentifrice formulations.

CONCLUSION: Using an *in vitro* breath VSC model, it has been demonstrated that two variants of a dentifrice containing triclosan, PVM/MA copolymer and fluoride have efficacy that is significantly better than a control fluoridated dentifrice and that there is no significant difference between the triclosan/PVM/MA copolymer/fluoride dentifrice variants.

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Keywords: copolymer; flow cell system; oral malodor; triclosan; dentifrice; saliva; *in vitro* method

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Introduction

The objective of the investigation was to document the *in vitro* efficacy of a triclosan/PVM/MA copolymer/fluoride (TCF) dentifrice against the formation of volatile sulfur compounds (VSC) as well as the growth of H_2S-producing bacteria. Clinical studies using organoleptic judges, gas chromatography, or a portable sulfide monitor have generally been employed in the assessment of treatments for the control of oral malodor. However, these studies are not appropriate for screening purposes because of the expense and time required.

Materials and methods

Products tested

Two triclosan/copolymer/sodium fluoride variants, the original Colgate® Total® formula (TCF-O) and a new formula with a high impact flavor, Colgate® Total® Advanced Fresh® (TCF-AF), were compared with a control dentifrice, Colgate® Anti-cavity dentifrice (Colgate-Palmolive Company, Piscataway, NJ, USA). Slurries of the dentifrices were prepared by mixing dentifrice and water in a 1:2 (w/w) ratio.

Growth inhibition

Saliva was collected from healthy human subjects and clarified by centrifuging for 20 min at 7840 *g*. Hydroxyapatite (HAP) disks were coated with clarified saliva for 30 min then incubated with 5 ml of dentifrice slurry in a 37°C water bath for 30 min. Subsequently, each disk, after rinsing twice with deionized sterile water was placed in 5 ml of trypticase soy broth inoculated with *c.* 10^6 cells of *Actinomyces naeslundii* (ATCC no. 43146). The optical density at 610 nm of the bacteria mixture was read after incubating for 0, 4 and 24 h at 37°C. The percentage reduction in bacterial growth was calculated *vs* the water control.

Zone of inhibition

Saliva-coated HAP disks were treated with dentifrice slurries and rinsed with water as described above. Bacteria from the back of the tongue were collected from the same healthy individual at time of use and

plated onto 'oral H_2S organisms' agar (OHO-C) containing lead acetate (Anaerobe Systems, Morgan Hill, CA, USA) to detect oral bacteria producing hydrogen sulfide (El-Halabi *et al*, 1999; Paryavi-Gholami *et al*, 1999). Each dentifrice slurry treated-HAP disk was placed in the center of the lawned agar plate, which was then incubated anaerobically at 37°C for 48 h. The distance from the edge of the clear area to the edge of the disk (zone of inhibition) was measured. The edge of the clear area was defined as the junctions between the clear zone and the growth area of the dark pigmented colonies. Products were tested in triplicate.

In vitro *VSC assessment*

The flow cell system, previously described by Pilch *et al* (2004), was modified by adding an air-tight headspace vial to collect gases formed from bacterial putrefaction (Figure 1). There were four sample chambers per flow cell with each having a total volume of about 5 ml. The system was housed in a 37°C incubator. Saliva from healthy individuals, diluted twofold with water and supplemented with 1% fluid thioglycollate broth (Becton, Dickinson, and Company, Sparks, MD, USA) served as the bacterial seed. To evaluate the effect of the test dentifrices on VSC formation, saliva-coated HAP disks were treated with dentifrice slurries as described above and then transferred to the sample chambers of the flow cell. The disks were then rinsed by flowing artificial saliva at a flow rate of 1 ml min^{-1} for 1 h (single pass through) to remove loosely bound materials. Supplemented saliva was then circulated through the flow cell system overnight at a steady flow rate of 0.1 ml min^{-1}. The generated VSC was trapped in the headspace vial and analyzed using an Agilent 6890 gas chromatography equipped with a flame photometric detector (Agilent Technologies, Palo Alto, CA, USA). An untreated cell was included in each study and used as a negative control. Efficacy was assessed by semi-quantitative means using the change (reduction) in the sum of the observed response values of the three volatile sulfur gases (hydrogen sulfide, methyl mercaptan, and dimethyl sulfide) for the test dentifrices compared with the negative control.

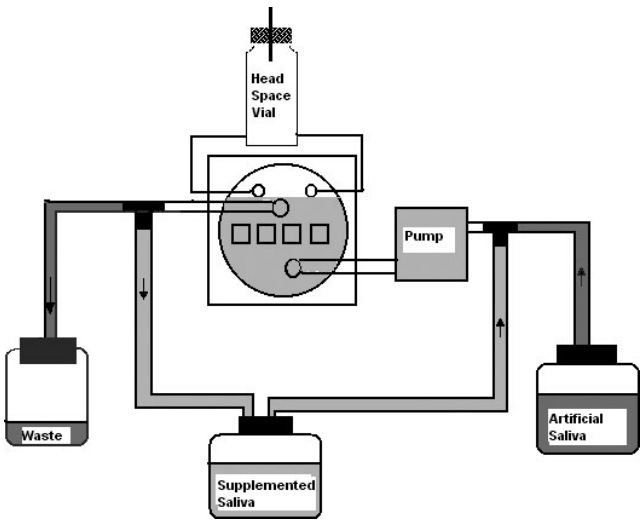

Figure 1 Schematic representation of the individual flow cell system. Gases produced by bacterial putrefaction are trapped in the tightly-sealed headspace vial

Post-reaction mixture assessment

The resulting supplemented saliva mixture from the *in vitro* VSC experiment was serially diluted with phosphate-buffered saline solution. The 10^{-3} and 10^{-4} dilutions were plated in duplicate onto OHO-C plates and incubated for 48 h at 37°C. The dark pigmented colonies were counted and expressed as log CFU.

Results

Antimicrobial efficacy of Colgate® Total® Advanced Fresh

Growth inhibition: The ability of the tested dentifrices to affect bacterial growth was evaluated using *A. naeslundii*, an early colonizer in dental plaque. Although there is no reported link between *A. naeslundii* and oral malodor formation, similar bacteria reduction profile has been observed between *A. naeslundii* and *F. nucleatum*, a known oral malodor former (data not shown), when treated with triclosan-containing products. The TCF-AF and the TAF-O formula reduced bacterial growth 59 and 51%, respectively, compared with water; whereas, the control dentifrice provided a 10% reduction in bacterial growth compared with water (Table 1). There was no significant difference ($P > 0.05$) between the two TCF variants. However, both were significantly ($P < 0.05$) better than the control dentifrice.

Zone of inhibition: The antimicrobial activity of the two triclosan/copolymer/sodium fluoride dentifrice variants was also observed on OHO-C plates using an *in vitro* diffusion method. The negative control dentifrice produced a zone that was 3.1 mm in diameter (Table 2). Both TCF variants produced similar zone of inhibition, 8.2 mm for TCF-O and 8.4 mm for TCF-AF (Table 2). There was no significant difference ($P > 0.05$) between the two variants, although both were significantly ($P < 0.05$) better than the control dentifrice.

Table 1 Percent bacterial growth inhibition after treatment with two variants of the triclosan/copolymer/ fluoride dentifrice and a control dentifrice

Product	*% Growth inhibition*[a]	*Statistical significance P*-value[b]
TCF dentifrice	51	< 0.05
TCF-AF dentifrice	59	< 0.05
Control dentifrice	5	> 0.05

[a]Within-treatment percent reduction between untreated cell values and post-treatment values, expressed as a percentage of the untreated cell values.
[b]Significance of within treatment paired *t*-test comparison of the untreated cell values *vs* post-treatment values.

Table 2 Zone of tongue bacterial growth inhibition after treatment with two variants of the triclosan/copolymer/fluoride dentifrice and a control dentifrice (mean ± s.d.)

Product	*Zone of inhibition (mm)*
TCF	8.2 ± 1.7
TCF-AF	8.4 ± 1.2
Control	3.1 ± 1.5

VSC formation in vitro

A modification of the *in vitro* model, first reported by Pilch *et al* (2004), was employed in the evaluation of two triclosan/copolymer/sodium fluoride dentifrice variants for the ability to reduce the formation of VSC associated with oral malodor. The TCF-AF formula displayed a similar percentage reduction in VSC formation compared with the TCF-O formula. The respective reduction in VSC formation for the TCF-AF and TCF-O dentifrices was 49 and 42% compared with the negative control (Figure 2). Both were significantly better ($P < 0.05$) than the control dentifrice which reduced VSC by approximately 3%.

Post-reaction mixture

The resulting bacterial mixture was analyzed for H_2S-producing bacteria using OHO-C agar which reveal H_2S-producing bacteria as dark pigmented colonies. The results, shown in Figure 3, indicated that the TCF-AF dentifrice provided bacterial reduction of 36% compared with the negative control, which was similar to the TCF-O formula (33%). Both variants were significantly better in reducing H_2S-producing bacteria than the control dentifrice.

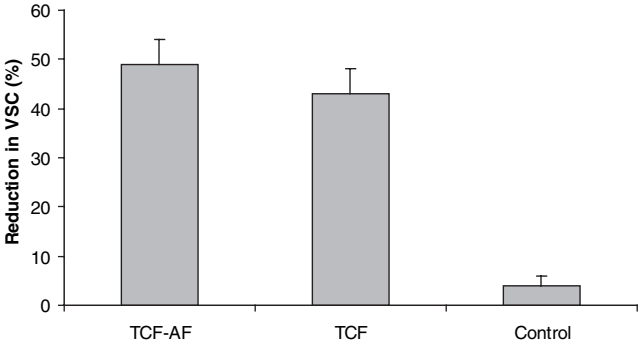

Figure 2 Percent reduction of VSC after treatment with two variants of the triclosan/copolymer/fluoride dentifrice and a control dentifrice *in vitro*

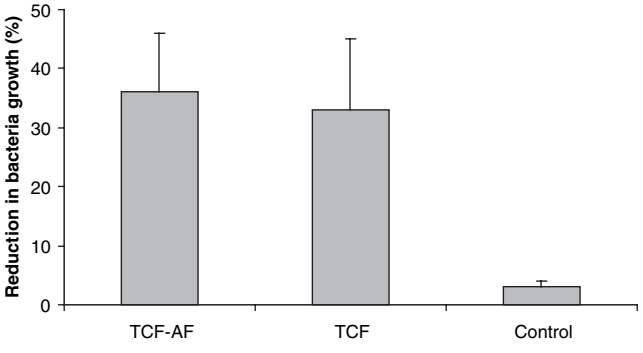

Figure 3 Percent reduction of H_2S-producing bacteria in the postreaction mixture after treatment with two variants of the triclosan/copolymer/fluoride dentifrice and a control dentifrice *in vitro*

Discussion

Three *in vitro* models were used to evaluate the efficacy of a new triclosan/copolymer/sodium fluoride dentifrice variant compared with the original formula and a control toothpaste. The triclosan/copolymer/sodium fluoride technology has been shown to reduce the common dental infections, caries and periodontal disease, as well as control common oral conditions such as tartar and oral malodor (Sharma *et al*, 1999; Mann *et al*, 2001; Allen *et al*, 2002; Mankodi *et al*, 2002). The TCF-AF dentifrice was shown to provide similar antibacterial activity to the original TCF formula and both were significantly better that a control dentifrice.

Many *in vitro* models developed to evaluate the effect of treatment for the control of oral malodor employ static conditions (Richter and Tonzetich, 1964; Solis-Gaffar *et al*, 1979; Kleinberg and Codipally, 1999) or in the case of dynamic models, concurrent entrapment of generated volatile sulfur gases is not achieved (Pratten *et al*, 2003). An *in vitro* breath VSC model, which incorporates flow dynamics and concurrent entrapment of volatile sulfur gases, was used previously (Pilch *et al*, 2004) to demonstrate the efficacy of a new triclosan/copolymer/fluoride variant (TCF-O) in reducing the formation of VSC. In that study (Pilch *et al*, 2004), the TCF-O dentifrice was shown to reduce VSC formation on average 35% *vs* a control dentifrice in a series of five experiments. It was expected that the TCF-AF dentifrice would behave similarly and the results of this study indicate that there was no significant difference between the TCF-AF and TCF-O variants in reducing VSC and H_2S-producing bacteria. Both variants were significantly more effective than a control dentifrice in reducing the measured factors. In a clinical study, Williams and Niles (2003) have demonstrated there was no significant difference between TCF-AF and TCF-O in their ability to control breath VSC measured by GC. Additionally, the results observed using the *in vitro* model were also supported by findings of Niles *et al* (1999) which showed that the triclosan and copolymer technology provided long-lasting control of breath VSC compared with a control dentifrice. These results are further supported by two *in vitro* microbiological methods which showed that the two TCF variants provided sustained antibacterial activity against oral bacteria. Together, these findings indicate that the *in vitro* breath VSC model may be a useful screener of developing formulations for the treatment of oral malodor before initiating expensive clinical trials.

In conclusion, a predictive *in vitro* breath VSC model, which incorporated flow dynamics, has been developed and used to demonstrate that two variants

of a dentifrice containing triclosan, PVM/MA copolymer and sodium fluoride have efficacy that is significantly better than a control fluoridated dentifrice and that there is no significant difference between the triclosan/PVM/MA copolymer/fluoride dentifrice variants.

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