The effect of a triclosan/copolymer/fluoride liquid dentifrice on interproximal enamel remineralization and fluoride uptake

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ABSTRACT

Background. The authors measured the enamel remineralization of dental interproximal surfaces by a triclosan/copolymer/fluoride liquid dentifrice to test its performance against that of a standard toothpaste.

Methods. In a randomized, crossover, blinded, in situ protocol, the authors fitted 19 healthy adult subjects with mandibular appliances holding bilateral, interproximal bovine enamel slabs. They measured initial abrasion levels and fluoride content. In a two-phase crossover protocol, they evaluated the percentage of surface mineral recovery, or SMR, and fluoride uptake caused by the experimental dentifrice and a fluoride-containing traditional control toothpaste.

Results. Abrasion depths were similar and not different statistically ($P > .05$). The interproximal blocks exposed to the triclosan-containing liquid dentifrice had a mean fluoride uptake that was nearly 100 parts per million (13.1 percent) greater than that achieved with the American Dental Association–accepted control dentifrice. This finding, while not statistically significant, may indicate enhanced performance of the experimental dentifrice because the initial enamel slab abrasion depths were shown to be quite uniform. Moreover, the blocks exposed to the experimental toothpaste had a 49.8 percent SMR, while the positive control blocks had an SMR of only 36.9 percent. This enhanced remineralization performance was statistically significant ($P < .05$).

Conclusions. This is the first study to show enhanced performance of a triclosan-containing liquid dentifrice in preventing interproximal dental caries. The difference in percentage of SMR was s statistically significant and is likely to be clinically relevant.

Clinical Implication. The results of this preliminary study offer evidence supporting clinicians' recommendation of the new liquid dentifrice to patients who are prone to developing interproximal carious lesions.

Fluoride works to inhibit incipient dental caries in several ways. It can prevent lesions by affecting the activity of cariogenic bacteria, and it can drive remineralization of demineralized tooth surfaces.[1] When remineralization overcomes the demineralization process, incipient caries actually can be reversed and the lesion can be repaired.[2]

A new liquid dentifrice may aid patients who are prone to developing interproximal carious lesions.

There are several sources of fluoride, an agent that is well-known to influence the process of remineralization. Bacteria on teeth metabolize carbohydrates and produce acid. As a result, the remineralization process begins when fluoride is released from the surrounding dental plaque in response to lowered pH at the tooth-plaque interface.[3] The released fluoride and the fluoride present in saliva then are adsorbed, along with salivary calcium and phosphate, by demineralized enamel to establish an

DISCLOSURE
Drs. DeVizio and Zhang are employees of Colgate-Palmolive, New York, manufacturer of the experimental dentifrice described in this article.

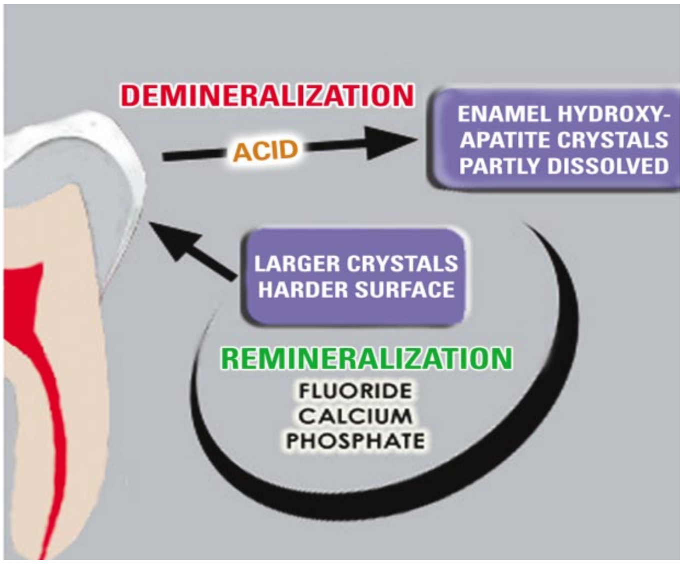

Figure 1. Demonstration of demineralization and remineralization processes. Remineralization of enamel leaves a surface with mineral crystals that are rich in fluoride and lower in solubility. Figure reproduced with permission of Dr. Martin Giniger, Martin Giniger & Company, New York.

improved enamel crystalline structure.[4] The apatite crystals get larger and the surface becomes harder (Figure 1). This improved structure is more acid-resistant, contains more fluoride and has less carbonate than the original structure.

In light of all of this, it should not be surprising that toothpaste containing fluoride, used twice daily, can be an effective way to enhance the remineralization process. In fact, publications in the past decade[5,6] have stated that fluoridated products are considered to be efficient therapeutic agents for caries control. Specifically, it is believed that the frequent use of low-concentration fluoride (such as toothpaste) by humans has been shown to be an efficient public health measure to combat caries.[6,7]

Examination of teeth exposed to a lifetime of fluoride dentifrice has shown that the outer few micrometers of enamel can have fluoride levels as high as 1,000 to 2,000 parts per million, or ppm, but typically less interproximally.[8] This may be because interproximal areas between teeth only rarely are affected by the mechanical action of toothbrushing owing to bristle size in relation to the tight contact area; in addition, it may be because the foam of standard toothpaste is too viscous and therefore unable to penetrate completely between teeth that are in tight contact with one another. Hence, bacteria tend to accumulate on interproximal surfaces and can demineralize them, further reducing effective fluoride concentration.[8]

Because of this tendency, and the fact that most people rarely floss,[8] new dentifrice technologies with lighter foaming properties and finer, more abundant bubbles need to be developed to carry fluoride to interproximal and other hard-to-reach areas, enhancing the acid resistance of vulnerable enamel. New commercially available “liquid” dentifrice formulations that recently have been introduced by several manufacturers may be able to fill this role. (The term “liquid” is placed in quotes because while the new formulations are not as runny as a true liquid such as water, they are less viscous and pastelike than traditional dentifrices.) This is because in comparison with standard pastes, liquid formulations with lighter, thinner foam should be able to penetrate tight spaces better and thereby more efficiently deliver a local fluoride dose.

Consequently, we undertook a study to assess whether a new liquid dentifrice—Colgate Total Liquid Dentifrice (Colgate-Palmolive, New York), containing 1,100 ppm fluoride, 0.3 percent triclosan and 2 percent copolymer in a mildly foaming silica base—demonstrated enhanced performance at interproximal tooth surfaces compared with a standard, paste-style positive control dentifrice that held the American Dental Association Seal of Acceptance. We determined potential anticaries activity in situ by evaluating surface microhardness and enamel fluoride uptake in partially demineralized enamel specimens.

The use of an in situ model also permitted direct evaluation of the interproximal areas, which could not be performed with an in vivo examination. We believed that if we found significant differences in surface mineralization, fluoride uptake or both as a result of use of the new dentifrice, it would indicate that further large-scale clinical trials would be warranted. It also would indicate that the experimental toothpaste might be useful for patients who are prone to developing interproximal carious lesions.

SUBJECTS, MATERIALS AND METHODS

Subjects. We collected a database of potential volunteers from people responding to fliers posted at the Universidade Federal de Alagoas, Maceió, Brazil. We chose 19 study subjects who fit the study’s exclusion/inclusion criteria to participate. They all lived in Maceió, where there is no community water fluoridation (the concentration of fluoride ion, or F-, in tap water is < 0.1 ppm). The medical histories and the oral soft tissue health status of the subjects all were good, and none of the subjects had significant periodontal disease.

All subjects were required to have a history of more than five carious lesions in the past, but they also were required to have no more than four currently active lesions to enter the study. Informed consent also was required. The key inclusion criteria required each subject to be missing both mandibular second premolars and all mandibular molars; have a history of interproximal dental caries; have a measured salivary flow rate within normal limits (parafilm stimulated ≥ 1.0 milliliter per minute; unstimulated, ≥ 0.2 mL/minute); and wear a Kennedy Class II bilateral mandibular partial denture.

In comparison with standard toothpastes, liquid formulations with lighter, thinner foam should be able to penetrate tight spaces better and thereby more efficiently deliver a local fluoride dose.

Materials and methods. *Abrasion depth and mineral density.* We prepared enamel blocks or slabs (2.2 × 2 × 2 millimeters) from bovine molars stored in 2 percent formaldehyde solution, pH 7.0, for at least a month. When ready for preparation, we weighed these slabs and then abraded 200 µm from their exterior-most surfaces to remove the high-fluoride external layer. During the study, the abraded side of the slab would serve as the "exposed" surface in the oral cavity, situated interproximally between two posterior teeth.

We then measured the width and depth of each polished slab using a micrometer to calculate the final reference surface area of the exposed surface in square millimeters. To check that approximately the same amount of enamel had been abraded from the external surface of all slabs, we calculated the exact baseline abrasion depth, or AD, using the following equation (as formulated by Iijima and Koulourides[9]):

AD (grams/cubic centimeters) = [mass of enamel specimen (µg)] / [reference surface area (mm^2) × 2.57 (bovine enamel density)]

We then used the AD to calculate the mineral density, or MD, of each slab. Using the slab's weighed mass and its surface area, we used the following equation (again, as formulated by Iijima and Koulourides[9]):

MD (g/cm^3) = [mass of test specimen enamel (µg)] / [reference surface area (mm^2) × AD (g/cm^3)]

Fluoride content of enamel slabs. A controlled demineralization process of the abraded enamel surfaces initiated the calculation of fluoride content, or FC, of each enamel slab. First, we painted the nonexposed sides of each enamel block with clear nail polish. After the polish had dried, we exposed the remaining abraded surface to 0.25 mL of 0.25-molar perchloric acid solution for 15 seconds. After acid exposure, we removed the enamel blocks and neutralized the remaining acid solution supernatant with 250 microliters of Total Ionic Strength Adjustment Buffer (Sigma-Aldrich Brasil, São Paulo, Brazil) containing 10 grams of sodium hydroxide per liter. We then determined the supernatant's fluoride concentration with an Orion 96-09 Fluoride (F-) Sure-Flow combination electrode (Thermo Electron, Beverley, Mass.), which the manufacturer states is accurate within 1 ppm. We then dried, weighed and saved the resulting demineralized enamel blocks for testing of surface microhardness (see below).

Since we knew the volume of the supernatant and fluoride atomic mass, we could calculate the fluoride mass, or FM (in micrograms), of the supernatant from its measured concentration. Ultimately, we calculated the FC of the slabs using the following formula:

FC (ppm) = FM (µg) / [initial mass of block (µg) × 10^6]

Microhardness testing. After each determination of FC, we repolished the blocks for 10 seconds with 1-micrometer aluminum oxide particles in solution and assessed them for baseline surface microhardness, or BMH, with a Knoop indenter and a 50-g load.

We made indentations using a microhardness tester with a Knoop diamond indenter (HMV-2000 Microhardness Measurement Unit, Shimadzu, Kyoto, Japan) set at a 50-g load for 30 seconds. We made six indentations at 10, 20, 30, 50, 70 and 90 µm, successively, from the outer enamel surface in three different regions: above, below or directly at the center region of the

TABLE 1

MAJOR CHARACTERISTICS OF THE EXPERIMENTAL AND POSITIVE CONTROL PRODUCTS.*

DENTIFRICE GROUP	BRAND NAME†	CONTENT AND FORM
Experimental	Colgate Total Liquid Dentifrice	0.3 percent triclosan 2 percent copolymer 1,100 parts per million, or ppm, fluoride ion Silica base "Liquid" format
Positive Control	Colgate Winterfresh Fluoride Dentifrice	1,100 ppm fluoride ion Silica base Traditional paste format

* Both were used for 10 days each, with a seven-day washout period between treatments.
† Both are products of Colgate-Palmolive, New York.

enamel block. We then averaged the mean values of all regional measuring points. We calculated the areas under the curves (Knoop hardness number, or KHN × μm) using a trapezoidal rule.

We next subjected these baseline pretreatment blocks to a six-hour demineralization procedure using an acetate-caustic potash buffer of pH 5.0, and then reassessed them for demineralized microhardness, or DMH. We subsequently sterilized and stored these slabs until placing them in the subject's partial dentures.

Exposure of slabs to test products. The goal of our study was to measure fluoride uptake and mineral uptake in the interproximal area between mandibular molar and premolar denture teeth bilaterally as influenced by two different dentifrices. We compared the effects of an experimental triclosan/copolymer/fluoride liquid dentifrice, or LD) to a commercially available, clinically proven ADA–accepted toothpaste, or TP, containing 1,100 ppm fluoride as a positive control (Table 1). The investigator involved with measuring fluoride and mineral uptake (M.F.D.S.) was blinded to this variable.

We randomly placed, by means of a coin flip, eligible subjects aged 18 to 60 years (mean age = 36.7 years) into one of two dentifrice treatment groups (LD or TP) and then fitted them with a special mandibular partial denture appliance (Figure 2) that contained two of the previously demineralized, assayed and measured bovine enamel blocks as described above. We fitted these highly polished squares of enamel flush into premilled 2-mm–deep recesses on the mesial aspect of the first molar prosthetic teeth and placed them in tight contact with the adjacent distal aspect of the second premolar.

The subjects received both oral and written oral hygiene instructions. We instructed them not to use floss at all and to brush twice daily for two minutes each time. Because liquid toothpaste has the consistency of syrup and sinks readily into the toothbrush bristles, the ADA-recommended "pea-sized" amount of dentifrice could not be used. Instead, we instructed the subjects to use a ribbon of either paste or liquid, covering the full length of the brush head. After brushing, the subjects were to swish the dentifrice between and all over their teeth for 15 seconds before expectorating. Subjects were allowed to use only tap water for this purpose, and we also asked them to drink only tap water, not bottled water, for the duration of the study. No other dietary instructions were given.

We conducted this study in two 10-day phases during a three-week period. After a washout period that followed the first phase, we assigned each subject to use the other dentifrice for the second phase. After completion of each phase, we recovered the enamel blocks, again assessed them for treatment microhardness, or TMH, and further analyzed FC using the methods described above.

Phase two of the crossover design was preceded by a seven-day washout period using a fluoride-free placebo dentifrice. After the washout, the subjects again reported to the clinical facility and had two new predemineralized enamel blocks placed in their mandibular removable partial dentures. At this appointment, they were provided with the opposite, crossover dentifrice for use during the final 10-day cycle. At the end of the experimental period, the blocks were recovered and the subjects were dismissed.

Percentage of surface mineral recovery. There is a linear relationship (r = 0.9) between the KHN and mineral content in enamel.[10] Therefore, determination of the KHN allows for determination of percentage of surface mineral recovery, or SMR, using a simple calculation (as established by Zhang and colleagues[10]). The blocks recovered after 10 days of exposure to the oral cavity were subject to one last assay for TMH using the method described above. Ultimately, we calculated percentage of SMR by using the method of Zhang and colleagues[10]:

$$\Delta_1 = (BMH) - (DMH)$$
$$\Delta_2 = (DMH) - (TMH)$$
$$\text{percentage of SMR} = (\Delta_1/\Delta_2) \times 100$$

Statistical analysis. We expressed results as means ± standard deviations, or SDs. We tested differences between the groups for significance using an unpaired Student *t* test. Differences were regarded as statistically significant when $P < .05$.

RESULTS

ADs and baseline FC. Two sets of enamel slabs were used on each subject. Phase 1 slabs were those placed at baseline in subjects of both groups; phase 2 slabs were the second set, placed after the washout period, just before dentifrice crossover. We determined the ADs of both sets to be extremely similar. The phase 1 slabs had a mean AD of 1.89 µm ± 0.06 in the LD group and 1.90 µm ± 0.04 in the TP group. Similarly, the phase 2 slab AD means of both groups were within .07 µm of each other. Because bovine enamel is known to be extremely consistent in FC at any given AD,[9] the close ADs yielded enamel slabs with a baseline FC that was not statistically different ($P > .05$) between any of the groups or sets.

FC. We measured fluoride uptake caused by the treatment dentifrice as the difference between the baseline FC and the FC of the slabs after 10-day exposure to either the LD or the TP dentifrice. This is an important measure of the potential anticaries activity of a test product, because remineralization has been shown to have excellent correlation with clinical efficacy.[10] Table 2 shows the FC of the enamel blocks (in ppm) after

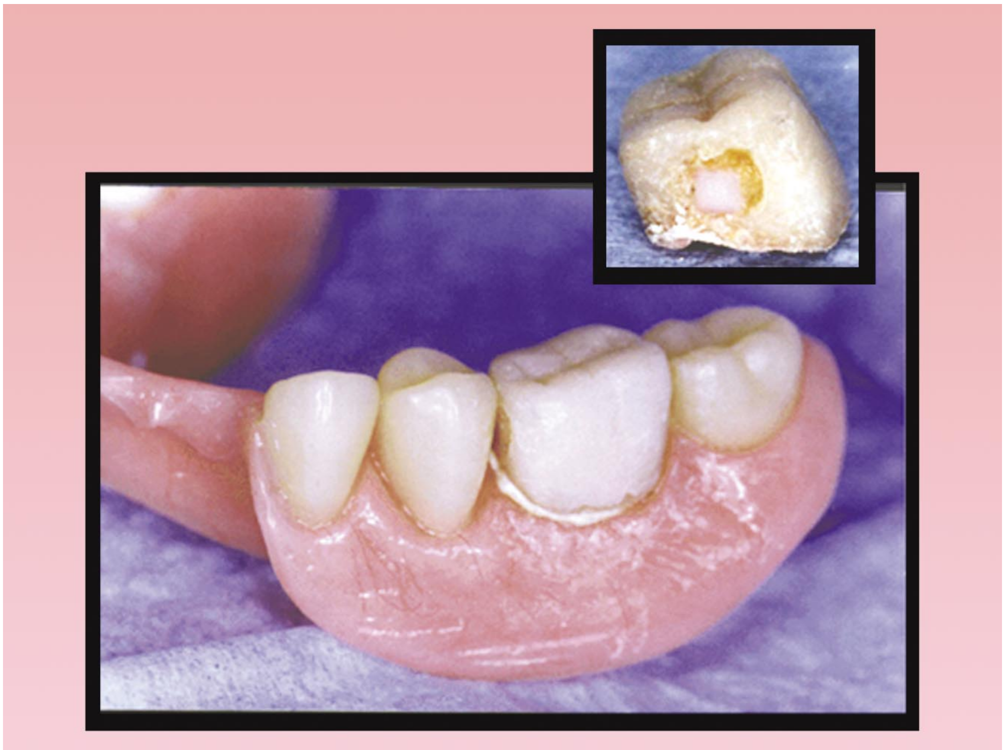

Figure 2. Mandibular Kennedy Class I distal extension prosthesis with an embedded, demineralized enamel block on the mesial surface of tooth 30. Inset: Mesial surface of tooth no. 30 showing the in situ enamel block in place after 10 days of contact with distal aspect of tooth no. 29. Figure reproduced with permission of Dr. Milton Fernando de A. Silva, Universidade Federal de Alagoas, Maceió, Brazil.

TABLE 2

SUMMARY OF THE MEAN FLUORIDE UPTAKE FOR EACH GROUP AFTER 10 DAYS OF TREATMENT.

GROUP	N	MEAN F-* UPTAKE IN PPM† ± SD‡	DIFFERENCE
LD§ (Experimental)	38	746.2 ± 97.3	13.1 percent NS# ($P > .05$)
TP¶ (Positive Control)	38	659.9 ± 78.8	

* F- : Fluoride ion.
† ppm: Parts per million.
‡ SD: Standard deviation.
§ LD: Liquid dentifrice (experimental dentifrice) (Colgate Total Liquid Toothpaste, Colgate-Palmolive, New York).
¶ TP: Toothpaste (positive control dentifrice) (Colgate Winterfresh Dentifrice, Colgate-Palmolive, New York).
# NS: Not significant (according to unpaired Student *t* test).

TABLE 3

SUMMARY OF MEAN PERCENTAGE OF SURFACE MINERAL RECOVERY, OR SMR, FOUND FOR THE TWO GROUPS TESTED.

GROUP	N	MEAN % SMR ± SD*	DIFFERENCE
LD† (Experimental)	38	49.80 ± 16.32	35.0 percent Significant§ ($P < .05$)
TP‡ (Positive Control)	38	36.96 ± 14.20	

* SD: Standard deviation.
† LD: Liquid dentifrice (experimental dentifrice) (Colgate Total Liquid Toothpaste, Colgate-Palmolive, New York).
‡ TP: Toothpaste (positive control dentifrice) (Colgate Winterfresh Dentifrice, Colgate-Palmolive, New York).
§ According to unpaired Student *t* test.

10 days of treatment with either an experimental or a control dentifrice. Enamel exposed to the liquid toothpaste had a mean fluoride uptake nearly 100 ppm (13.1 percent) greater than that of the positive control. This observed trend in favor of enhanced fluoride uptake performance by the LD group, while not statistically significant at the preset level of $P< .05$, cannot be discounted because the ADs, and thus the initial FC of the baseline slabs, were extremely uniform.

Percentage of SMR. The percentage of SMR, shown in Table 3, is an objective, ADA-recommended method of measuring anticaries effectiveness.[11] In our study, we measured a significant difference between the experimental and positive control groups ($P < .05$). Group LD had a mean 48.8 percentage of SMR and group TP a mean of 36.96 percent.

DISCUSSION

The in situ denture chip model described in this report is an ADA-validated model system for the assessment of a dentifrice's potential anticaries activity. In 1989, the ADA Council on Dental Therapeutics (now the ADA Council on Scientific Affairs) awarded its Seal of Acceptance to a new, modified fluoride dentifrice largely on the basis of data from intraoral appliance models,[11] thus acknowledging that denture chip remineralization studies could be used as a measure of dentifrice's clinical effectiveness.

Because most of today's toothpastes contain similar levels of fluoride and other inactive ingredients, their properties tend to be equivalent. While many dentifrices make marketing claims to reach in between the teeth, few if any ever have been shown to have an enhanced activity in that crucial area. Because many dental carious lesions form interproximally, a dentifrice that has demonstrated activity at these sites would be expected to have the potential to decrease overall caries prevalence.

In that regard, the results of this study are promising, as they show a progressive increase in the fluoride and MD of the interproximal in vivo tooth enamel when exposed to an experimental liquid dentifrice (Colgate Total Liquid Toothpaste). Also, the changes in both FC and MD we saw with the experimental dentifrice were greater than the changes we measured for the positive control toothpaste.

There are several mechanisms that could provide a possible explanation for the results and that warrant further study. One credible explanation is related to the liquid toothpaste's light foaming properties and lower surface tension. These attributes, in theory, could allow the liquid dentifrice to be carried into the depths of pits, fissures and interproximal spaces better than a traditional toothpaste. The very presence of dentifrice in these narrow caries-prone locations could be sufficient to enhance the dentifrice's anticariogenic properties, especially because few people floss effectively.[4]

In the present study, Colgate Total Liquid Dentifrice produced a 13.1 percent higher fluoride uptake in the interproximal enamel. And, most importantly, the difference in percentage of SMR was sufficiently large to be statistically significant and is likely to be clinically relevant. Therefore, ours is the first study to show that a triclosan-containing liquid dentifrice may prevent interproximal dental caries somewhat more than a standard fluoride dentifrice.

CONCLUSION

On the basis of the above arguments, we believe our study provides preliminary evidence that there are likely clinical implications of using this new liquid dentifrice. At the very least, the results provide preliminary evidence supporting clinicians' recommendation of the product to patients who are prone to developing interproximal carious lesions. We also believe the study

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provides sufficient evidence to justify further large-scale clinical trials of this product. ■

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