

Efficacy of Natural Toothpastes in an *In situ* Caries Model



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ABSTRACT

Objective: To evaluate the efficacy of two NaF/silica base (whitening gel and whitening liquid gel) all-natural dentifrices in an *in situ* caries model clinical trial.

Methods: Two gauze-covered human enamel blocks, one sound (DEMIN model) and one partially demineralized (REMIN model), were placed in the buccal flange area of subjects' mandibular partial dentures. Forty-one subjects completed the 5 leg, double blind, crossover study. Test dentifrice treatments (see Table below) were applied by brushing twice per day for 14 days. The DEMIN and REMIN specimens were each evaluated for surface microhardness (SMH) using a Wilson 2100 Hardness Tester and fluoride uptake using the microdrill enamel biopsy technique. Statistical analyses included ANOVA and pair-wise comparisons between treatments. PCK tests were employed by estimating $X_{50} - 1/2(X_{10} + X_{90})$ and testing at a 5% significance level.

Results:

Dentifrice	REMIN Model		DEMIN Model	
	% SMH Recovery	Fluoride Uptake $\mu\text{g}/\text{cm}^2$	% SMH Change	Fluoride Uptake $\mu\text{g}/\text{cm}^2$
0ppm F Placebo	20.8±12*	5.6±4	36.2±33	2.2±1
250ppm F Control	25.0±11	9.6±5	27.9±31	3.1±2
1100ppm F USP Ref.	39.0±17	16.1±11	17.6±22	3.8±2
Tom's Whitening Gel	40.3±18	18.2±12	18.2±16	4.2±2
Tom's Whitening Liquid Gel	42.7±20	18.3±10	18.2±21	4.8±4

*Mean ± SD

A dose response to increasing fluoride concentration was observed for all test parameters. In both the DEMIN and REMIN models, both test products exhibited significantly better efficacy than Placebo and 250ppm F Control, but were not significantly different from USP Reference Standard. Fluoride uptake for both test products was found to be "at least as good as" the USP Reference. In the REMIN model only, both test products passed the robust demands of the PCK test.

Conclusions: Tom's of Maine Natural Anticavity plus Whitening Gel and Natural Anticavity plus Whitening Liquid Gel Toothpastes were found to provide clinical anticaries efficacy as determined by *in situ* remineralization and fluoride uptake.

INTRODUCTION

FDA testing requirements¹ to demonstrate the bioavailability of fluoride toothpastes are outlined in the anticaries monograph as Animal Caries Reduction and either Enamel Solubility Reduction or Fluoride Enamel Uptake. Because Tom's of Maine does not support the use of animal testing, Tom's previously petitioned and was subsequently granted permission by FDA to substitute an *in situ* caries model in lieu of the required animal caries test. Tom's of Maine has once again made use of an *in situ* caries model to comply with requirements set forth in the caries monograph for marketing silica/sodium fluoride toothpastes.

OBJECTIVE

The purpose of this study was to compare the efficacy of two new fluoride dentifrice formulations with a clinically proven fluoride product (USP Reference Dentifrice) in an *in situ* caries clinical trial, thus fulfilling FDA monograph requirements without the use of animal testing.

MATERIALS & METHODS

Study Population

Forty-two subjects were recruited to participate in the study involving five test legs with different dentifrice treatments, each lasting two weeks. Forty-one subjects completed the study. All subjects were fitted with mandibular partial dentures modified to hold two enamel specimens on the buccal flange (Figure 1).

Inclusion Criteria

1. Provided written informed consent and medical history information;
2. Were between 18 and 75 years old and in good health;
3. Lived in a community with a fluoridated water supply (1 ppm F);
4. Wore a removable mandibular partial denture with sufficient room in the posterior buccal flange area to accommodate two enamel specimens;
5. Had no evidence of gross active caries or moderate to severe periodontal disease;
6. Had a salivary flow rate in the range of normal values (unstimulated whole saliva flow rate ≥ 0.2 mL/min; stimulated whole saliva flow rate ≥ 1.0 mL/min); and
7. Agreed to comply with all subjects' responsibilities as stated in the protocol.

In situ Caries Model

Two gauze-covered human enamel blocks, one sound and one partially demineralized, were placed in the buccal flange area of the subjects' mandibular partial dentures. The use of the gauze-covered sound enamel specimen served as a demineralization (DEMIN) model, while the gauze-covered partially demineralized enamel specimen served as a remineralization (REMIN) model. Changes in mineral content of the enamel specimens were assessed using the surface microhardness (SMH) test². The DEMIN and REMIN specimens were each evaluated for SMH using a Wilson 2100 Hardness Tester. The extent of demineralization was calculated based on the % change in SMH:

$$\% \text{ SMH change} = \frac{D-B}{B} \times 100$$

Figure 1



Figure 2

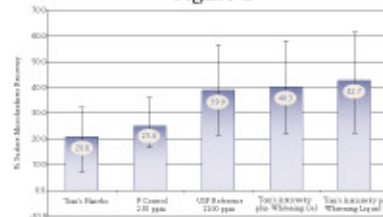


Figure 3

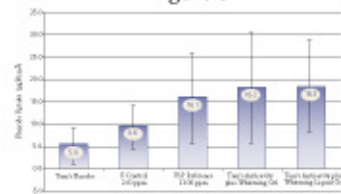


Figure 4

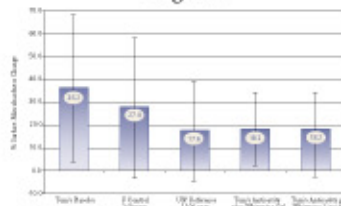
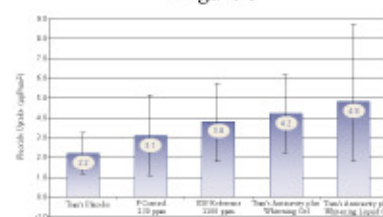


Figure 5



± margin of error

B = indentation length (μm) of the sound enamel specimen at baseline
D = indentation length (μm) after *in situ* demineralization

The extent of remineralization was calculated based on the method of Gelhard³ et al.

$$\% \text{ SMH recovery} = \frac{D-R}{D-B} \times 100$$

d = indentation length (μm) after *in vitro* demineralization
R = indentation length (μm) after intra-oral exposure.

Enamel fluoride uptake was determined using the microdrill biopsy technique⁴.

Study Design

The 5-leg study was conducted double blind with all subjects completing all aspects of the study in a crossover design. There was a one-week "wash-out" period between each two-week test leg to minimize any carry-over effects. Subjects received a dental cleaning two to three days prior to placement of the specimens. Once placed, subjects wore their partial dentures twenty-four hours a day and used their assigned toothpaste until their next visit.

Treatments

The following dentifrice treatments were applied by brushing twice/day for 14 days:

1. 0 ppm sodium fluoride/silica placebo dentifrice
2. 250 ppm sodium fluoride/silica control dentifrice
3. 1100 ppm sodium fluoride/silica dentifrice (USP Reference)
4. Tom's of Maine Anticavity plus Whitening Gel Toothpaste (1100ppm NaF)
5. Tom's of Maine Anticavity plus Whitening Toothpaste Liquid Gel (1100ppm NaF).

Statistical Analysis

Statistical analyses included ANOVA and pair-wise comparisons between treatments. For the SMH data, PCK tests⁵ were employed by estimating $X_{50} - 1/2(X_{10} + X_{90})$ and testing at a 5% significance level the one-sided hypothesis that the true mean difference was significantly lower than zero for % SMH Change and significantly greater than zero for % SMH Recovery. For the fluoride uptake data, a 90% confidence interval for the ratio (true mean fluoride uptake for test dentifrice / true mean fluoride uptake for USP Reference) was calculated in order to investigate whether the fluoride uptake associated with the test dentifrice was "at least as good as" that associated with the USP Reference.

SUMMARY OF RESULTS

Remineralization Model: %SMH Recovery (Figure 2).

- %SMH recovery was significantly lower for placebo than USP and the two Tom's dentifrices.
- 250ppm control was also significantly lower than USP and the two Tom's dentifrices.
- The USP and the Tom's dentifrices were not significantly different from each other.
- The PCK tests were statistically significant for both Tom's dentifrices.

Remineralization Model: Fluoride Uptake (Figure 3)

- Fluoride uptake was significantly lower for placebo than all other treatments. Fluoride uptake was significantly lower for the 250ppm control than USP or the Tom's dentifrices.
- The USP and Tom's dentifrices were not significantly different from each other.
- The 90% confidence interval for the ratio of test dentifrice fluoride uptake to USP fluoride uptake indicated that the two Tom's dentifrices were both "at least as good as" the USP for fluoride uptake.

Demineralization Model: %SMH Change (Figure 4)

- %SMH change was significantly higher for placebo than all other treatments. %SMH change was significantly higher for 250ppm control than USP and the two Tom's dentifrices.
- The USP and Tom's dentifrices were not significantly different from each other.
- The PCK tests were not statistically significant for either Tom's dentifrice.

Demineralization Model: Fluoride Uptake (Figure 5)

- Fluoride uptake was significantly lower for placebo than all other treatments.
- Fluoride uptake was significantly lower for 250ppm control than USP or the Tom's dentifrices.
- The USP and Tom's dentifrices were not significantly different from each other.
- The 90% confidence interval for the ratio of test dentifrice fluoride uptake to USP fluoride uptake indicated that the two Tom's dentifrices were both "at least as good as" the USP for fluoride uptake.

CONCLUSIONS

Based on all results of this study, the findings suggest the plausibility of concluding that Tom's of Maine Natural Anticavity plus Whitening Gel and Natural Anticavity plus Whitening Liquid Gel Toothpastes both provide a clinically efficacious level of anticaries efficacy through the action of remineralization.

REFERENCES

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