An *in vitro* caries cycling study of fluoride bioavailability from dentifrices

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Introduction

The overall efficacy of fluoride dentifrices in the prevention of dental caries is well established, with fluoride bioavailability being one of the most important parameters.

The purpose of this *in vitro* caries cycling study was to investigate the activity of three fluoride toothpastes to:

- promote enamel fluoride uptake (EFU)
- promote early caries lesion remineralisation (REM)
- diminish subsequent demineralisation (DEM)

Furthermore, the results of the present study are compared to an *in situ* caries study [1].

Methods

Specimen Preparation
Enamel specimens (3 mm in diameter) were removed from extracted human teeth and mounted in rods. The specimens were ground and polished using standardised methods (n=18).

Initial Decalcification
Artificial lesions were formed in the enamel specimens by a 96h immersion in a solution of 0.1M lactic acid and 0.2% Carbopol C907 which was 50% saturated with hydroxyapatite and adjusted to pH 5.0. All specimens had Vickers' surface hardness between 25 and 45 (average lesion depth was estimated to be approximately 70µm).

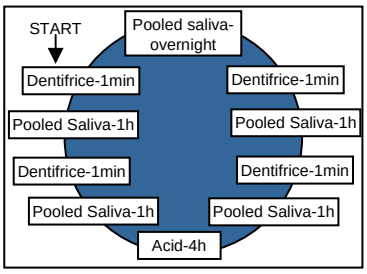
Saliva Collection
A 50:50 mixture of pooled human and artificial saliva (gastric mucin-2.2g/l; NaCl-0.381g/l; $CaCl_2$*$2H_2O$-0.213g/l; KH_2PO_4-0.738g/l; KCl-1.114g/l) was used as the remineralisation medium. Wax-stimulated saliva was collected from at least five individuals and refrigerated until used. Fresh pooled saliva was used each day.

Treatment Slurries
During the treatment period, the specimens were immersed in dentifrice slurries. The slurries were prepared by adding 5g of dentifrice to 10g of the fresh pooled saliva. A fresh slurry was prepared just prior to each treatment.

Treatment Products - Dentifrices

USP	USP sodium fluoride (NaF)/silica reference standard; 1450ppm F
NaF	Aquafresh Fresh & Minty (NaF/silica); 1350ppm F
MFP	Colgate Cavity Protection; dicalcium phosphate, sodium monofluorophosphate (MFP), NaF; 1450ppm F (1000ppm F as MFP, 450ppm F as NaF)
FFP	fluoride-free placebo, silica

Treatment Regimen
The daily cyclic treatment regimen was repeated for 20 days as followed:



*On the first day, the first dentifrice treatment was not given; the test began with one hour in pooled saliva to permit pellicle development prior to any treatments.
After the treatments, the specimens were rinsed with running deionized water and then placed back into the pooled saliva.

Fluoride Analysis (EFU)
At the end of the 20-day treatment regimen, the fluoride content of each enamel specimen was determined using the microdrill technique to a depth of 100µm.

Remineralisation Measurements (REM)
Following fluoride analysis all samples were measured again for surface microhardness. As all treatment groups were balanced for initial lesion hardness, surface microhardness measurements after the 20 day cycling period reflect the ability of the analyte to enhance remineralisation.

Determination of Enamel Resistance to Demineralisation (DEM-2h; DEM-16h)
Resistance of treated enamel to a subsequent acid challenge was determined by placing the treated specimens into the lesion formation solution (with no remineralisation phase) for one 2-hour and one 16-hour period of simulated plaque acid challenge (SPAC). Following each acid challenge, the surface hardness of the specimens was measured. The difference between the hardness following each subsequent demineralisation and the hardness after the 20 day cycling period would reflect the degree of resistance to demineralisation provided by each dentifrice.

Statistical analysis
The mean and standard error (SEM) were calculated for each group and each measurement point. A one-way ANOVA at the 95% confidence interval was employed in this study to determine differences between treatment groups.

Results

The EFU data as a function of dentifrice treatment measured after the 20 day cycling period are shown in **Figure 1**.

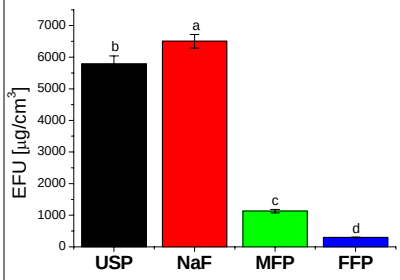


Figure 1. Enamel fluoride uptake of enamel samples post-cycling as a function of dentifrice treatment. Significant differences are indicated by different letters (p < 0.05)

Both sodium fluoride dentifrices caused a statistically significantly higher fluoride uptake than MFP, with NaF treatments resulting in a statistically significantly higher enamel fluoride uptake than USP.

The VHN data for all treatments and measurement points are shown in **Table 1**.

Table 1. Microhardness data (VHN) for all treatment groups and measurement points

	VHN (SEM)			
Dentifrice	initial	REM	DEM-2h	DEM-16h
USP	35 (1)	126 (7)	108 (6)	98 (6)
NaF	35 (1)	146 (5)	130 (4)	116 (4)
MFP	35 (1)	45 (2)	37 (2)	32 (2)
FFP	35 (1)	30 (2)	25 (2)	22 (2)

For clarity, the REM and DEM data are also shown graphically in **Figures 2 and 3**. For better comparison between treatments, the DEM data were transformed using the following equation:

$$DEM = \frac{VHN_{dem}}{VHN_{rem}} \bullet 100\%$$

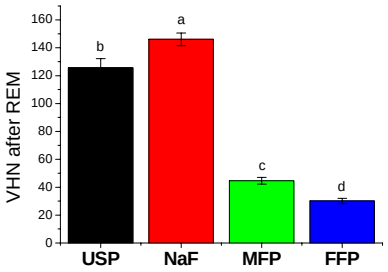


Figure 2. Carious enamel remineralisation as a function of dentifrice treatment. Significant differences are indicated by different letters (p < 0.05)

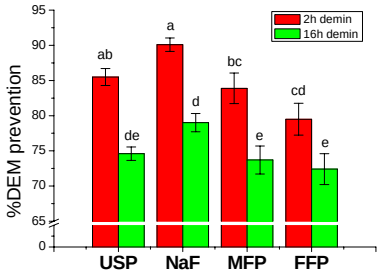


Figure 3. Enamel demineralisation protection as a function of dentifrice treatment. Significant differences are indicated by different letters (p < 0.05)

Figures 2 and 3 show that NaF treatments resulted in statistically significantly superior enamel remineralisation than any other dentifrice and statistically significantly better demineralisation protection than MFP and FFP, and directionally superior demineralisation protection than USP. Both sodium fluoride dentifrices showed statistically significantly superior remineralisation and directionally superior demineralisation protection than MFP.

In a related *in situ* caries study [1], the enamel fluoride uptake and enamel remineralisation potential of two commercially available dentifrices (NaF and MFP) were investigated. In this *in situ* study, NaF was found to be statistically significantly superior in EFU and REM compared to MFP.

Conclusions

- Both sodium fluoride dentifrices showed greater efficacy than MFP
- NaF was superior to USP, and this despite the higher fluoride concentration of USP
- EFU did correlate very well with REM and well with DEM and has proven to be a very good predictor of anti-caries activity
- Results of the present *in vitro* study correlate very well with a comparable *in situ* study. Results from both studies clearly highlight the superior anti-caries activity of NaF vs. MFP
- Data from this study support the usefulness of this human saliva based cycling model to investigate dentifrice anti-caries activity

References

[1] D. Zero, H.M. Proskin, R.M. Buch, C. Kollar, M.L. Bosma, S.R. Smith. Efficacy of marketed dentifrices using an in situ caries model. IADR 2007, abstract 0502.