Effect of CPP-ACP or a Potassium Nitrate Sodium Fluoride Dentifrice on Enamel Erosion Prevention

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Aim: *To investigate in situ/ex vivo the effect of remineralizing agents in the prevention of dental erosion in permanent and primary teeth.* ***Study design:*** *A randomized, controlled, double-blind study with crossover design with three treatment phases: Control, ProNamel® and Tooth Mousse™. Twenty adults and children wore removable palatal appliances containing two insets of permanent and primary human enamel and used the corresponding assigned toothpaste twice daily for 10 days. The enamel samples were then removed, mounted on acrylic bases and acid-challenged in demineralizing solution. Enamel surface microhardness (SμH) was measured pre and post acid challenge. Data were analyzed using two-way ANOVA and Tukey's post hoc test (P < 0.05).* ***Results:*** *The mean SμH values (Vicker's unit) prior to acid challenge were: Permanent teeth (Control 366.16 ± 12.28, ProNamel® 372.18 ± 14.75, Tooth Mousse™ 370.19 ± 11.88) and Primary teeth (Control 325.31± 11.90, ProNamel® 327.34 ± 9.90, Tooth Mousse™ 331.63 ± 10.55). Following the acid challenge, the mean %ΔSμH (±SD) were: Permanent (79.72 ± 1.59, 66.52 ± 2.45, 60.13 ± 4.98) and Primary (81.09 ± 2.90, 76.50 ± 3.13, 69.02 ± 4.23).* ***Conclusion:*** *The application of remineralizing agents reduced the significantly softening by acidic attack of enamel especially in the permanent dentition.*

Key words: *Prevention, remineralization, Tooth Mousse™, ProNamel®, dental erosion*

INTRODUCTION

Dental erosion is defined as the loss of tooth substance by chemical process of acid exposure and dissolution, but not involving bacterial plaque acid. The process of dissolution can be halted by remineralization using essential minerals such as calcium, phosphate, and fluoride to produce more acid resistant crystals than the original hydroxyapatite crystal.[1] Tooth Mousse™ (GC Corporation, Tokyo, Japan) and ProNamel® (GSK, Uxbridge, UK) are two commercially available products claimed to provide protection for teeth against the effects of acid erosion by remineralization of enamel and increasing its acid resistance.[2,3]

Tooth Mousse™ is an alkaline casein phosphopeptide amorphous calcium phosphate (CPP-ACP) based paste. The mechanism of its action is based on the ability of amorphous calcium phosphate (ACP) to bind to the tooth surface and plaque in case of an acidic attack.[2] The bond between the casein phosphopeptide (CPP) and the ACP is acid-dependent and reduces as the pH drops [Cross et al., 2007].[2] Thus, under acidic conditions, the localized CPP-ACP buffers the free calcium and phosphate ions, increasing the level of calcium phosphate in plaque and maintaining a state of supersaturation that inhibits demineralization and enhances remineralization, preventing also episodes of dental erosion.[3]

ProNamel® is a product recently launched on the market, and for this reason, little evidence is available on its efficacy in the prevention of dental erosion.[4,5] ProNamel™ is available as a white, mint flavored toothpaste, containing 5% w/w potassium nitrate and 0.32% w/w sodium fluoride as active ingredients. Sodium fluoride, its main component, is known to increase the enamel hardness values and inhibit subsequent softening following an erosive attack.[6] To the best of the authors knowledge, the effect of ProNamel® in the prevention of dental erosion in primary teeth has never been reported.

The aim of this study was to quantitatively assess the effectiveness of two remineralizing products, namely Tooth Mousse™ (GC Corporation, Tokyo, Japan) and ProNamel® (GSK, Uxbridge, UK) *in situ* against the development of erosive lesions in both primary and permanent enamel by measuring the surface microhardness of enamel treated with either products.

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MATERIALS AND METHOD

This was a randomized, controlled, double-blind study with a crossover design. Ethical approval was obtained from the Institutional Review Board (IRB) at Jordan University of Science and Technology (JUST). All subjects/subject's legal guardians received a complete explanatory document of the study, signed an informed consent and children signed an assent form.

For sample size determination, data from previous *in situ* studies were used.[7,8] Sample size calculations were based on an α-error of 5%, and power of 80% to detect an effect size of 15.0 (±8.7). Ten subjects were selected for the study in each group considering the possible loss inherent to *in situ* studies.

Ten healthy adults (aged 18-41years/ 5 males and 5 females) and ten healthy children (aged 8-10 years/ 5 males and 5 females) residing in the same optimally fluoridated area (0.70mg F/L) and needing extractions of at least three of their third molars or three of their primary second molars as a part of their dental care were recruited at the Dental Clinics/JUST. Inclusion criteria included having at least 22 natural teeth (in adults), adequate oral health with no caries or erosion lesions, and normal physiological salivary flow rates. Exclusion criteria were systemic illness, use of antibiotics, pregnancy or breastfeeding, use of remineralizing agents (excluding toothpaste) or any medications which could affect salivary flow rate or quality.

Three removable mid-palatal acrylic appliances extending from the first premolars to the last tooth in the arch and retained by four stainless steel clasps in adults were prepared for each adult subject. In children, appliances were extending from the first primary molars to the first permanent molars and retained posteriorly by two stainless steel clasps. Each appliance held two enamel slabs retained in bilateral troughs by sticky wax to produce 1 mm trough above the enamel surface. Each subject wore an appliance having his/ her own extracted teeth.

Sound, relatively plain buccal and lingual surfaces free of cracks, stains and hypomineralized areas were selected and rinsed with double-deionized water (DDW). Each tooth was cut to produce two enamel slabs yielding a total of 60 permanent enamel specimens and 60 primary enamel specimens. The outer enamel surface was removed and polished wet to a mirror finish using a series of Soflex™ disks (3M ESPE, USA). Each polished surface was then sawn from the tooth to 5 × 5 mm slab, using a water-cooled (DDW) diamond blade saw (Minitom, Struers, Denmark). Two test slabs were inserted into each of the removable palatal appliance. In each side, one sample of enamel is fixed with wax. Following each treatment period, the enamel slabs were removed, rinsed with DDW and stored in a humidified environment.

The study had one control group (no treatment) and two treatment groups: Tooth Mousse™ and ProNamel®. The two products were masked, coded and stored at room temperature. The code of the product was not released to the investigator until after the study was completed.

Each subject went through three treatment phases. The individual was randomly assigned to start with either the control (Meswak ™ toothpaste only) termed group 1, treatment1 (ProNamel®+ Colgate ™ fluoridated toothpaste 500ppm for children and 1500ppm for adults) termed group 2 or treatment 2 (Tooth Mousse™ + Colgate ™ fluoridated toothpaste 500ppm for children and 1500ppm for adults) termed group 3. Each phase lasted for 10 days with a wash-out period of 1 week between phases. Subjects were given a laminated detailed instructions sheet for the application of the paste and home care. A compliance record was collected from the subjects at the end of each period regarding the time of product application and the appliance wear.

Subjects were instructed to wear the appliance continuously throughout this phase of the study including at night and to remove it only during meals, drinking water and oral hygiene practices. During the treatment phases, subjects had to apply a pea size amount of the study paste on their teeth and enamel slabs of the appliances for 3 minutes 2 times daily and keep it undisturbed for 20 minutes. Good oral hygiene and dietary habits were reinforced and monitored during the study. Subjects were instructed to clean the appliances with a soft toothbrush and non-fluoridated toothpaste provided for this purpose. When the appliances were removed, they were rinsed briefly with distilled/ deionized water and then stored in sealed, humidified container in room temperature until they are reinserted. Following the 10-day trial period, appliances were collected and 1 week washout period was followed. Then, subjects crossed over to the other group of the three phases. The enamel slabs were removed from the appliances for processing.

At the completion of each treatment period, enamel slabs were mounted on acrylic circular blocks and surface microhardness of the slabs was measured by the surface microhardness tester (Turret Digital, Shanghai) in Vickers' Units (Vicker diamond, 200g, 5s, HMV-2000). This represented the baseline or the first surface microhardness number ($S\mu H_0$) before the acid challenge. After the 1st surface microhardness measurement, each of the permanent and primary mineralized slabs were individually acid eroded by immersion into 35±1 mL of 0.3% citric acid (pH 3.8) for 25 minutes and thoroughly rinsing with de-ionized water for 2 to 3 minutes.[9] After the acid challenge, surface microhardness was re-measured for each of the enamel slabs (final: $S\mu H_1$).

The percentage of surface microhardness change were calculated as follows $\%\Delta S\mu H= [(S\mu H_1-S\mu H_0/S\mu H_0)]*100$ for each couple of pre-acid and post-acid challenge. Then the mean percentage of surface microhardness change (mean $\%\Delta S\mu H$) was obtained.

All tested variables were verified for normality and constant variance. The mean values and standard deviation of the surface microhardness values were calculated. Vickers hardness numbers (VHNs) at baseline and after acid exposure for treatment groups were compared using one way ANOVA. When ANOVA revealed a difference between groups, Tukey's Post Hoc test was performed to compare the changes in microhardness by acid among the three groups.

RESULTS

The mean (± SD) surface microhardness of the primary and permanent teeth for the three groups prior to and following the acid challenge are shown in Table 1 ($S\mu H_0$, $S\mu H_1$). There were no statistically significant differences between microhardness mean values before the acid challenge (P = 0.179 and 0.210, respectively). The values of initial surface microhardness of permanent teeth were significantly higher than primary teeth (P=0.000). Following the acid challenge, surface microhardness in both dentitions dropped significantly.

Table 1: Surface microhadness (SμH0, SμH1) for different toothpastes treatments prior to and following the acid challenge and mean microhardness change in Vicker's unit

Type of Teeth	Treatment Group	Means of $S\mu H_0$ ± SD $S\mu H_0$ Range	Means of $S\mu H_1$ ± SD $S\mu H_1$ Range	t-test for Equity of Means			
				Mean SμH Difference ± Std. Error Difference	5% Confidence interval of the Difference		P Value* within the group
					Lower	Upper	
Permanent Teeth	Group 1 (N= 20)	366.16 ± 12.28 350.3 – 382.3	74.34 ± 7.58 67.3–89.1	291.82 ± 3.23	285.29	298.35	.000
	Group 2 (N= 20)	372.18 ± 14.75 348.2 – 401.1	124.82 ± 12.78 97.5–147.7	247.37 ± 4.37	238.53	256.20	.000
	Group 3 (N= 20)	370.19 ± 11.88 348.1 – 391.1	149.39 ± 18.65 112.1–177.2	226.25 ± 6.48	213.13	239.37	.000
P Value* between the groups		.210	.000	.000			
Primary Teeth	Group 1 (N= 20)	325.31 ± 11.90 299.9 – 350.3	61.79 ± 11.50 44.4–79.8	263 ± 3.70	256.03	271.02	.000
	Group 2 (N= 20)	327.34 ± 9.90 309.7 – 341.8	77.13 ± 12.04 57.8–103.2	250 ± 3.48	243.15	257.28	.000
	Group 3 (N= 20)	331.63 ± 10.55 312.00 – 350.3	103.00 ± 16.08 84.1–122.9	228 ± 4.30	219.87	237.37	.000
P Value* between the groups		.179	.000	.000			

* Significant if P < 0.05

The mean SμH difference ($S\mu H_0$–$S\mu H_1$) between pre- and post-acid challenge values are shown in (Table 1). The greatest difference was found in group 1 in both permanent and primary dentition, followed by group 2, then group 3. There was a statistically significant difference between the effect of the three treatment groups in the mean SμH (P=0.0000).

The percentage of surface microhardness change (%ΔSμH) was calculated (Table 2). Group 3 exhibited the least change, followed by group 2 then group 1 in both dentitions. The difference in the percentage of surface microhardness change between the groups was significant between the treatments and the control group. The overall change in surface microhardness was higher in the primary teeth.

Percentage surface microhardness change (%ΔSμH) of treatment groups 2 and 3 were statistically significantly higher in the permanent than in primary teeth. The toothpastes prevented the change in percentage of surface microhardness more significantly in permanent than primary teeth in groups 2 and 3 (Table 3). However, in group 1 there was no statistically significant difference in the percentage surface microhardness change between primary and permanent teeth.

DISCUSSION

This study is a randomized, controlled, double-blind with cross-over design. The *in situ/ ex vivo* protocol was designed to overcome the limitations of a total *ex vivo* protocol such as: inadequate simulation of biological aspects, difficulty in matching solid-solution ratios occurring *in vivo* and artifacts associated with substrate choice/reaction conditions.[10]

Although *in situ* testing protocols depend primarily on participants' compliance, the *in situ* erosion model is particularly suited for assessing the potential of various agents to provide protection against dental erosion. It enables monitoring of the entire process of erosion in a completely natural environment of saliva flow, pellicle development and routine oral care.[11, 12]

The intraoral appliance was fabricated similar to previous studies.[11-13] Each intraoral appliance held two enamel slabs on each side to standardize the effect of tooth position, tooth brushing, and salivary flow effect on dental erosion. The enamel slabs were inserted with 1 mm trough above the appliance surface to allow plaque to be established to mimic the intraoral environment. Although plaque is not directly related to dental erosion, the mechanism of action of many remineralizing agents may involve retention in plaque to be released during later acidic attack.[2,14]

Using surface microhardness techniques as a quantitative method of erosion assessment enabled the investigators to detect early stages of enamel and dentin loss with great reliability, simplicity and low cost.[6]

Table 2: Mean percentage of surface microhardness change %ΔSμH for permanent and primary teeth †

Type of Dentition			Group1	Group2	Group3	Group1-2	Group 1-3	Group 2-3
Permanent Teeth	Mean %ΔSμH		79.72	66.52	60.13	13.19	19.59	6.39
	Std. Deviation		1.59	2.45	4.98	1.05	1.05	1.05
	95% Confidence Interval	Min.	74.99	63.18	52.90	10.55	16.94	3.74
		Max.	81.46	72.59	75.44	15.85	22.24	9.04
	P Value *					.000	.000	.000
Primary Teeth	Mean %ΔSμH		81.09	76.50	69.02	4.59	12.07	7.48
	Std. Deviation		2.90	3.13	4.23	1.09	1.09	1.09
	95% Confidence Interval	Min.	75.88	69.54	62.27	1.84	9.32	4.73
		Max.	85.64	81.50	77.20	7.35	4.73	10.24
	P Value *					.000	.000	.000

† Tukey's Post hoc test

* Significant if P < 0.05

Table 3: Comparison of effects of different treatment groups between permanent and primary teeth †

Treatment Group	Type of Teeth	Mean %ΔSμH	Std. Deviation	P Value*
Group 1	Permanent Teeth	79.72	1.59	0.072
	Primary Teeth	81.09	2.90	
Group 2	Permanent Teeth	66.52	2.45	.000
	Primary Teeth	76.50	3.13	
Group 3	Permanent Teeth	60.13	4.98	.000
	Primary Teeth	69.02	4.23	
Mean	Permanent Teeth	68.79	8.85	.000
	Primary Teeth	75.54	6.06	

† Student t-test

* Significant if P < 0.05

Since enamel surface shows an intrinsic coarseness rendering detection of small changes due to erosion/abrasion difficult, most of the methods used to assess dental erosion need polished surfaces for precise assessment of the erosive defects and for creating reference surfaces. This indicates that the natural, often fluoridated surface of the tooth has to be removed to get a mirror-like finish. As enamel surface can differ according to patient age, tooth number, water fluoridation and mineral composition, using specimens with polished surfaces, would produce a similar surface for all enamel slabs making their depth and mineral content uniform within the study.[12]

The rate of the progression of dental erosion in primary teeth is still debatable. Some studies reported faster progression in primary than in permanent teeth; [15,16] others found no differences between the two types of dentition.[17,18] This may be due to the variations in the use with different developmental maturity stages and differences in acid and fluoride time exposures among the different dentition groups.[14,16] In accordance with previous studies [14,19], primary teeth had significantly lower initial surface microhardness measurements and higher percentage of change in surface microhardness due to acid. The unique composition of the primary teeth with more carbon dioxide and carbonate and less phosphorous and calcium phosphate in their composition than the permanent teeth may explain the findings.[14,19] In addition, deciduous enamel is thinner and smaller making the erosive process faster, reaching deeper structures earlier and leading to advanced lesions following shorter exposure to acids. [19]

ProNamel® was shown to be effective in reducing the microhardness change due to acids in both the permanent and primary teeth though its effect on permanent teeth was more pronounced. This is the first study in the literature to test the effect of ProNamel on primary teeth but in terms of permanent teeth. The results of this study are in agreement with many previous *in vitro* studies.[6,20-23] Recently, Hooper et al. (2014) in a randomized, blind, two-treatment, non-brushing, four-period crossover *in situ* study utilizing human permanent teeth demonstrated that ProNamel® did not show superior preventive potential over stannous-containing sodium fluoride dentifrices. [11, 24] There was 38% lower enamel loss in favor of stannous-containing sodium fluoride dentifrice compared to ProNamel®. This was in agreement with a study measuring enamel surface loss using transverse microradiography

that supported the potential for the stabilized, stannous-containing sodium fluoride dentifrice to provide erosion protection benefits that are significantly better than other dentifrice formulations including ProNamel®.[20] Specifically, Sn-containing NaF dentifrice showed only 6.5 μm of surface loss following the acidic attack compared to 20.5 μm in samples treated with ProNamel®. However, Kato et al. did not prove the effects for ProNamel® in the prevention of dental erosion.[25] This may be attributed to the difference in the methodology utilized; including the use of bovine enamel, and the application time of ProNamel® on the enamel surface.

Tooth Mousse™ has good preventive abilities in both permanent and primary teeth to prevent dental erosion in permanent teeth. [26-28] The use of permanent teeth in the previous studies may make the comparison to primary teeth in this study not applicable. Tooth Mousse™ and ProNamel® were found to be effective in the prevention of dental permanent and primary enamel erosion. [5] Our study results showed that Tooth Mousse™ performed better than ProNamel® upon application in both sets of teeth. However, when using surfometry, the mean amount of enamel removed in the ProNamel® group was 2.60 micron compared to 3.28 micron in the Tooth Mousse™, indicating that ProNamel® offers more protection than Tooth Mousse™.[5]

Based on the mechanism of action of both products, Tooth Mousse™ is expected to offer more preventive capability as the bond between CPP-ACP is acid dependent and declines as the pH drops buffering acidic and phosphate ions under acidic conditions, maintaining a state of supersaturation that inhibits enamel demineralization and enhances remineralization.[2] However, in the case of ProNamel®, even the more acid resistant fluoroapatite crystals are undersaturated when immersed in acidic solutions with low pH.[14] Moreover, the CaF_2 protective layer dissolves readily in acidic drinks offering little protection against dental erosion.[14]

CONCLUSIONS

- Tooth Mousse™ and ProNamel® *in situ* display increased enamel surface resistance to erosive acid attack in both primary and permanent teeth and are recommended to be used by both children and adults to prevent dental erosion.
- Tooth Mousse™ provided a better degree of protection and was more effective against demineralizing acidic attacks in both permanent and primary teeth than ProNamel®.
- The degree of protection offered by Tooth Mousse™ and ProNamel® against dental erosion is stronger and more pronounced in permanent teeth than in primary teeth.

Primary teeth are more prone to dental erosion and are more affected by acidic attacks than permanent teeth.

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