

THE EFFECT OF REPEATED APPLICATIONS OF ENAMEL SURFACE TREATMENT ON IN-VITRO BOVINE ENAMEL HARDNESS AFTER MULTIPLE EXPOSURES TO COLA DRINK

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ABSTRACT

The aim of this study was to test the impact of repeated applications of various enamel surface treatments on the remineralization process after multiple exposures to cola drink, and to compare their ability to resist demineralization. Enamel demineralization and remineralization were monitored using surface microhardness. Bovine incisor teeth were exposed to cola drink for 2 minutes, 3 times per day for 3 days in the presence of artificial saliva. Then, the following surface treatment materials were tested: 1) Tooth Mousse, 2) MI Paste Plus™, 3) Fluoraphat, and 4) Control (no treatment). The procedure was repeated every 3 days for 12 days. Results showed that cola soft drink significantly ($p < 0.05$) decreased enamel microhardness. All tested enamel surface materials significantly ($p < 0.05$) increased the hardness of eroded enamel when repeatedly applied. MI Paste Plus™ showed better remineralization and higher resistance to erosion when compared to other groups. It was concluded that repeated applications of enamel surface treatment were effective in increasing enamel microhardness after erosion process, caused by multiple exposure to soft drink in bovine incisor teeth.

Key words: Tooth Mousse, MI Paste Plus, Fluoraphat, Microhardness, Cola drink.

INTRODUCTION

Dental erosion is defined as localized loss of the tooth surface by a chemical process of acidic solution of nonbacterial origin.¹ Erosion prevalence rates in 34 % of 5-6-year-old boys and 26% in 12-14-year-old boys have been reported in Saudi Arabia.² Similar results were reported in 5-year-old Irish children.³ Although multiple factors seem to contribute to this process, excessive consumption of acidic drinks is one of the most common extrinsic factor that causes dental erosion.⁴ About 93% of Saudi adolescents consume carbonated soft drinks and 28% of them are on high frequent consumption.⁵ As it is difficult to control possible etiological factors, many strategies have been developed for the prevention of erosion. Different enamel surface treatment measures have been used for prevention of erosion and enhancement of remineralization, such as fluoride varnishes, casein phosphopeptide amorphous calcium phosphate pastes (CPP-ACP) and tooth pastes.⁶⁻⁸ The erosive potential of soft drinks on teeth has been well documented.^{9,10} In addition, the ability of fluoride and CPP-ACP to remineralize eroded teeth have also been investigated.^{6,7} In many studies, researchers treated enamel specimens once before or

after single immersion in soft drink or other erosive solutions. However, Maupome et al¹¹ evaluated the effect of frequent cola consumption on enamel over an interval of 8 days using surface microhardness testing. They found that microhardness decreased regardless of the frequency regime used. In a more recent study, Tantbirojn et al¹² evaluated the change of enamel microhardness after immersion of enamel specimens in cola drink for 8 minutes and then evaluated the changes after the contact with CPP-ACP paste for 3 minutes at 0, 8, 24 and 36 hours. They found significant effect of repeated applications of CPP-ACP paste on the changes in surface microhardness of the softened enamel. For those on continuous soft drink consumption and not willing to stop, enamel surface treatment should be tested for continuous remineralization against frequent and constant exposure to soft drink for longer period. Accordingly, more realistic soft drink consumption pattern replicated under experimental condition would be beneficial in determining the actual impact of interrupted enamel surface treatment during the consumption period. Thus, the aim of this study was to evaluate whether repeated applications of 3 different enamel surface treatments can rehardened softened

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bovine enamel, and to compare between these treatments in resisting the demineralization process caused by frequent exposure to cola drink every 3 days over a period of 12 days.

METHODOLOGY

Sample Preparation

Forty freshly extracted bovine incisors were cleaned and stored in deionized water for up to 2 weeks. The crowns were separated from the roots and were embedded in orthodontic resin cylinders (Harry Bosworth Co, IL, USA). The labial surfaces were ground wet using 200, 400, and 600 grit silicon carbide paper (Automata A, JearWirtz, West Germany) and polished with 1.0 and 0.05 μm alumina suspension (BUEHLER, IL, USA) to expose a flat enamel. The sample was randomly divided into four groups of ten teeth each. Baseline enamel microhardness (BL) of the sound enamel was measured with a microhardness tester (Micromet[®] 2100 Series, BUEHLER, IL, USA) using a Vickers indenter at 300 g load for 15 seconds. Four indentations in different regions of each crown were made. The microhardness value of each sample was calculated by averaging the value of all four indentations.

Demineralization Process

The pH of cola soft drink (Coca-Cola Bottling Co. of Saudi Arabia, Riyadh, SA) and artificial saliva were measured with pH meter (Ultra BASIC, Denver Instrument, Goettingen, Germany). Each sample was immersed in 33 ml of fresh cola for 2 minutes at room temperature and then in 33 ml of artificial saliva. This procedure was repeated 3 times per day at 8 hours intervals for 3 days. After the first erosion process was completed, the samples were washed with deionized water and blotted dry. Enamel microhardness (De1) of each sample was then measured. Four indentations in different regions of each crown were made. The hardness value of each sample was calculated by averaging the value of all four indentations.

Remineralization Process

In TM group, a thin layer of CPP-ACP paste (Tooth Mousse, GC Corporation, Tokyo, Japan) was applied using a microbrush on the enamel surfaces of the samples, and left undisturbed for three minutes and then stored in artificial saliva for six hours. In MI group, a thin layer of CPP-ACP paste (MI Paste Plus[™], GC Corporation, Tokyo, Japan) was applied using a microbrush on the enamel surfaces of the samples, and left undisturbed for three minutes and then stored in artificial saliva for six hours. In FP group, a thin layer of fluoride varnish (Fluoraphat, PROMEDICA, Ger-

many) was applied using a microbrush on the enamel surfaces of the samples, and left undisturbed for three minutes and then stored in artificial saliva for six hours. In control group, no surface treatment was used, the teeth kept in artificial saliva for six hours. All samples were stored at 25°C. After the remineralization process was completed, the samples were washed with deionized water and left in artificial saliva for 18 hours. Enamel microhardness (Re1) of each sample was then measured using the same measurement protocol.

The samples were subjected again to the demineralization and remineralization processes which were repeated every 3 days using the same protocol, giving a total of 27 exposures to cola soft drink and 3 times of enamel surface treatment application. Enamel microhardness was measured after each demineralization and remineralization processes (De2, Re2, De3, Re3). The compositions of all materials are presented in Table (1).

Data Analysis

The data were subjected to one-way ANOVA and Tukey HSD test for comparison among different groups and at different time intervals. The statistical analysis was carried out using SPSS version #16. The significance level was set at 0.05.

RESULTS

The mean pH value for cola and artificial saliva was 2.6 and 6.9 respectively. Table 2 shows that at baseline, enamel microhardness values of sound enamel ranged between 346.2-357.4 Vickers Hardness Number (VHN) and no significant difference was found among the experimental groups ($p=0.342$).

Figure 1 shows that after the first immersion in cola drink for 2 minutes, 3 times per day for 3 days, the microhardness values sharply decreased by a mean of 120 VHN; a 33% reduction from baseline which was highly significant ($p < 0.01$). No statistical ($p=0.926$) difference in enamel microhardness was found among the groups after cola immersion.

A significant ($p < 0.05$) increase in enamel microhardness was noticed in all treatment groups after the first enamel surface treatment, with significant differences when compared to the control group. After first surface application, MI paste rehardened the enamel and the microhardness value increased by 35.5%, compared to 20.8% and 24.2% in FP and TM groups respectively.

After the second exposure to cola drink, enamel microhardness slightly decreased in all the groups without significant differences between treatment

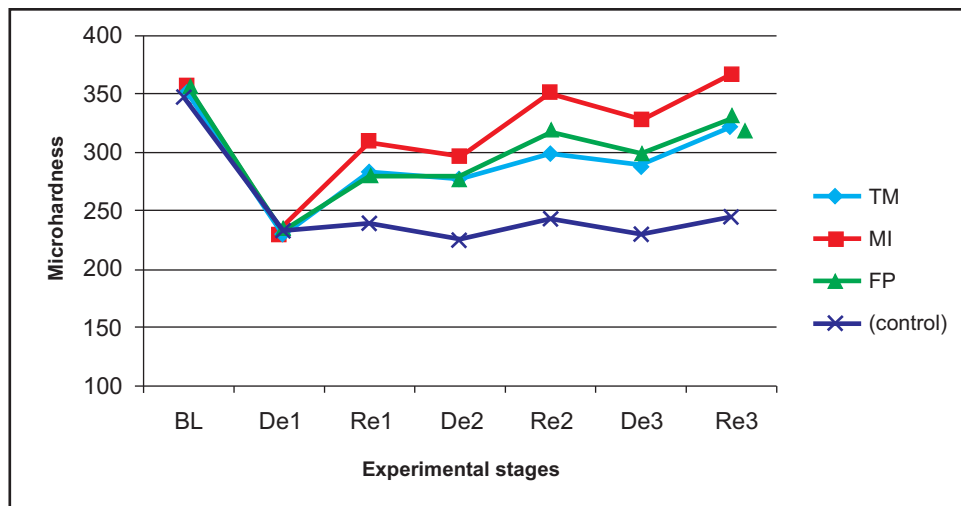


Fig 1: Changes in enamel microhardness in bovine teeth at different time intervals during the experiment

groups. The decrease in enamel microhardness of treatment groups was significantly ($p < 0.05$) less than the decrease occurred after the first exposure to cola drink. After the second surface application, significant ($p < 0.05$) increase in enamel microhardness of MI group

compared to TM and FP groups, however, the FP group showed better behavior than TM group. Compared with microhardness values after the second demineralization, enamel microhardness increased by 19.3%, 14.2% and 8.2% in MI, FP and TM groups respectively, after the second enamel surface treatment

TABLE 1: THE COMPOSITIONS OF ALL MATERIALS USED IN THE STUDY

Material	Composition
Cola	Carbonated water, Sugar, Caramel color, Caffeine, Phosphoric acid, cola flavouring
Artificial Saliva	Deionized water, KH_2PO_4 , $2H_2O$, $KHCO_3$, NaCl, $MgCl_2 \cdot 6H_2O$, Citric acid, $CaCl_2 \cdot 2H_2O$
CPP-ACP Paste (TM)	Pure water, Glycerol, CPP-ACP, D-sorbitol, Silicon Dioxide, CMC-Na, Propylene glycol, Titanium dioxide, Xylitol, Phosphoric acid, Flavoring, Guar gum, Zinc Oxide, Sodium Saccharin, Ethyl p-hydrobenzoate, magnesium oxide, Butyl p-hydroxybenzoate, Propyl p-hydroxybenzoate
CPP-ACP Paste (MI)	Pure water, Glycerol, CPP-ACP, D-sorbitol, Silicon Dioxide, CMC-Na, Propylene glycol, Sodium fluoride Titanium dioxide, Xylitol, Phosphoric acid, Flavoring, Sodium Saccharin, Ethyl p-hydrobenzoate, Butyl p-hydroxybenzoate, Propyl p-hydroxybenzoate
Fluoride Varnish (FP)	5% sodium fluoride, fruity flavor, alcoholic solution of natural resins

At the end of the experiment, no significant ($p > 0.05$) difference in enamel microhardness was noticed after the third surface treatment when compared to enamel microhardness at baseline. However, compared to the first demineralization process, significant ($p < 0.05$) increase in enamel microhardness occurred in all treatment groups after the third enamel surface treatment. MI group showed the best remineralization and the most resistance to demineralization with significant ($p < 0.05$) difference when compared to all other groups. The enamel microhardness increased by 12%, 11% and 11.3% in MI, FP and TM groups respectively compared with microhardness values after the third demineralization.

Along the experiment, artificial saliva group showed slight increase in enamel microhardness after each demineralization process, however, it showed significantly ($p < 0.05$) less resistance to demineralization process when compared to all treatment groups. All the groups failed to restore the initial microhardness values except the MI group.

DISCUSSION

The methodology of the present study was designed to mimic soft drink consumption of frequent cola consumers in the presence of saliva. Bovine incisor teeth were used, as the study design required a sufficiently large and flat area to allow multiple microhardness measurements. Mineral distribution in

TABLE 2: MEAN ENAMEL MICROHARDNESS VALUES OF BOVINE TEETH TREATED WITH DIFFERENT MATERIALS AT EACH STAGE OF THE EXPERIMENT

Surface Treatment	CPP-ACP Paste (MI Paste) MI	Fluoride Varnish (Fluoraphat) FP	CPP-ACP Paste (Tooth Mousse) TM	Artificial Saliva (No Treatment) Control
Enamel Microhardness	Mean (SD)VHN	Mean (SD)VHN	Mean (SD)VHN	Mean (SD)VHN
Baseline Hardness(BL)	357.4(63.2) ^{a,*}	355.5(59.5) ^a	346.2(63.6) ^a	348.2(61.3) ^a
After 1 st demineralization(De1)	228.4(43.1) ^a	235.5(32.2) ^a	227.2(58.4) ^a	232.3(29.7) ^a
After 1 st remineralization(Re1)	309.5(25.6) ^a	281.0(67.2) ^a	282.3(35.0) ^a	239.4(53.6) ^b
After 2 nd demineralization(De2)	294.7(28.2) ^a	278.2(28.9) ^a	276.6(43.2) ^a	225.8(19.8) ^b
After 2 nd remineralization(Re2)	351.5(64.1) ^a	317.7(57.2) ^b	299.4(59.0) ^b	242.3(16.3) ^c
After 3 rd demineralization(De3)	327.0(59.4) ^a	298.7(63.8) ^a	288.3(56.2) ^b	229.4(32.1) ^c
After 3 rd remineralization(Re3)	366.4(46.9) ^a	331.6(61.4) ^b	321.1(43.9) ^b	244.3(32.5) ^c

SD = Standard Deviation; VHN = Vickers Hardness Number

*Same superscript letters indicate no significant difference among treatment groups, at the same time period, $p > 0.05$.

the lesions of bovine teeth is reported similar to that found in human teeth, and structural changes in human and bovine teeth are also similar.¹³ Mineral loss or gain in enamel can be measured as hardness change after demineralization or remineralization processes.¹⁴ Erosive challenge prior to enamel surface treatment was followed in the present study, as it seems the real practice, where it would be difficult to motivate the patient or the parents of children prone to the erosion to implement surface treatment regime before acidic attack, such as soft drink consumption or vomiting. Pastes and varnish were completely removed after 6 hours to simulate the clinical situation in which they might be removed by tooth brushing or mastication after some hours. This was done to focus on the chemical effects of surface treatment rather than mechanical protection.

In this study, baseline microhardness values for enamel ranged from 346.2 to 357.4 VHN which were similar to previous studies.^{11,12} The sharp drop in enamel microhardness occurred after the first demineralization process. The first exposure of the teeth was to cola drink for 2 minutes, which might explain the sharp reduction in enamel microhardness from the baseline.

Artificial saliva introduced during the erosion processes might buffer the acidity from cola drink and limit the softening of the enamel surfaces. This was clear in control group during the remineralization processes after the second and third demineralization processes, where the enamel microhardness slightly increased when the teeth were kept in artificial saliva for 24 hours. Devlin et al⁹ evaluated the hardness of

enamel exposed to cola and artificial saliva and found that cola reduced the mean indentation enamel hardness but the hardness was partially restored with artificial saliva.

In the present study, all tested surface treatment materials restored the decrease in enamel microhardness in varying degrees after cola exposure when repeatedly applied. It is difficult to compare between the results of this study and others' due to the difference in methodology and study design. However, Tooth Mousse has been shown to reduce the demineralization process of tooth structure and enhance remineralization by other studies.^{7,12,15} Tooth Mousse paste consists of casein phosphor-peptide amorphous calcium phosphate complex (CPP-ACP). Casein, an amino acid, can adjust to acid base environment. In acidic pH, ACP will separate from CPP, thereby increasing salivary calcium and phosphate level.¹⁶ For clinical application, clinicians should consider potential side effects from ingestion of casein derivatives in people with immunoglobulin E allergies to milk products.¹⁷

Fluoride varnish treatment, Fluoraphat, was able to remineralize softened enamel after repeated applications. Fluoraphat was comparable to Tooth Mousse paste in restoring demineralization. One gram of Fluoraphat contains 50 mg sodium fluoride corresponding to 22.6 mg fluoride ions. Sorvari et al⁶ showed that treatment of enamel with sodium fluoride solution or Duraphat fluoride varnish prior to its immersion in cola inhibits erosion. In addition, Seppa¹⁸ in an in vitro study reported that fluoride varnishes were more effective in remineralization of enamel than a repeat-

edly used less concentrated NaF solution. In the present study, fluoride varnish showed the ability to harden softened enamel but less than MI paste even though it was hardly removed from the enamel surface. This might be due to the fact that the protective layer would be removed over time after multiple erosive challenges. In clinical situation, this varnish might be less effective in preventing dental erosion for long time especially in patients with high soft drink consumption. Although fluoride varnish has the ability to harden soften enamel and resist erosion, one should consider the risk of fluoride toxicity if the patient ingests a significant amount of fluoride especially if it is repeatedly applied.¹⁹

Treatment of softened enamel with MI Paste produced the highest remineralization when compared to Fluoraphat and Tooth Mousse paste. This probably due to the combined action of fluoride and calcium-phosphate. This is consistent with Elsayed et al²⁰ study which found that combining fluoride and ACP with CPP-ACP can give a synergistic effect on enamel remineralization. MI Paste Plus is a product that contains 900 ppm fluoride. It is designed to increase enamel remineralization through the deposition of fluoride-containing calcium-phosphate precipitates. However, at 900 ppm fluoride, this product is not considered ingestible and therefore, children younger than 6 years should not use it.¹⁷ Within the limitations of this study and based on its results, it is recommended that people on frequent consumption of acidic drinks should be put on a recall program to receive enamel surface treatment applications depending on their age and frequency of acidic drinks consumed.

CONCLUSIONS

- The hardness of bovine enamel decreased after multiple exposures to cola drink. The softened enamel became hardened after 3 applications of different surface treatment along with artificial saliva for 12 days.
- Artificial saliva alone did not enhance the hardness of demineralized enamel.
- MI Plus paste application showed the highest resistance against demineralization process.

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