The Effect of Casein Phosphopeptide-Amorf Calcium Phosphate and Acidulated Phosphate Fluoride Gel on Dental Erosion in Primary Teeth: An *in Vitro* Study

Eda Arat Maden*/ Özge Acar**/Ceyhan Altun***/ Günseli Güven Polat****

Objective: *This study aimed to investigate the effect of acidulated phosphate fluoride (APF) gel and casein phosphopeptide/amorphous calciumphosphate (CPP-ACP) on the dental erosion produced by carbonated soft drink in primary teeth* **.** ***Study Design:*** *This study evaluated by an in vitro model the effect of APF gel and CPP-ACP on the dental enamel previously subjected to erosive challenge with carbonated soft drink. Sixty sound human primary molars were prepared by embedding the crown sections in acrylic resin blocks leaving the enamel surfaces exposed. The surface roughness of the enamel was measured with prophilometry at baseline. Specimens were randomly divided into three treatment groups (n:20): artificial saliva, CPP-ACP, 1.23% APF gel. All specimens were then exposed to an erosive challenge of carbonated soft drink and artificial saliva for 20 cycles of 20 seconds each. Demineralization-remineralization cycles was repeated twice at eight-hour intervals and roughness values were measured. Enamel samples were treated with artificial saliva, CPP-ACP, 1.23% APF gel applied for 10 min after erosive challenge. The arithmetic average roughness (Ra) readings were recorded after remineralization agents were applied.* ***Results:*** *The mean surface roughness in all groups increased significantly after erosion process and decreased after remineralization treatment. After treatment, the mean surface roughness of the 1.23% APF gel group was significantly less than the other groups and the mean surface roughness of the artificial saliva group was significantly more than the other groups. 1.23% APF gel showed the highest protective effect against erosive enamel loss.* ***Conclusions:*** *Under the conditions of this study, artificial saliva, CPP-ACP and 1.23% APF treatments were able to reduce erosive enamel loss produced by carbonated soft drink in primary teeth* **.** *However, 1.23% APF gel showed the highest protective effect against erosive enamel loss.*

Key words: *CPP-ACP paste, dental erosion, fluoride gel, primary, roughness.*

INTRODUCTİON

Dental erosion is irreversible hard tissue loss resulting from any kind of chemical effect in the absence of bacterial, mechanical and traumatic causes. This process may be caused by extrinsic or intrinsic agents. Extrinsic agents include acidic foodstuffs, beverages, snacks and may also occur following environmental exposure to acidic agents. The prevalence of erosion is thought to be increasing, reflecting frequent consumption of acidic food and beverages.[1] The intrinsic origin for acid is gastric juice, with its high concentration of hydrochloric acid (HCl).[2] In developed countries the extrinsic factor is becoming more important due to the increased consumption of acid drinks such as soft drinks, sport drinks, fruit juices and fruit teas. Children and adolescents are the most involved age groups in the consumption of these acid drinks.[3] The frequent intake of acid beverages poses risk of erosion damage in primary and permanent teeth. Erosive effects can be seen due to acid juices, soft drinks and carbonated beverages show erosive effects.[4] Dental professionals have a responsibility to identify individuals at risk of erosion and to offer appropriate preventive

From the Department of, Pediatric Dentistry, Center of Dental Sciences, Gulhane Medical Academy, Ankara, Turkey.
*Eda Arat Maden, DDS, PhD.
**Özge Acar, DDS, PhD.
***Ceyhan Altun, Associate Professor.
****Günseli Güven Polat, Professor.

Send all correspondence to:
Ceyhan Altun,
Deparment of Pediatric Dentistry
Center of Dental Science
Gülhane Medical Academy
Etlik/Ankara
Turkey
Phone: +90(312)3046041
E-mail: ceyhanaltun@yahoo.com

measures. Professional recommendations for prevention of erosion include use of fluoride products, modification of toothbrushing habits and application of protective resin coating to teeth.[5]

The best approach to prevent erosive tooth wear is primary prevention: the treatment and elimination of causative factors. These preventive strategies provide some degree of protection against dental erosion, but improved strategies are needed to further diminish the risk of abrasive⁄erosive insult on teeth. It has been suggested that clinical control of tooth wear should be based on early diagnosis and prevention, rather than complex treatment modalities.[6]

For the prevention of caries, it is evident that fluoride promotes the remineralization and inhibits the demineralization of dental hard tissues[13], while the effectiveness of reducing erosive tooth wear by fluoride is discussed controversially in the literature.[7] Recent evidence indicates that topical fluoride can protect enamel and dentine from severe erosion at pH 2.35 and pH 3.0.[8] Several studies have reported the effectiveness of topical fluoride as a cariostatic agent in enhancing enamel remineralization.[8] A similar anti-erosive capability of different topical fluorides was tested.[9]

Hove *et al* [9] observed that intensive application of fluoride provided significant protection against enamel erosion under conditions simulating gastric reflux at pH 2.0, but Willumsen et al.[10] noted that fluoride did not protect enamel against erosion at pH 1.2.

Recent *in vitro* studies have indicated that Tooth Mousse, containing a phosphopeptide that stabilizes amorphous calcium phosphate (CPP-ACP) may reduce dental erosion caused by citric acid and an acidic sports drink.[11] Tooth mousse is a water based, sugar free cream containing casein phosphopetide and amorphous calcium phosphate. The casein based carrier vehicle delivers calcium and phosphate ions to the tooth surface in the optimum ratio necessary to encourage remineralisation. Some preliminary studies have shown that tooth mousse encourages remineralisation of enamel following erosion by cola based drinks, protects dentine against acid erosion induced wear.[11]

A controversial issue in dental erosion literature involves differences in erosion progression rates between primary and permanent teeth. Some authors stated that primary enamel is more susceptible to erosion than permanent enamel[12] while others found no differences between these two types of substrates.[13] Several studies have reported the effectiveness of topical fluoride as an anti-erosive cariostatic agent in enhancing enamel remineralization.[9] However, the protective effect of different remineralizing agents especially CPP-ACP on primary enamel did not receive much attention.

Softening of the enamel surface is an early manifestation of the erosion process. Increased surface roughness which accompanies erosion of the enamel surface by acidic beverages can be assessed using a physical measurement such as the roughness test.[9]

In view of the above considerations, the aim of the present study was to evaluate the degree to which a CPP-ACP-containing crème (Tooth Mousse) and 1.23% APF gel can be used to prevent erosive tooth wear on artificially induced dental erosion in human primary enamel. It was hypothesized that a CPP-ACP- containing crème and 1.23% APF can reduce tooth wear due to erosion caused by carbonated soft drink.

MATERIALS AND METHOD

In the study, sixty human primary mandibular incisors (n = 60) free of caries and defects extracted from children 6-7 years old were used. **The protocol for the study was reviewed and approved by the ethical committee of Gulhane Medical Academy in Ankara. The nature and aim of the study were explained verbally and in writing to the subjects who signed an approved informed consent form.**

The enamel sections that were free of any caries or any enamel defects were mounted in acrylic resin with the outer buccal enamel surface exposed. After setting of the resin, the labial surfaces were grounded using silicon carbide papers (grades 600–1200) under water irrigation to remove 50–100 mm and produce flat surfaces. Specimens were randomly assigned to three groups each of 20 according to the protective agent used: group 1; (1.23% APF gel (dentsply professional 1301 Smile Way, York PA 17404)), group 2; (Tooth Mousse (GC Corp.,Tokyo, Japan)), and group 3; (artificial saliva) (Table 1).

Table 1. Treatment groups

Group 1	1.23% APF gel (dentsply professional 1301 Smile Way, York PA 17404
Group 2	Tooth Mousse (GC Corp.,Tokyo, Japan)
Group 3	Artificial saliva

Experimental Procedure

The surface roughness of each sample was determined in Ra (arithmetic average roughness–µm) with a portable profilometer device (Mahr Perthometer M2/M3, Gottingen, Germany) was used to measure the surface roughness. The initial surface roughness values of the samples were measured and evaluated in the profilometer device. 3 measurements for each sample was performed, and their averages were taken. Demineralization–remineralization cycles of 10 seconds of immersion in carbonated soft drink (pH 2.3) and 10 seconds in artificial saliva were conducted for 20 cycles. This procedure was repeated twice at eight-hour intervals. The carbonated soft drink was changed at every cycle. All specimens were stored in artificial saliva between cycles. After the erosion process, roughness values were measured. 1.23% APF gel was applied to the enamel surfaces in group 1 for ten minutes. The enamel surfaces of the specimens in group 2 were remineralized by treatment with the Tooth Mousse for ten minutes. Specimens in group 3 were stored in artificial saliva for ten minutes. Specimens were washed with deionized water after treatment with the remineralizing agents. Finally, the roughness of each sample was determined on the enamel surface.

Statistical analysis

SPSS v.15.0 (Chicago, IL, USA) software package was used for statistical analysis and calculations. Differences in the averaged values among the groups after remineralization treatment were analyzed by Kruskal-Wallis test. The Bonferroni correction Mann Whitney U test was used to find differences in groups. $P < 0.05$ was adopted for statistical significance.

RESULTS

Statistically significant increase was found for surface roughness values measured after erosion process in all groups. Statistically significant decrease was observed in surface roughness of all groups after remineralization treatment. However, 1.23% APF gel performed better when compared to the other groups. The mean surface roughness of the artificial saliva group was significantly more than the other groups. Tooth Mousse showed more protective effect against erosive enamel loss compared to artificial saliva (Fig.1, Table 2).

Statistical analysis showed that all of the three treatments were able to diminish the amount of enamel roughness and provide a significant protective effect against erosive enamel loss . However, 1.23% APF gel showed the highest protective effect against erosive enamel loss.

DISCUSSION

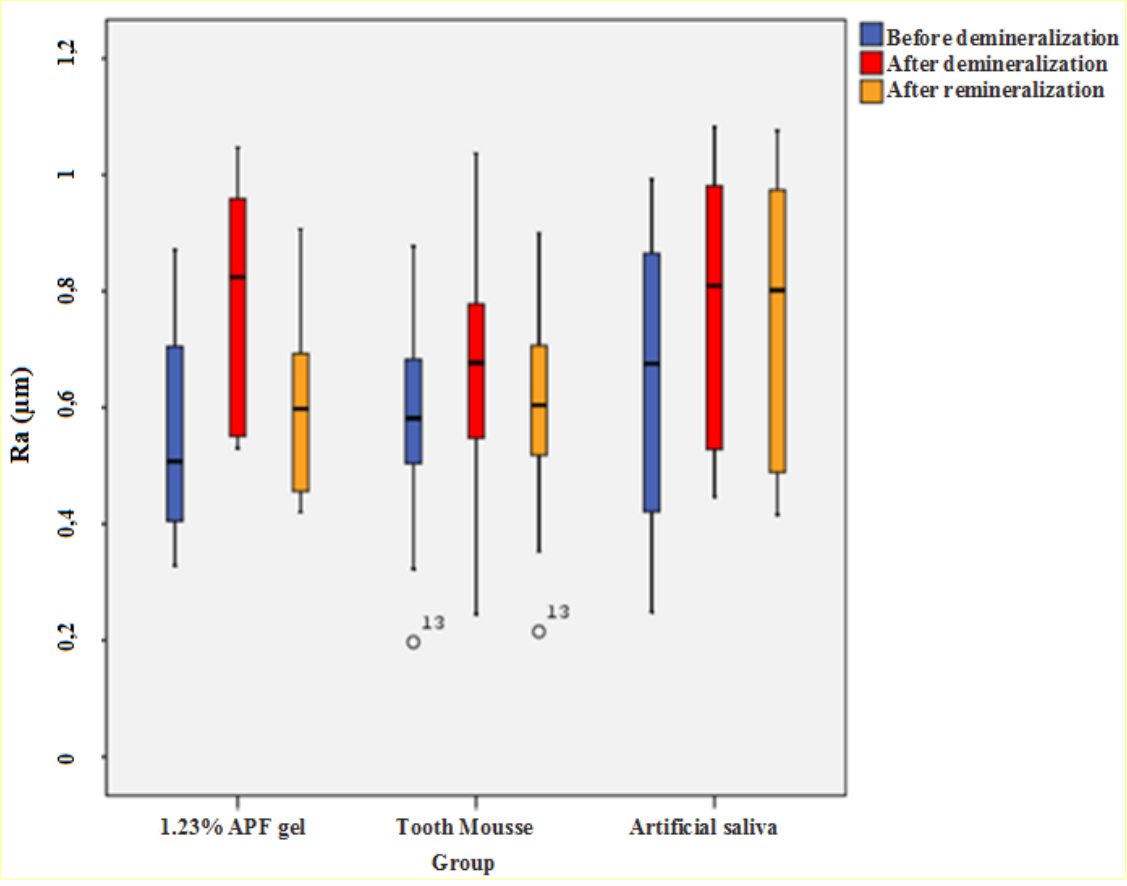
This *in vitro* study was designed to compare anti-erosive effect of Tooth Mousse with one of the most commonly used fluoride product in dental practice nowadays: the 1.23% APF gel on primary teeth. So far, to our knowledge, no study has studied the protective anti-erosive effect of these materials on primary teeth. In this study, as it is one of the most commonly consumed carbonated soft drink was used to induce artificial erosive effect as in other studies.[14]

Epidemiological studies have reported that dental erosion is common in adolescents, 37% in the UK and 41% in the US, and that its incidence is increasing with time.[15] Among children aged 11 years or older, a quarter or more were found to have some erosion of the palatal surfaces of the upper permanent incisors. There seems to be an increase in erosion in children particularly from the higher socioeconomic groups.[16]

Table 2. Enamel surface roughness values.

	Ra values before demineralization			Ra values after demineralization			Ra values after remineralization		
	Mean ±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max
APF gel group	0,54±0,18	0,33	0,87	1,05±0,20	0,53	1,05	0,61±0,17	0,42	0,91
Tooth Mousse group	0,56±0,19	0,20	0,88	0,66±0,21	0,25	1,04	0,58±0,19	0,22	0,90
Artificial saliva group	0,65±0,23	0,25	0,99	0,77±0,22	0,45	1,08	0,75±0,23	0,42	1,08

Figure 1. Distribution of enamel surface rougness values.



In the present in vitro study, the profilometer (Mahr Perthometer M2/M3, Gottingen, Germany) was used to measure changes in enamel surface roughness. It was used as a verification to the protective effect of a CPP-ACP paste, 1.23% APF gel and artificial saliva on enamel exposed to erosive action of a carbonated soft drink. This method has been used in numerous other studies concerning dental hard tissue wear due to erosion, abrasion or erosion/abrasion.[17] The most frequently reported surface roughness paramete is Ra in the dental literature.[17] Ra value has been conventionally evaluated on enamel as an indicate of acid-erosion using the contact stylus surface profilometry (SSP).[18]

Previous work has shown the potential of CPP-ACP as an anticariogenic agent both in- vitro and in-situ, with CPP-ACP preventing demineralization and promoting remineralization of sub-surface carious lesions in enamel and dentine.[19,20] A recent study suggests that microhardness of enamel can be increased and erosion by cola drinks can be decreased by CPP-ACP, implying that CPP-ACP can remineralize eroded lesions.[14] How the process of remineralization of eroded lesions is unclear; however, it most probably involves a repair process through deposion of mineral into porous zone rather than involving crystal regrowth.[20] It is hypothesized that Tooth Mousse remineralizes (repairs) eroded enamel and dentine crystals in addition to the prevention of erosive demineralization. This hypothesis is supported by an observation that superficial granular structures, probably representing remineralized enamel crystals, formed on the enamel surface after treatment with a sports drink containing CPP-ACP.[21]

Recent reports have indicated that frequent application of fluoride protects enamel against erosion by soft drinks. Intensive application of fluoride has also been shown to reduce enamel and dentine erosion by strong acids, even under conditions simulating gastric reflux.[10]

The results of this study showed that it is possible to to diminish the amount of enamel roughness and provide protective effect against erosive enamel loss in primary teeth through all of the three treatments applied. These results are in accordance with those of previous studies that investigated the effect of APF gel on dental erosion. Moreover, similar to our study, these studies have shown a reduction of hardness in all treated groups. Although it is found that fluoride products can inhibit dental erosion, they do not completely prevent it.[22] In the present *in vitro* study, 1.23% APF gel did not completely **repair** dental erosion. Contradictory results ranging from no to limited protection or to almost complete protection of topical fluoride against dental erosion are found in literature.[9] There might be different factors such as differences in study design, particularly regarding the type of dental substrate, the frequency of application, the pH and concentration of the different fluoridated substances used, leading to such conclusions.[8]

The results obtained in this experiment clearly showed that post-treatment with Tooth Mousse, 1.23% APF gel and artificial saliva after being exposed to an acidic drink, were unable to reduce tooth surface and mineral loss.

In primary teeth, the results of the study showed that APF gel provided the highest protective effect against erosive enamel loss compared to Tooth Mousse and artificial saliva. This can be explained with the fact that the acidic pH of APF gel may have etched the enamel surface and helped to increase the incorporation of fluoride into enamel. A further explanation may be that the free negative fluoride ions become more reactive in acidic media, thus enhance the formation of CaF_2. Several studies have also agreed with the enhancement of the formation of the CaF_2 layer under acidic conditions when comparing neutral to acidic fluoride solutions and when comparing neutral to acidic fluoride gels.[23]

The formation of a CaF_2 like layer on the tooth surface, which acts as a fluoride reservoir, seems to be thre reason for the protective effect of fluoride. During an acidic attack, fluoride released from the CaF_2 deposit can be incorporated into the mineral by forming fluoroapatite or fluorohydroxyapatite resulting in a decreased susceptibility to further dissolution. A similar mode of action is assumed for the anti-erosive capability of fluorides. Additionally, the CaF_2 layer might act as a mechanical barrier hampering the contact of the acid with the underlying enamel or as a mineral reservoir, which is attacked by the erosive challenge, thus leading to a buffering or depletion of hydrogen ions from the acid. The formation of the CaF_2 layer depends on the pH and the concentration of the fluoride agent and the duration of application.[24] Since high concentrated fluoride agents or a prolonged application time might possibly lead to a thicker and more stable CaF_2 precipitate, an intensive fluoridation is considered to be most effective for prevention of erosive enamel loss.[24]

As there are some issues regarding the collection, storage and degradation of human saliva, artificial saliva is commonly used instead of human saliva. To make sure that the artificial saliva is similar to the clinical situation, it is vital to quantify its remineralisation potential and to compare this to human saliva. In previous experiments, it was found that the saliva remineralised erosive lesions.[25]

Further studies are needed to clarify the nature of lubricating and remineralizing properties of CPP-ACP paste in reducing tooth wear, and to compare its effectiveness with nightguards and other remineralizing products (including topical fluoride). Such information will be crucial in designing better strategies for tooth wear prevention. The findings of the present study also point out that there is need for clinical trials to investigate the effect of CPP-ACP in **healing** tooth wear *in vivo*.

CONCLUSION

Considering the results of the present *in vitro* study, it can be concluded that the application of APF gel, CPP-ACP paste and artificial saliva are effective repairing dental erosion produced by a soft drink. Further studies are needed to compare the relative benefits of Tooth Mousse and fluoride, when used individually or in combination, repairing dental erosion.

REFERENCES

1. Asher C., Read MJF. Early enamel erosion in children associated with the excessive consumption of citric acid. Br Dent J 162: 384–7, 1987.
2. Hunt JN. The composition of gastric juice. J Physiol 113: 419–424, 1951.
3. Lussi A., Jaeggi T., Zero D. The role of diet in the aetiology of dental erosion. Caries Res 38: 34-44, 2004.
4. Amoras DR., Corona SA., Rodrigues AL Jr., Serra MC. Effect of beverages on bovine dental enamel subjected to erosive challenge with hydrochloric acid. Braz Dent J 23(4): 367-372, 2012.
5. Lussi A, Hellwig E. Risk assessment and preventive measures. Monogr Oral Sci 20: 190–199, 2006.
6. Ganss C., Klimek C., Brune V., Effects of two fluoradation measures on erosion progression in human enamel and dentine in situ. Caries Res 38: 561-566, 2004.
7. Tehrani MH, Ghafournia M, Samimi P, Savabi O, Parisay I, Askari N, Abtahi SH. Effect of casein phosphopeptide-amorphous calcium phosphate and acidulated phosphate fluoride gel on erosive enamel wear. Dent Res J (Isfahan) 8(1): 64-70, 2011.
8. Wiegand A., Bichsel D., Magalhães AC., Becker K., Attin T. Effect of sodium, amine and stannous fluoride at the same concentration and different pH on in vitro erosion. J Dent 37(8) : 591-5, 2009.
9. Hove L., Holme B., Ogaard B., Willumsen T., Tveit AB. The protective effect of TiF4, SnF2 and NaF on erosion of enamel by hydrochloric acid in vitro measured by white light interferometry. Caries Res 40: 440–3, 2006.
10. Willumsen T., Ogaard B., Hansen BF., Rolla G. Effects from pretreatment of stannous fluoride versus sodium fluoride on enamel exposed to 0.1 M or 0.01 M hydrochloric acid. Acta Odontol Scand 62: 278–81, 2004.
11. Sukasaem H., Panich M., Poolthong S. Effect of CPP-ACP on hardness of enamel eroded by Cola-drink. IADR meeting, abstract no 1673, 2006.
12. Willershausen B., Callaway A., Azrak B., Duschner H. Influence of apple juice on human enamel surfaces of the first and second dentition—an in vitro study. Eur J Med Res 13: 349–54, 2008.
13. Deery C., Wagner ML., Longbottom C., Simon R., Nugent ZJ. The prevalence of dental erosion in a United States and a United Kingdom sample of adolescents.J Pediatr Dent 22: 505–10, 2000.
14. Tantbirojn D., Huang A., Ericson MD., Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP–ACP paste. J Dent 36: 74–79, 2008.
15. Ganss C., Klimek J., Giese K. Dental erosion in children and adolescents—a cross-sectional and longitudinal investigation using study models. Community Dent & Oral Epidemio 29: 264–71, 2001.
16. O'Brien M. Children's Dental Health in the United Kingdom 1993. London: HMSO; 1994.
17. Field J., Waterhouse P., German M. Quantifying and qualifying surface changes on dental hard tissues in vitro. J Dent 38: 182-190, 2010.
18. Fujii M., Kitasako Y., Sadr A., Tagami J. Roughness and pH changes of enamel surface induced by soft drinks in vitro-applications of stylus profilometry, focus variation 3D scanning microscopy and micro pH sensor. Dent Mater J 30(3): 404-410, 2011.
19. Kumar VL., Itthagarun A., King NM. The effect of casein phosphopeptide–amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. Aust Dent J 53: 34–40, 2008.
20. Rahiotis C., Vougiouklakis G. Effect of a CPP–ACP agent on the demineralization and remineralization of dentine in vitro. J Dent 35: 695–8, 2007.
21. Ramalingam L., Messer LB., Reynolds EC. Adding casein phosphopeptide amorphous calcium phosphate to sports drinks to eliminate in vitro erosion. Pediatr Dent 27: 61–67, 2005.
22. Murakami C., Bönecker M., Corrêa MS., Mendes FM., Rodrigues CR. Effect of fluoride varnish and gel on dental erosion in primary and permanent teeth. Arch Oral Biol 54(11): 997-1001, 2009.
23. Saxegaard E., Rolla G. Fluoride acquisition on and in human enamel during topical application in vitro. Scandinavian J Dent Res 96: 523-35, 1988.
24. Lagerweij MD., Buchalla W., Kohnke S., Becker K., Lennon AM., Attin T. Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 40: 148–53, 2006.
25. Hara AT., Gonzalez-Cabezas C., Creeth J., Zero DT. The effect of human saliva substitutes in an erosion-abrasion cycling model. Eur J Oral Sci 116(6): 552-556, 2008.