Original

Evaluation of Enamel Surface Morphology and Microhardness after the Application of Different Protective Agents

Huseyin Simsek¹, Sera Derelioğlu⁰, Suleyman Kütalmış Büyük⁰ and Ebru Emine Sukuroğlu⁰

¹ Department of Pediatric Dentistry, Faculty of Dentistry, Ordu University, Ordu, Turkey
² Department of Pediatric Dentistry, Faculty of Dentistry, Atatürk University, Erzurum, Turkey
³ Department of Orthodontics, Faculty of Dentistry, Ordu University, Ordu, Turkey
⁰ Department of Mechanical Engineering, Faculty of Engineering, Gümüşhane University, Gümüşhane, Turkey

(Accepted for publication, December 18, 2017)

Abstract: The aim of this in vitro study was to compare the effect of different protective agents on the surface microhardness and morphology on enamel eroded by a cola drink. Sixty sound anterior teeth were randomly divided into five groups. The baseline surface microhardness of the enamel was measured. For demineralization procedures, all teeth were immersed in cola for 8 minutes and surface microhardness was remeasured. The five groups for remineralization procedures were defined as: Group 1: Artificial saliva, Group 2: 1.23% Acidulated Phosphate Fluoride gel (APF), Group 3: 5% NaF varnish, Group 4: Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP), and Group 5: Probiotic yogurt. After each experimental procedure, a third microhardness measurement was repeated. Surface morphological changes were observed using SEM.

All data were recorded for each group and statistically analyzed using two-way analysis of variance and one-way repeated-measures analysis of variance. All tests were performed at a significance level of p < 0.05. No significant differences for baseline microhardness values were found (P> 0.05). The mean surface microhardness in all groups decreased significantly after immersion in cola (P< 0.05). After remineralization, NaF varnish and APF gel significantly increased the surface microhardness values. There were no significant increases in the surface microhardness values of artificial saliva, CPP-ACP and probiotic yogurt. This study concludes that NaF varnish is more effective in re-hardening of eroded enamel.

Key words: Surface morphology, Microhardness, Enamel, Tooth erosion, CPP-ACP, Probiotic

Introduction

Teeth are exposed to many physical and chemical effects that can cause erosion throughout life². Hard tissue loss occurs in teeth without caries is classified as abrasion, attrition, abfraction, and erosion³,⁴. Dental erosion is an irreversible loss of tooth surface due to a chemical process without bacterial invasion⁵.

It is reported that time and frequent exposure to a solution below the critical pH of 5.5 causes erosion in dental enamel⁶-⁸. Tooth erosion may be due to internal and external factors⁹. For this reason excessive consumption of acidic beverages and food are one of the most common extrinsic factors causing irreversible erosive wear of dental enamel⁹. Saliva provides a protective effect by cleansing and neutralizing acids⁹. Simultaneously, it is a source of inorganic ions which are necessary for the remineralization process⁹, ¹⁰. Enamel, softened by acidic drinks, hardens after exposure to saliva or artificial saliva¹¹. Patients with low or reduced salivary flow are more susceptible to erosion of the teeth¹¹,¹².

Different enamel surface protective agents, such as fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), are used to activate remineralization of enamel and protect it from erosion¹³. CPP-ACP nanocomplexes are casein-derived peptides stabilized by the CPP of ACP, and serve as a calcium and phosphate reservoir when incorporated into dental plaque and tooth surface¹⁴. CPP-ACP has been shown to reduce demineralization, promote remineralization of caries lesions¹⁵. Fluoride increases mineralization by adsorbing early caries’ lesions of the partially solved crystal lattice, and attracts calcium and phosphate ions to accelerate mineralization¹⁶. Fluoride similarly improves the remineralization process of enamel eroded lesions¹⁷. The positive effects of probiotics on remineralization suggest there may be an alternative to enamel erosion treatment.

The aim of this study was to evaluate the remineralization effect of eroded enamel by artificial saliva, different fluoride agents, CPP-ACP and probiotic yogurt using scanning electron microscopy (SEM) and surface microhardness (SMH) analysis.

Materials and Methods

Preparation of samples

In this study, we used 60 permanent maxillary central teeth, which were extracted due to periodontal reasons. Teeth with caries, restoration, hypoplasia, fractures, and cracks were not included in the study. Soft tissue debris were cleaned with a periodontal scaler. Teeth were stored in artificial saliva until the experimental stage, while their crowns were separated from the roots with a low-speed saw in the cemento-enamel junction (Isomet 1000, Buehler, Lake Bluff, IL, USA). All crowns were embedded in the acrylic epoxy resin with the labial surfaces were parallel to the horizontal plane and labial surface levelled on top.

Enamel microhardness assessment and erosion procedure

The baseline surface hardness values of all teeth were measured with microhardness tester (Leitz, Model 8331, Wetzlar, Germany) using a Vicker’s indenter. The diamond-shape abrasion of the test device was applied to enamel surfaces at 25 gr (F= 0.225 N) for 15 seconds. Measurements were made from 3 different regions for each specimen in the one-third incisal portion of the labial surface of the teeth.
Table 1. Protective agents used in this study

| Materials Type     | Chemical Composition                                                                 | Products | Manufacturer                  |
|--------------------|---------------------------------------------------------------------------------------|----------|-------------------------------|
| Artificial Saliva  | Calcium Chloride, Magnesium Chloride, Potassium Chloride, Potassium Phosphate Dibasic, Potassium Phosphate Monobasic, Sodium Chloride, Azote, Sorbitol, Carboxymethyl Cellulose, Para Hydroxybenzaldehyde Methylene | Hypozalix | Biocodex Inc., Gentilly, France |
| 1.23% APF Gel      | Sodium Fluoride, Phosphoric Acid, Titanium Dioxide                                     | Topex    | Sultan, NJ, USA                |
| 5% NaF Varnish     | Sodium Fluoride, Ethanol                                                               | Proflurid | VOCO GmbH, Cuxhaven, Germany   |
| CCP-ACP            | Glycerol, CPP-ACP, D-Sorbitol, Silicon dioxide, Propylene glycol, Titanium dioxide, Sodium fluoride, Ethyl-p-hydroxybenzoate, Butyl-p-hydroxybenzoate, Propyl-p-hydroxybenzoate | Tooth Mousse | GC Corporation, Tokyo, Japan |
| Probiotic Yogurt   | Water, Sodium Citrate, Calcium, Vitamin D3, Malic Acid, Agar Agar, Carrageenan.        | Activia  | Danone, Istanbul, Turkey      |

Table 2. Mean surface microhardness values at baseline, after demineralization and remineralization

| Enamel Treatment     | Group 1               | Group 2               | Group 3               | Group 4               | Group 5               |
|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Baseline             | 981.33±114.04         | 1028.83±156.36        | 958.00±144.91         | 976.75±173.15         | 1080.91±102.59        |
| Post-Erosion Stage   | 250.00±22.74          | 226.41±25.34          | 232.83±25.58          | 254.50±19.13          | 254.50±19.13          |
| After remineralization | 267.16±19.73        | 328.50±31.60          | 679.41±38.35          | 278.75±30.59          | 271.66±24.57          |

Different superscript letters are significantly different according to Tukey test P<0.05

Hardness measurements were performed with micron magnification of 50× in the form of stars that appear on the resulting sample. Average hardness values were calculated by averaging results of each sample, which was then subjected to a second surface hardness test after 8 minutes in 5 ml of cola (Coca-Cola, Coca-Cola Co. Istanbul, Turkey) (pH 3.4).

Remineralization of eroded enamel

Different protective agents were applied to the teeth. The chemical composition, characteristics, and manufacturer of the products are reported in Table 1. The teeth were randomly divided into 5 groups with 12 samples in each group.

Group 1: Artificial saliva (Hypozalix, Biocodex Inc., Gentilly, France).
Group 2: 1.23% Acidulated Phosphate Fluoride gel (APF gel, Topex, Sultan, NJ, USA).
Group 3: 5% NaF varnish (Profluride varnish, VOCO, GmbH, Cuxhaven, Germany).
Group 4: Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP, GC Corporation, Tokyo, Japan)
Group 5: Probiotic yogurt (Activia, Danone, Istanbul, Turkey)

Group samples were stored in artificial saliva during the experimentation. The protective agents (2 ml) were applied on the labial surface of groups 2, 3, 4, and 5 with brushing for 5 min, then washed with distilled water until being wiped off. Finally, vickers surface microhardness values was obtained. The hardness measurements of the enamel surface from baseline, after 8-min of cola immersion, and after remineralization for 5 min were calculated.

SEM observation

SEM was used to observe surface morphology of samples. Three samples from each group were prepared to examine the change in surface morphology; they were stored at room temperature and thoroughly dried before SEM evaluation. The labial surfaces of the specimens were fixed on both sides using carbon bands and surfaces were covered with gold. SEM evaluation was performed at 15 kV and 250× magnification in initial, after cola application, and remineralization agents (Fig. 1).

Statistical Analysis

The data were statistically analysed using a computerized statistical software program (SPSS Statistics for Windows, Version 20.0, SPSS Inc., Chicago, IL, USA). The results were analysed by one-way repeated-measures analysis of variance to compare the surface microhardness values at baseline, after demineralization and after remineralization. We analysed the homogeneity among groups at baseline and after demineralization by using one-way analysis of variance at the 5% probability level. We analysed the differences among groups after remineralization by using two-way analysis of variance at the 5% probability level.

Results

Table 2 shows that the mean surface microhardness at baseline, after demineralization and after remineralization procedures. Statistical evaluation of one-way analysis of variance showed that homogeneous distribution and no statistically significant difference among the groups at baseline (P= 0.642).

After demineralization, there were a significant decreased in all groups (P< 0.05). Surface microhardness values were not significantly different among the groups (P= 0.371).

After remineralization, the mean surface microhardness increased by higher group 3 (NaF Varnish) and then in group 2 (APF gel) (P< 0.05).
Group 1 (Artificial saliva), group 4 (CPP-ACP), and group 5 (Probiotic yogurt) which have shown very similar microhardness values did not show any significant difference statistically (P= 0.249).

**Discussion**

Mineral changes that occur due to demineralization and remineralization procedures affect the hardness of the teeth. Initial microhardness values in our study were found to be higher than similar studies. Cuy et al. found that the degree of enamel hardness was related to the degree of mineralization, location of enamel rods and tufts. In other studies, measurements were performed in the middle or cervical one-third of the labial surface whereas microhardness measurements were performed in the incisal one-third of enamel in our study. The microhardness values were found to be higher due to greater enamel thickness of the incisal part.

Microhardness values of treated and polished enamel may change. Removal of the outer layer of enamel, which is hypermineralized, makes the enamel more susceptible to erosion. Anterior teeth imitated the oral environment, and as such, we had more accurate results in the demineralization-remineralization processes, with normal anatomic structure.

The etiology of dental erosion is multifactorial, but diet is the most important factor. If erosion-causing factors cannot be removed or preventive measures cannot be taken, dental erosion of the enamel...
which increased saturation of the minerals’ microhardness. In our study, a decrease of approximately 70% in microhardness values was observed after the samples were exposed. A similar decrease of about 40% was observed in similar studies. In the SEM images, changes in porosities, depressions, and enamel prisms were seen on the surface of the exposed enamel. We noted that the more visible decrease was due to the uncomplicated exposure of the specimens for a longer time and that the mineral was more affected by the collet than the dentin.

Possible protective effects of different agents on erosive enamel were evaluated and compared in this present study. Artificial saliva (Hypoalix), APF gel (Topex), NaF varnish (Profluorid varnish), a CPP-ACP paste (Tooth Mousse), and probiotic yogurt (Danone) were used in this study. Compared to other groups, artificial saliva did not significantly increase the enamel’s microhardness. The cracks and depressions on the enamel surface were mildly repaired in the SEM images. Some studies restricted the remineralization feature in the eroded enamel, while other studies were able to re-establish artificial saliva in the enamel. These differences were thought to be due to the composition of the artificial saliva, the study design, and duration of exposure.

In our study, the microhardness values of NaF varnish significantly increased compared to the other groups. In similar studies, NaF-containing agents were found to provide remineralization and increase enamel microhardness values. This was probably due to the substitution of calcium and phosphate ions on the fluoride, and the erosive enamel to form fluorapatite crystals, which are resistant to demineralization. It was seen that the protective layer was formed in the rich regular structure of the fluoride in SEM images. Microhardness values were not significantly increased, although they contained fluoride in the APF-induced group. The natural acidic structure of APF is thought to affect solubility of the enamel. A thin enamel layer may become soluble and the resulting mineralized mineral phases are continuously displaced with fluoride-rich calcium and phosphate mineral phases during APF application. APF appears to form a very thin and superficial irregular fluoride layer on the erosive enamel surface in SEM images.

Dental erosion and caries lesions have different surface and subsurface properties. While dental erosion is often characterized by a shallow subsurface lesion and surface demineralization, initial caries lesions have a relatively firm enamel surface and a much deeper subsurface lesion. Fluoride provides rapid hardness in the enamel surface layer and prevents mineral migration to the lower surfaces in caries lesions. However, Ca and P-containing prophylaxis agents provide remineralization in deeper regions and form a protective hydroxyapatite layer on the enamel surface. Studies show that CPP-ACP is more effective in deep caries lesions. CPP-ACP promoted the formation of a mineral deposition and complete layer on enamel surface in SEM. In our study, the NaF group microhardness values were found to be higher than CPP-ACP. Fluorapatite crystals formed in the enamel surface region are more resistant to demineralization than hydroxyapatite crystals, so measurements may be due to how they are performed in the topmost enamel layer.

Although there are many studies on the efficacy of probiotics in preventing tooth caries, there are no studies on the effects of microhardness of dental enamel. In our study, probiotic yogurt increased the microhardness of minerals numerically, although it was not significant increased compared to other groups. In the SEM images, erosive enamel overlaps slightly with the Ca and P layers. The probiotic yogurt in our study stuck to the surface of the tooth as well as to the Ca and P contents, which increased saturation of the minerals’ microhardness.

In conclusion, it is advisable to rinse the mouth with a mineral additive such as Ca, PO₄, F, or a probiotic without brushing after consumption of acidic beverages, this would be for the prevention of dental erosion and microhardness reduction of the teeth.

Conflict of Interest

The authors declared that they have no conflict of interest.

References

1. Murrell S, Marshall TA, Moynihan PJ, Qian F and Wefel JS. Comparison of in vitro erosion potentials between beverages available in the United Kingdom and the United States. J Dent 38: 284-289, 2010
2. Laurance-Young P, Bozec L, Gracia L, Rees G, Lippert F, Lynch RJ and Knowles JC. A review of the structure of human and bovine dental hard tissues and their physicochemical behaviour in relation to erosion challenge and remineralisation. J Dent 39: 266-272, 2011
3. Moss SJ. Dental erosion. Int Dent J 48: 529-539, 1998
4. Valinoti AC, Pierro DS, Santos V, Silva D, Moreira E and Maia LC. In vitro alterations in dental enamel exposed to acidic medicines. Int J Paediatr Dent 21: 141-150, 2011
5. Gotouha H, Nasu I, Kono T, Ootani Y, Kanno T, Tamamura R, Kuwada-Kusunose T, Suzuki K, Hirayama T, Hirayama T, Sakae T and Okada H. Erosion by an acidic soft drink of human molar teeth assessed by x-ray diffraction analysis. J Hard Tissue Biol 26: 81-86, 2017
6. Lussi A, Schlüter N, Rakhmatullina E and Ganss C. Dental erosion—an overview with emphasis on chemical and histopathological aspects. Caries Res 45: 2-12, 2011
7. Srinivasan N, Kavitha M and Loganathan S. Comparison of the remineralization potential of CPP–ACP and CPP–ACP with 900ppm fluoride on eroded human enamel: An in situ study. Arch Oral Biol 55: 541-544, 2010
8. Hayashi O, Chiba T, Shimoda S and Momoi Y. Demineralization and remineralization phenomena of human enamel in acid erosion model. J Hard Tissue Biol 25: 27-34, 2016
9. Buzałaf MAR, Hamas AR and Kato MT. Saliva and dental erosion. J Appl Oral Sci 20: 493-502, 2012.
10. West NX and Joiner A. Enamel mineral loss. J Dent 42: S2-S11, 2014
11. Ionta FQ, Mendonça FL, de Oliveira GC, de Alencar CR, Honório HM, Magalhães AC and Rios D. In vitro assessment of artificial saliva formulations on initial enamel erosion remineralization. J Dent 42: 175-179, 2014
12. Rios D, Honório H, Magalhães A, Delbem AC, Machado MA, Silva SM and Buzałaf MA. Effect of salivary stimulation on erosion of human and bovine enamel subjected or not to subsequent abrasion: an in situ/ex vivo study. Caries Res 40: 218-223, 2006
13. Correr GM, Alonso RCB, Correa MA, Campos EA, Baratto-Filho F and Puppin-Rontani RM. Influence of diet and salivary characteristics on the prevalence of dental erosion among 12-year-old schoolchildren. J Dent Child (Chic) 76: 181-187, 2009
14. Poggio C, Grasso N, Ceci M, Beltrami R, Colombo M and Chiesa M. Ultrastructural evaluation of enamel surface morphology after tooth bleaching followed by the application of protective pastes. Scanning 38: 221-226, 2016
15. Hamita H, Nikaido T, Inoue G, Sadr A and Tagami J. Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel remineralization: a quantitative assessment using micro-computed tomography. J Dent 39: 405-413, 2011
16. Murakami C, Bonecker M, Correa MSNP, Mendes FM and Rodrigues CRMD. Effect of fluoride varnish and gel on dental erosion in primary and permanent teeth. Arch Oral Biol 54: 997-1001, 2009
17. Ren Y-F, Liu X, Fadel N, Malmstrom H, Barnes V and Xu T. Preventive effects of dentifrice containing 5000ppm fluoride against dental erosion in situ. J Dent 39: 672-678, 2011
18. Cuy JL, Mann AB, Livi KJ, Teaford MF and Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. Arch Oral Biol 47: 281-291, 2002
19. Panich M and Poolthong S. The effect of casein phosphopeptide–amorphous calcium phosphate and a cola soft drink on in vitro enamel hardness. J Am Dent Assoc 140: 455-460, 2009
20. Huysmans M, Chew H and Ellwood R. Clinical studies of dental erosion and erosive wear. Caries Res 45: 60-68, 2011
21. Arnadottir IB, Holbrook WP, Eggertsson H, Gudmundsdottir H, Jonsson SH, Gudlaugsson JO, Saemundsson SR, Eliasson ST and Agustsdottir H. Prevalence of dental erosion in children: a national survey. Community Dent Oral Epidemiol 38: 521-526, 2010
22. Tantbirojn D, Huang A, Ericson M and Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 36: 74-79, 2008
23. Zero DT and Lussi A. Erosion—chemical and biological factors of importance to the dental practitioner. Int Dent J 55: 285-290, 2005
24. Devlin H, Bassioumy M and Boston D. Hardness of enamel exposed to Coca-Cola® and artificial saliva. J Oral Rehabil 33: 26-30, 2006
25. Vyavhare S, Sharma DS and Kulkarni V. Effect of three different pastes on remineralization of initial enamel lesion: an in vitro study. J Clin Pediatr Dent 39: 149-160, 2015
26. Huang S, Gao S and Yu H. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomed Mater 4: 034104, 2009
27. Banda NR and Shashikiran N. Evaluation of primary tooth enamel surface morphology and microhardness after Nd: YAG laser irradiation and APF gel treatment—an in vitro study. J Clin Pediatr Dent 35: 377-382, 2011