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The Impact of Selected Fluoridated Toothpastes on Dental Erosion in Profilometric Measurement

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Some fluoridated toothpastes, available commercially, are described to have a protective effect against dental erosion.

Objectives. To evaluate the influence of the selected marketed toothpastes on the human enamel exposed to acid beverages.

Material and Methods. Enamel specimens from extracted human teeth were prepared (n = 40). Specimens were randomly divided into 10 experimental groups, 4 specimens each, which were subjected to acid challenge for 10 min using orange juice (pH 3.79) or Pepsi Cola (pH 2.58) and then immersed for 2 min into a slurry of five marketed toothpastes with distilled water (1 : 3 w/w). The tested toothpastes contained 1450 or 5000 ppm fluoride, CPP-ACP with 900 ppm fluoride, 1450 ppm fluoride with potassium nitrate 5%, all of them as sodium fluoride, and 700 ppm fluoride as amine and sodium fluoride with 3500 ppm SnCl₂. Enamel roughness (Ra parameter) by contact profilometer at baseline and after exposure onto soft drinks and slurry was measured.

Results. Exposure to both beverages caused a similar increase of enamel surface roughness. After the specimens immersion into slurries of toothpastes with 1450 or 5000 ppm fluoride, 1450 ppm fluoride with potassium nitrate 5% and CPP-ACP with 900 ppm fluoride the significant decrease of Ra values were found, reaching the baseline values. However, toothpaste with 700 ppm fluoride and 3500 ppm SnCl₂ did not cause any fall in Ra value, probably due to other mechanism of action.

Conclusions. Within the limitation of the study we can conclude that the sodium fluoride toothpastes are able to restore the surface profile of enamel exposed shortly to acidic soft drinks (Adv Clin Exp Med 2016, 25, 2, 327–333).

Key words: dental erosion, fluoridated toothpastes, profilometry.

Brushing with toothpaste plays an important role in the maintenance of oral health due to dental plaque removal. The components of toothpastes enhance caries prevention or provide additional benefits such as control of gingivitis and calculus formation, reduction of teeth hypersensitivity and bad breath, whitening teeth as well as protection against the initiation and progression of dental erosion. Dental erosion is defined as loss of hard dental tissues, which is caused by chemical etching and dissolution resulting from nonbacterial acids of extrinsic or intrinsic origin [1].

Extrinsic acids mainly come from diet containing acidic food (e.g., citrus fruits, grapes, sour apples, vinegar) and drinks (fruit juices, soft drinks, carbonated beverages, ice tea, herbal teas, cider,

white wine) or acidic oral medication, whereas intrinsic are from acidic gastro-esophageal reflux and vomiting [2, 3]. Acidic agents cause erosive lesion by chemical dissolution or chelation of dental minerals leading to complete removal of outermost layers of the tooth surface. They act in two ways: first, by softening the tooth surface as a result of partial demineralization and then, by destruction of this surface and layer by layer, irreversible loss of dental hard tissues. It has been stated that their pH should be lower than critical pH for fluorhydroxyapatite, i.e. under 4.5.

However, erosive potential of acidic agents is also determined by other chemical factors as titrable acidity, type of acid (pKa values) and acid concentration, chelating properties as well as cal-

cium, phosphate and fluoride concentration and adhesion of the product to the dental surface. Although both dental caries and erosion are acid driven processes, the causes, clinical and histological presentations and symptoms are clearly differentiated. The causes of erosion are acids produced extra-orally (extrinsic or intrinsic) being unsaturated with respect to hydroxyapatite (pH under 5.5) and fluorhydroxyapatite (pH under 4.5), whereas for caries acids are produced by dental plaque bacteria making the tooth environment with pH under 5.5 and over 4.5.

Erosion occurs on the tooth surface as localized and irreversible mineral loss, but the remaining enamel can be hardened by fluoride, whereas in carious spot lesions (incipient caries) mineral loss starts beneath the tooth surface and the lesion is reversible by remineralization with use of fluoride [3, 4]. In a clinical situation, the erosive process can be modified by several biological factors such as saliva (flow rate and acquired salivary pellicle), teeth chemical composition, and also by the anatomy of soft tissues, soft tissues movements (tongue and buccal mucosa) as well as behavioral factors such as swallowing pattern, which can influence the risk of erosion by controlling the retention or clearance pattern of erosive agents [5, 6]. Parameters influencing the initiation, progression and prevention of dental erosion are often studied *in vitro* conditions, however some efforts are taken to mimic, in some extent, the clinical situation. Softening and loss of hard dental tissues occurring due to acidic attack are assessed with use of various techniques, mostly by profilometric or microhardness measurements.

The aim of the study was to evaluate the influence of the selected marketed toothpastes on the human enamel exposed onto acidic beverages.

Material and Methods

Preparation of Enamel Specimens

Caries-free human permanent third molars were selected from a pool of extracted teeth due to surgical reasons. Prior to extraction, the patients were asked about the use of their teeth for the study purpose, and consent was obtained. After extraction, the teeth were cleaned of debris, examined under good light and magnification ($\times 2.5$) to detect any enamel cracks, caries incipient, developmental defects of enamel or discolourations. The teeth with any enamel abnormality were discarded. The crowns were cut from the roots at cemento-enamel junction and two slabs of enam-

el (buccal and lingual) supported by dentin were prepared from one tooth. The surfaces of the stabilized slabs were flattened and polished using decreasing abrasives (up to 3 μm aluminum oxide) under constant cooling and after each polishing step they were sonicated in water and then rinsed. The slabs were stored in thymolized (1%) saline in room temperature.

Tested Toothpastes

Five commercially available fluoridated toothpastes were used: Colgate Total Pro-Gum-Health (1450 ppm F as sodium fluoride), Colgate Duraphat 5000 (5000 ppm F as sodium fluoride), MI Paste Plus (Casein Phosphopeptide – Amorphous Calcium Phosphate – CPP-ACP – Recaldent™ and 900 ppm F as sodium fluoride), Sensodyne Pronamel (1450 ppm F as sodium fluoride, potassium nitrate 5%) and ElmexErosionProtection (1400 ppm F, out of which 700 ppm from amine fluoride and 700 ppm from sodium fluoride, 3500 ppm Sn^{2+} from stannous chloride).

Used Soft Drinks

Two commercially available soft drinks were used – orange juice 100% (Cappy), group A and Pepsi Cola (PepsiCo), group B. Both drinks were analyzed chemically in respect to the following parameters: pH (pHametrically), buffering capacity (pHametrically titrated with 0.1N HCl), titrated acidity (pHametrically titrated with 0.1M NaOH to pH 7.0), calcium (colorimetric method with use of Arsenazo III), inorganic phosphate (colorimetric method with use of ammonium molybdate) and fluoride (by Orion 9609 BNWP electrode).

Test Procedure

Slabs were randomly divided into 10 experimental groups, 4 specimens each, immersed in orange juice or Pepsi Cola for 10 min, then rinsed with distilled water and immersed into the tested toothpastes slurry with distilled water (1 : 3 w/w) for 2 min. Measurements of enamel surface topography were conducted before (measurement 0) and after the slabs immersion into drinks (measurement 1) followed by the immersion in the tested toothpastes slurries with use of contact profilometer (Form Talysurf 120L Series S5-CD with Ultra Software). The measurements were carried out by diamond stylus tip with radius 2 μm , 0.01 N load and 0.5 mm/s movement for 0.8 mm measurement length. Each slab was measured 3-fold and a mean value was calculated as a one measurement. Among 29 parameters describing a surface

roughness, 3 parameters Ra, Rz and Rt were analyzed. However, it has turned out that the values of the Ra parameter are strongly positively correlated with Rz and Rt values (Ra vs. Rz, $R^2 = 0.7474$; Ra vs. Rt, $R^2 = 0.8314$) and hence the analysis of the obtained data was limited to the Ra values.

Statistical Analysis

The obtained data was analyzed by Kolmogorov-Smirnov and Shapiro-Wilk tests, one-way ANOVA, *post-hoc* and Kruskal-Wallis tests at $p < 0.05$ level of significance using STATISTICA 8.0 software.

Results

Chemical analysis of the used soft drinks as the erosive agents showed some differences in levels of the chemical parameters influencing their erosive potential (Table 1). The orange juice compared to Pepsi Cola had higher initial pH, buffering capacity, titrated acidity to pH 7.0, and concentrations of calcium and inorganic phosphate.

The exposure of all enamel slabs onto orange juice (group A) caused some increase in Ra values and indicated the enhancement of the enamel roughness (measurement 1 vs. 0). After the immersion in slurries of the pastes (measurement 2) in comparison to the measurements, 1 significant decrease ($p < 0.05$) in the Ra values was found, except the ElmexErosionProtection paste, where the Ra mean value was on the same level. Consequently, the surface roughness of the enamel was reduced significantly ($p < 0.05$) due to immersion into slurries of Colgate Total, Duraphat 5000, MI Plus and Sensodyne Pronamel pastes. Comparing the data from measurement 2 with measurement 0, a significant ($p < 0.05$) reduction in Ra values for Colgate Total and Duraphat 5000 pastes and only a slight reduction for MI Plus paste were found, which could suggest more flattened enamel surface than at the baseline. However, after immersion into slurry of Sensodyne Pronamel, the sur-

face roughness returned to the baseline (the same Ra values), but in the case of ElmexErosionProtection paste it remained rough as after exposure on orange juice (Table 2).

Like orange juice, the slabs exposure on Pepsi Cola (group B) revealed some increase of the roughness of enamel surface, i.e. higher Ra values (measurement 1 vs. 0). Similarly to group A the immersion into the pastes slurries led to a significant diminution of Ra values with the exception for ElmexErosionProtection paste, which seemed to be unaffected and remained on the same level (measurement 2 vs. 1). However, only after being immersed in Colgate Total and Duraphat 5000 slurries, the Ra values were significantly lower when compared to the baseline. MI Plus and Sensodyne Pronamel pastes had mean values which were almost on the same levels. Contrary, the immersion of the slabs into slurry of ElmexErosionProtection paste did not change the enamel roughness at all (Table 3).

Discussion

The relationship of acidic soft drinks with the development of dental erosion is widely and well-documented. In numerous studies acidic drinks were assessed in the aspect of causative factor of enamel and dentin erosion [7–10]. However, the same drinks produced in different countries showed some differences in their erosiveness [10]. The erosive potential of soft drinks can be predicted by some chemical properties determined by acids which they contain as the initial pH, the buffering capacity, titrated acidity as well as calcium and phosphate concentrations. They can modify the erosive attack on hard dental tissues, as determining the saturation degree in reference to dental minerals, which is a driven force of the dental minerals dissolution. However, a rise of fluoride concentration can influence the speed of the erosive loss of enamel. The erosive potential of soft drinks is verified *in vitro*, *in situ* or *ex vivo* studies in respect to specimens of human or bovine hard dental tissues or hy-

Table 1. Chemical parameters of the used soft drinks

Soft drinks	pH	Buffering capacity [mol/L]	Titrated acidity to pH 7.0 [mmol OH ⁻ /L]	Calcium [mmol/L]	Inorganic phosphate [mmol/L]	Fluoride [mg/L, ppm]
Orange juice (Cappy) Group A	3.79	$4.17 \cdot 10^{-2}$	133.00	24.50	60.61	0.20
Pepsi Cola (PepsiCo) (Group B)	2.58	$2.27 \cdot 10^{-2}$	43.00	3.40	45.59	0.18

Table 2. Ra values after enamel exposure on orange juice and immersion into slurry of the tested toothpastes

Sub-groups	toothpaste	Ra (μm)			Δ Ra (μm)		
		0	1	2	1 vs. 0	2 vs. 0	2 vs. 1
		x $\pm$ SD (Me)	x $\pm$ SD (Me)	x $\pm$ SD (Me)	x (Me)	x (Me)	x (Me)
A-1	Colgate Total	0.138 $\pm$ 0.023 ^{ab} (0.137)	0.198 $\pm$ 0.051 ^{abc} (0.217)	0.106 $\pm$ 0.007 ^{ac} (0.108)	0.060 (0.080)	−0.032 (−0.32)	−0.092 (−0.109)
A-2	Duraphat 5000	0.057 $\pm$ 0.002 ^d (0.057)	0.059 $\pm$ 0.003 ^e (0.058)	0.053 $\pm$ 0.002 ^{de} (0.053)	0.002 (0.001)	−0.004 (−0.004)	0.006 (0.005)
A-3	MI Plus	0.071 $\pm$ 0.005 (0.069)	0.074 $\pm$ 0.008 ^f (0.072)	0.064 $\pm$ 0.003 ^f (0.064)	0.003 (0.003)	−0.007 (−0.005)	−0.010 (−0.008)
A-4	Sensodyne Pronamel	0.056 $\pm$ 0.001 ^g (0.055)	0.064 $\pm$ 0.005 ^{gh} (0.064)	0.056 $\pm$ 0.003 ^h (0.056)	0.008 (0.009)	0.000 (0.001)	−0.008 (−0.008)
A-5	Elmex Erosion Protection	0.062 $\pm$ 0.003 ^{ik} (0.062)	0.074 $\pm$ 0.001 ⁱ (0.075)	0.074 $\pm$ 0.001 ^k (0.075)	0.012 (0.013)	0.012 (0.013)	0.000 (0.000)

Significant difference within subgroups between a-a, b-b, c-c, d-d and f-f at level $p < 0.05$, and between e-e, g-g and h-h at level $p < 0.001$, and between i-i and k-k at level $p < 0.001$

Table 3. Ra values after enamel exposure on Pepsi Cola and immersion into slurry of the tested toothpastes

Sub-groups	toothpaste	Ra (μm)			Δ Ra (μm)		
		0	1	2	1 vs. 0	2 vs. 0	2 vs. 1
		x $\pm$ SD (Me)	x $\pm$ SD (Me)	x $\pm$ SD (Me)	x (Me)	x (Me)	x (Me)
B-1	Colgate Total	0.152 $\pm$ 0.007 ^a (0.153)	0.162 $\pm$ 0.004 ^b (0.162)	0.130 $\pm$ 0.012 ^{ab} (0.132)	0.010 (0.009)	−0.022 (−0.021)	−0.032 (−0.030)
B-2	Duraphat 5000	0.089 $\pm$ 0.007 ^c (0.077)	0.115 $\pm$ 0.018 ^{cd} (0.116)	0.077 $\pm$ 0.012 ^d (0.075)	0.026 (0.039)	−0.012 (−0.002)	−0.038 (0.012)
B-3	MI Plus	0.064 $\pm$ 0.003 ^e (0.064)	0.076 $\pm$ 0.003 ^{ef} (0.077)	0.065 $\pm$ 0.006 ^f (0.065)	0.026 (0.013)	0.001 (0.0010)	−0.011 (−0.012)
B-4	Sensodyne Pronamel	0.086 $\pm$ 0.005 ^g (0.086)	0.111 $\pm$ 0.010 ^{gh} (0.113)	0.087 $\pm$ 0.002 ^h (0.088)	0.025 (0.027)	0.001 (0.002)	−0.024 (−0.025)
B-5	Elmex Erosion Protection	0.069 $\pm$ 0.002 ^{ik} (0.069)	0.095 $\pm$ 0.017 ⁱ (0.094)	0.095 $\pm$ 0.014 ^k (0.092)	0.026 (0.025)	0.026 (0.023)	0.000 (0.002)

Significant difference within subgroups between e-e, f-f, g-g, i-i and k-k at level $p < 0.05$, and between a-a, b-b, c-c, d-d and h-h at level $p < 0.01$.

droxyapatite. However, it has been assumed that the erosive potential depends mainly on the initial pH and the titrable acidity of the erosive agent [3].

Orange juice (100%) is commonly used as a model in erosion study *in vitro* and *in vivo*. The orange juice used in our study compared to Pepsi Cola revealed a greater initial pH, titrated acidity as well as calcium and phosphate content.

The increase in prevalence and severity of dental erosion along with the increase in consumption of acidic soft drinks reinforce the need to develop an efficient protective measure. There are some

possible ways to prevent the initiation and progression of dental erosion: by reducing the amount of dietary acids ingestion, controlling the mode and frequency of intake, strengthening enamel against acids, and coating exposed dental surface with protective barrier layer [11, 12].

Fluorides have been described as a possible agent for reduction of the erosive potential of soft drinks and constitutive the protection measure against the erosion development [13]. In this aspect, marketed fluoridated toothpastes and fluoride preparations are evaluated as well as prod-

ucts based on experimental formulations. Most of the marketed toothpastes contain sodium fluoride (NaF) and amine fluoride as the source of fluoride, which provides the fluoride concentration up to 1500 ppm. The benefit of those toothpastes can be related to the formation of precipitated calcium fluoride (CaF_2) that makes up a reservoir of fluoride from which fluoride ions gradually are released upon acid attack [14]. However, the results from the studies of the fluoride impact on erosion are inconsistent. Moretto and al. [15] showed that fluoride toothpastes (1100 ppm F) were not able to stop completely the erosive process *in vitro* evaluated by profilometry. Austin et al. [16] found the protective influence against erosion of high fluoride concentration (5000 and 19 000 ppm), but not for lower ones (225 ppm and 1450 ppm) that was measured with the use of non-contact profilometer. However, a negative result was received by Rios et al. [17] with toothpastes containing 5000 ppm of fluoride. Contrarily, Hara et al. [18] found a reduction of erosion and abrasion after the use of fluoridated paste (1100 ppm) in comparison to the use of non-fluoridated paste. Likewise, Magalhães et al. [19] *in situ/ex vivo* showed that toothpaste with 1100 ppm fluoride significantly reduced the loss of dentin exposed to acid, however the reduction did not rise along with the rise of fluoride concentration (toothpaste with 5000 ppm).

Our data confirmed the results as we did not observe a greater reduction of surface roughness by toothpastes containing only fluoride in concentration of 1450 and 5000 ppm. Kato et al. [20] studied some marketed desensitizing toothpastes with (Sensodyne Pronamel, 1450 ppm F, 5% KNO_3 and Colgate Sensitive, 1450 ppm F) and without fluoride (Sensodyne Original, 10% SrCl_2), and experimental pastes containing fluoride (1100 ppm F, 10% SrCl_2 , 5% KNO_3) or 5% potassium citrate alone and in combination with fluoride in reference to erosion. They found that the presence of fluoride or desensitizing agent in pastes, appearing independently or conjointly, can reduce the enamel erosion, but used simultaneously occurrence did not appear any additive effect. Our data is consistent with the results, as we also did not notice any additive effect of potassium nitrate on the measurements of the enamel surface roughness.

The presented study was aimed to evaluate the influence of marketed fluoridated toothpastes on the surface profile of human enamel previously subjected to two commonly consumed soft drinks (orange juice and Pepsi Cola) for the short time. The tested toothpastes were Colgate Total Pro-Gum-Health (1450 ppm F), Duraphat 5000 (5000 ppm F), Sensodyne Pronamel (1450 ppm F, 5% KNO_3), MI Plus (CPP-ACP, 900 ppm F) and ElmexEro-

sionProtection (1400 ppm F, 3500 ppm Sn^{2+}). The surface roughness were measured by contact profilometer with use of Ra parameter at baseline, after exposing it to acidic soft drinks and followed by the immersion into the tested pastes slurry. The averaging Ra value for all enamel specimens ($n = 20$) exposed to orange juice was $0.017 \mu\text{m}$ and for Pepsi Cola ($n = 20$) $0.026 \mu\text{m}$. The immersion of the eroded specimens into slurry of all pastes with the exception of EnamelErosionProtection revealed the significant decrease in enamel roughness (Ra value), which could suggest deposition of calcium fluoride granules compensating the enamel surface profile. The registered falls in Ra value suggested also that the application of Colgate Total and Duraphat 5000 pastes not only restored the surface profile to the baseline, but also planished it. Contrarily, the application of ElmexErosionProtection paste did not level the resultant surface roughness after acidic agent exposure compared to the baseline and, moreover, made the surface roughness slightly greater. This could be the result, probably, of the different working mechanism of the toothpaste. According to an *in situ* study performed, this toothpaste promotes the formation of a protective stannous layer on dental surfaces and, in the course of erosive attack; insoluble stannous compounds are incorporated into the softened enamel [21].

It is known that the application of tin-containing fluoride solutions leads to deposits on the tooth surface that is relatively resistant to acids. Stannous fluoride (SnF_2) is known to be not only anticaries, but is also on erosion protection agent. Cooley [22] stated that tin (II) is deposited as a uniform coating layer on the dental surface, while fluoride ion penetrates the enamel. Jordan et al. [23] showed that stannous fluoride reacting with dental minerals formed a stannous fluorophosphates complex coating the dental surface. Recently, Baig et al. [24] comparing the ability of NaF and SnF_2 to the inhibition of powdered hydroxyapatite dissolution in buffered acidic solution, found the greater acid resistant of hydroxyapatite after the use of SnF_2 than NaF solutions and similar results after the immersion into slurries of marketed toothpastes containing those chemical compounds. In several studies simulating the oral condition, the efficacy of tin ions against erosion was assessed on enamel specimens coated by salivary pellicle [12, 25–29].

Dental pellicle is a film formed by selective adsorption of salivary protein and glycoprotein on the dental surface. It provides a natural protective coating against acid attack. In the absence of the pellicle, the dental surface directly contact with harmful acids.

Faller and Eversole [25] studied *in vitro* condition three marketed toothpastes containing alone

fluoride compounds and one with SnF₂. They found, based on the use of calcium-selective dye technique, significantly higher protection of enamel surface against acid challenge for SnF₂ product. SnF₂ deposited a barrier layer onto pellicle coated enamel surface which was different from any obtained by other fluoridated toothpastes. In another study Eversole et al. [26] using daily cycling demineralization-remineralization protocol, based on transverse microradiography results, presented that the toothpaste with stabilised SnF₂ provided significantly greater protection of the tooth from acidic challenge compared to other fluoridated toothpastes.

Faller et al. [25] in model mimicking the dynamics of the dental erosion process compared the protective benefit against acid attack of 12 marketed toothpastes. They observed the potential in erosion protection of the Sn-containing sodium fluoride toothpaste that was not significantly lower than stabilised SnF₂ paste but was significantly better in comparison to other toothpastes formulations. The randomized *in situ* study comparing the

anti-erosive efficacy of Sn-containing sodium fluoride toothpaste with a sodium fluoride and potassium nitrate toothpaste showed the lower enamel loss after use of the stabilised Sn-containing sodium fluoride toothpaste, which was measured by contact profilometry [28]. Also Bellamy et al. [29] in contact profilometry readings using modified model *in situ* confirmed the greater erosion protection benefit of toothpaste with stabilised SnF₂ compared to sodium fluoride toothpaste.

Contrary to the mentioned above results, our data showed that the fluoridated toothpaste with SnF₂ did not change the surface profil of the enamel exposed to acid attack. Probably it results from the difference of study design. We did not produced coating of enamel slabs by salivary pellicle, which seemed to be an essential for formation of stannous-layer giving protection against the next acid attack. Moreover, it may be suggested that the formed layer is a thin enough to reflect the previous surface roughness or is irregularly distributed making the enamel surface more rough.

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