Comparative Study Regarding the Effect of Remineralizing Products on Primary Teeth Dissolution Induced by Acidic Drinks

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The aims of this study were to investigate the surface topography and to compare the calcium and phosphorus ions concentration in primary teeth enamel when two commercial remineralizing products were used before the contact with two acidic drinks. Thirty-five caries-free enamel samples were randomly assigned to seven groups (1-7). In group 1 the slices has been stored in distilled water (control group). In groups 2 and 3 the slices have been immersed four times a day, 5 min each, for fourteen days, in Pepsi®(PepsiCo) and lemon juice, respectively. Between demineralizing cycles, the samples have been stored in artificial saliva. In groups 4 and 5, before immersion in acidic beverages, a commercial fluoride gel (PreviDent® brush-on gel, Colgate®) was applied for 3 min In groups 6 and 7, before immersion in acidic beverages, an ACP-CPP cream (MI Paste Plus, GC Corporation*) was applied for 3 min. The samples were analyzed using a scanning electron microscope and an EDX detector. The mean calcium and phosphorus ion concentration in enamel significantly decreased after the immersion in both tested beverages (mean calcium ion concentration (wt%): 25.45 in control group, 16.47 in lemon juice, 20.24 in Pepsi®; mean phosphorus ion concentration (wt%) was: 10.45 in control group, 8.77 in lemon juice, 9.12 in Pepsi®. The decrease of mineral ion concentrations in enamel was significantly lower when both remineralizing products have been used before the immersion in acidic drinks (p=<0,05, ANOVA and Bonferroni test). In the conditions of this study, both remineralizing products offered to primary enamel a protective effect on acidic challenge of the tested drinks.*

Keywords: enamel, acidic beverages, SEM, calcium ions, phosphorus ions

Many studies indicated a direct correlation between acidic beverages intake and the onset and progression of dental erosion [1-3]. The excessive consume of these beverages is one of the most important external factors for dental chemical dissolution [4]. In the past few years a dramatic increase of acidic beverages and fruit juices consumption was recorded in young patients. Some studies showed that in USA the consumption of such beverages raised by 300 times in the last 20 years, while the consumption quantities increased from 185 g in 1950-1960, to 240 g in 1960-1970 and over 500 g in 1990-2000 [5]. The prevalence of dental erosion is ranging from 30%[5] to 68% [6], especially among children and adolescents [7].

In children erosion could be associated with some clinical problems: eating and aesthetic disorder, dental hypersensitivity, altered occlusion, pulp exposure and abscesses [8,9]. Different commercial dental products on the market have the potential to remineralize the tooth structure affected by the acidic aggression. Regarding the effect of topical fluoride products, some studies showed no or limited protection of the enamel [10-12]. On the other hand, some results found a complete protection [13].

The aims of this study were to investigate the surface topography and to compare the calcium and phosphorus ion concentration in primary teeth enamel when two commercial remineralizing products were used before the contact with two acidic drinks.

Experimental part

In this study seventeen primary extracted molars have been chosen. Enamel samples were obtained by cutting the teeth from buccal and lingual surfaces using diamond discs (Komet Dental, Brasseler GmbH&Co, Germany), under watercooling. The thirty-five caries-free enamel samples were randomly assigned to seven groups (1-7). In group 1 the slices have been stored in distilled water (control group). In groups 2 and 3 the slices have been immersed four times a day, 5 minutes each, for fourteen days, in Pepsi® (PepsiCo) and Nestea Lemon (Coca Cola & Nestle Company), respectively. At room temperature, in separate containers, 15 mL of each acidic beverage were used for each sample. Between the demineralizing cycles, the samples have been stored in artificial saliva (AFNOR NF S90-701). In groups 4 and 5, before immersion in acidic beverages, a commercial fluoride gel (PreviDent® brush-on gel, Colgate®) was applied for 3 min. In groups 6 and 7, before immersion in acidic beverages, an ACP-CPP cream (MI Paste Plus, GC Corporation) was applied for 3 min. All the samples were then washed with distilled water. The surface topography has been analyzed using a scanning electron microscope VEGA II LSH (TESCAN, Czech Republic) and the quantitative and qualitative chemical composition has been evaluated using an EDX detector (QUANTAX QX2, BRUKER/ROENTEC, Germany).

Results and discussions

The surfaces topography of primary enamel after SEM evaluation of some samples after their immersion in the tested beverages and when PreviDent® and MI Paste plus were applied before immersion are presented in figure1. All the samples in groups 2 and 3 presented aspects of irregular enamel erosion (fig. 1 - 1a, 2a). For the samples in groups 4 and 5, rare areas of dissolution were observed (fig.1 - 1b, 2b). In groups 6 and 7, very rare and small areas of demineralization were present. The samples immersed in Pepsi and in lemon juice seem to present comparable demineralization pattern.

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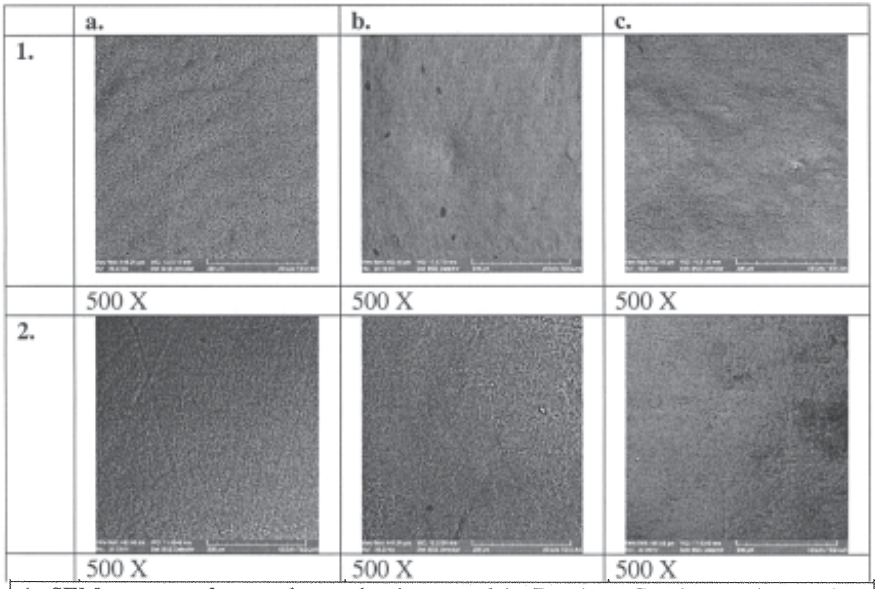

Fig.1. Enamel SEM aspects after continuous immersion in the tested solutions and alternative immersion in the tested solutions and saliva

1. SEM aspects of enamel samples immersed in Pepsi. a. Continuous immersion (group 2); b. PreviDent® application before immersion (group 4); c. MI Paste Plus application before immersion(group 6)
2. SEM aspects of enamel samples immersed in Nestea Lemon a. Continuous immersion (group 3); b. PreviDent® application before immersion (group 5); c. MI Paste Plus application before immersion (group 7)

The qualitative chemical analysis of the enamel showed that the highest concentration of enamel ions was represented by calcium and phosphorus ions. For that reason, only calcium and phosphorus ions were reported as a result of enamel samples quantitative chemical analysis. The mean values of calcium and phosphorus ions in enamel, expressed as weight percents (wt%), are presented in table 1.

A decreasing tendency of calcium and phosphorus ion concentrations was recorded in groups 2-7 when compared to group 1. The highest differences of calcium ions concentration in enamel were recorded in group 2, when the mean of calcium ion concentration of 25.45% in control group decreased to 16.47% after immersion in lemon juice (table 1). Pepsi beverage also decreased the mean calcium ion concentration in primary enamel when compared to control group (table 1). The lowest variation of calcium ion concentration was recorded in group 7, when MI Paste Plus was applied before immersion in Pepsi beverage (table 1). The highest differences of phosphorous ion concentration in enamel were also recorded in group 2, when the samples were immersed in lemon juice (from a mean of 10.45% in control group to 8.77% after immersion). In group 7 the lowest variation of phosphorous ion concentration was recorded (from 10.45 to 10.23%).

The data were statistically analyzed using ANOVA and post-hoc Bonferroni tests, with a 95% confidence interval, and *p* value 0,05. Statistically significant differences were recorded when the mean calcium and phosphorous concentration was compared in the groups where the samples were immersed in lemon juice and Pepsi beverage to control group. When both remineralizing products were used before immersion in acidic beverages, no statistically significant differences were obtained when compared to control group, but significant differences were obtained when compared to groups where the samples were immersed in both acidic beverages.

Some previous studies demonstrated similar effects of the tested acidic beverages on permanent teeth enamel [14–16]. Data in the literature showed that the progression of dental erosion is higher in primary teeth than in permanent ones [17–21] due to the fact that primary enamel is more porous [22,23], have less phosphorous [24] and calcium phosphate ions [17] in composition and more carbon dioxide and carbonate [18, 21]. Also, in primary enamel the microcrystals are less organized [23], the enamel has a greater diffusion coefficient [24] and presents a superficial aprismatic enamel layer [25]. In our study the extern, aprismatic enamel layer was not removed in order to reproduce the conditions in the oral cavity. As a result, because this layer is less permeable than the underlying enamel, the action of the acidic beverages could be influenced. Intact tooth surfaces have been shown to soften at slower rates than ground tooth surfaces, being less soluble as well [26]. In this study the scanning electron microscope evaluation of the primary enamel surface after the samples immersion in different acidic beverages showed enamel prism demineralization. In other studies a gradual mineral loss has also been reported after exposing primary teeth enamel to cola-type soft drink [27].

One of the major factors that can directly influence the dissollution rate of dental enamel. is the *p*H of beverages.

Table 1
MEAN VALUES OF ENAMEL CALCIUM AND PHOSPHORUS IONS CONCENTRATIONS (WT%) ± SD FOR CONTROL AND STUDY GROUPS

Ions concentration (wt%)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Calcium	25.45±0.03	16.47±0.07*	21.24±0.04*	22.56±0.11**	23.63±0.08**	23.44±0.12**	24.58±0.05**
Phosphorous	10.45±0.12	8,77±0.05*	8.92±0.05*	9.53±0.09**	9.87±0.04**	10.05±0.07**	10.23±0.06**

* Statistically significant result when compared to control group (ANOVA and post-hoc Bonferroni, $p<0.05$)
**No statistically significant result between groups (ANOVA and post-hoc Bonferroni, $p>0.05$)

A low level of saturation related to enamel leads to an initial surface demineralization, followed by an increase of *p*H and an increase of mineral content in the liquid localised nearby dental surface. This surface layer will become saturated when compared to enamel and will not produce further demineralisation. In present study the highest loss of calcium and phosphorus ions in enamel was obtained after the samples immersion in lemon juice, the acidic solution with the lowest *p*H (*p*H 2,4). The erosive potential of the beverage could also be influenced by the acid type. Phosphoric acid and citric acid are the most frequently used agents in carbonated beverages. Both acids are triprotic and can release up to three hydrogen ions in solution, while phosphate and citrate can sequester calcium ions [28]. This kind of acids are capable to cause dissolution even at high pH levels [29]. Studies have shown that up to 32% from salivary calcium ions can be complexed by citric acid. The result would be an increase of dental minerals dissollution [30]. Previous studies demonstrated that at similar levels of acidity citric acid can produce more pronounced erosions than phosphoric acid [31].

As remineralizing commercial products in this study were used an ACP-CPP cream and a fluoride gel. MI Paste Plus contains amorphous calcium phosphate (CPP-ACP) stabilised by a phosphopeptide. Previous studies demonstrated a protective effect of the product against acidic beverages [32,33] due to its capacity to inhibit erosive enamel loss [34,35]. The preventive effects of fluoride against dental erosion are related to the formation of precipitates on the tooth surface, which acts as a protective barrier against acid challenge [36,37]. Unfortunatly this fluoride layer has a big disadvantage. Into acidic environments the fluoride layer is rapidly dissolved [36].

Conclusions

Beverages like Pepsi and lemon juice have an erosive effect on primary enamel surface. The calcium and phosphorus ions concentrations significantly decrease after immersion in both acidic beverages. Application of ACP-CPP cream and fluoride gel before erosive challenge provides an efficient protection to primary enamel.

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