Comparative Study Regarding the Impact of Saliva on Chemical Disolution of Enamel Induced by Various Acidic Beverages

COSMIN ARNAUTEANU[1]* SIMONA STOLERIU[1], GIANINA IOVAN[1], ANDREI VICTOR SANDU[2], ALEXANDRU ANDREI ILIESCU[3], SORIN ANDRIAN[1]

[1] „Gr.T.Popa" University of Medicine and Pharmacy, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iaşi, Romania
[2] „Gh.Asachi" Technical University, Materials Science and Engineering Faculty, 53A Dimitrie Mangeron Str., 700050, Iaşi, Romania
[3] University of Medicine and Pharmacy, Faculty of Dental Medicine, 2-4 Petru Rares Str., 200349 Craiova, Romania

The aims of this study were to investigate the surface topography and to compare the calcium and phosphorus ions concentration of enamel following the contact with five acidic drinks in the presence or absence of saliva. 25 caries free extracted teeth were used in this study. All the teeth were longitudinally sectioned in three slices. One slice had been stored in distilled water (control group). The second slice has been continuously immersed for 12 h in one of the tested beverages: Red Bull, Lipton Green Tea, a commercial apple juice, a natural carbonated mineral water and lemon juice. The third slice has been subject to 3 cycles of immersion in one of the tested beverages for 1 minute, followed by storage in artificial saliva (AFNOR NF S90-701) for 4 h. The samples were analyzed using a scanning electron microscope and an EDX detector. The specimens continuously stored in acidic beverages showed severe erosion of enamel. The calcium and phosphorus ions concentration in enamel significantly decreased following continuous storage in all the tested beverages (mean calcium ion concentration (wt%)): 32.65 in control group, 30,13 in apple juice, 30.39 in Lipton Green Tea, 29.58 in natural carbonated mineral water, 18.67 in lemon juice, 23.13 in Red Bull; mean phosphorus ion concentration (wt%) was: 12.87 in control group, 12.20 in apple juice, 12.24 in Lipton Green Tea, 12.82 in natural carbonated mineral water, 9.90 in lemon juice, 10.25 in Red Bull. The decrease of mineral ions concentrations in enamel was significantly lower when saliva has been used as a storage medium between immersions in acidic beverages ($p=<0,05$, ANOVA and Bonferroni test). In the conditions of this study, saliva offered to enamel a protective effect on acidic challenge of tested beverages.

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

Last years a dramatic increase of acidic beverages and fruit juices was recorded both for young patients and adults. In USA the consume of such beverages raised by 300 times in last 20 years, while consumed quantities increased from 185 g in 1950-1960, to 240 g in 1960-1970 and over 500 g in 1990-2000 [1]. Many epidemiologic studies showed a direct correlation between the consume of acidic beverages and fruit juices and the apparition of dental tissues demineralisations [2-6]. The excessive consume represents the most important extrinsec factor implied in the initiation of dental erosions (chemical disolution) [7]. Most of comercial drinks present erosive potential against dental tissues [8,9]. The *p*H value of acid beverages, buffering capacity, calcium, phosphats and fluor content are factors that determine the saturation related to minerals concentration in dental tissues and are responsible for the initiation of disolution processes [10].

Any solid substrate exposed to oral environment is rapidly covered by acquired pellicle, a bacteria-free biofilm [11], composed of calcium-binding proteins [12]. The physiological roles of the acquired pellicle are lubrefiation and protection [13].

The protective level of acquired pellicle is determined by some elements as follows: composition, thickness and maturation time. The distribution way of the dissolution processes is influenced by the variation of thickness. The areas with a higher thickness of the acquired pellicle present a lower rate of demineralisation [14]. The acquired pellicle can also store the remineralisation electrolits with direct effects regarding the apparition and progression of demineralisation. In vitro studies demonstrated that salivary mucins increase the protection of enamel surface against demineralisation [15]. Saliva and its components represent a physiological response of the host to occasional and mild episodes of acidic attack inside oral cavity. The pathological consequences will not appear as long as the acidic attack will not exceed some levels of force and frequence and the host will have a proper reaction. If acidic aggresion will exceed a certain limit, the normal parameters of saliva will not be sufficient to protect the teeth.

Experimental part

25 caries free extracted teeth were used in this study. The teeth presented unaffected surfaces and were extracted from orthodontic or periodontal reasons. After extraction teeth were stored in distilled water. The teeth were divided in five study groups. All the teeth were longitudinally sectioned in three slices with diamond discs (Komet Dental, Brasseler GmbH&Co, Germany), under watercooling. One slice had been stored in distilled water (control group). The second slice has been continuously immersed for 12 h in one of the tested beverages: Red Bull, Lipton Ice Green Tea, a commercial apple juice (Auchan apple juice), a natural carbonated mineral water (Borsec mineral water) and natural lemon juice. The composition of these beverages is presented in table 1. The third slice has been subject to 3 cycles of immersion in one of the tested beverages for 1 min, followed by storage in artificial saliva (AFNOR NF S90-701) for 4 h. The samples were then washed with distilled water and analyzed using a scanning electron microscope VEGA II LSH (TESCAN, Czech Republic) to analyse the surface topography and an EDX detector QUANTAX QX2 (BRUKER/ROENTEC, Germany) to evaluate quantitative and qualitative chemical composition.

* *email: cosmin.arnauteanu@yahoo.com*

Beverage	Composition
Red Bull	coffeine, taurine, glucuronolactone, B vitamins, glucose, sucrose
Lipton Ice tea green	coffeine, tea (green tea extract), sucrose, citric acid
Mineral water Borsec	calcium 325,9mg/l, magnesium 113.4 mg/l, Na 72 mg/l, HCO_3^- 1634 mg/l
Apple juice Auchan	apples concentrate juice

Table 1
COMPOSITION OF THE BEVERAGES USED IN STUDY GROUPS

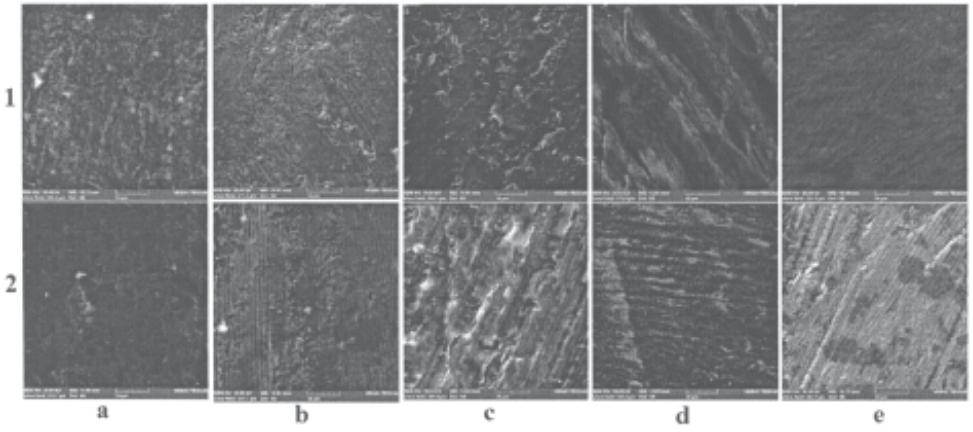

Fig. 1. Enamel SEM aspects after continuous immersion (1) in the tested solutions and alternative immersion (2) in the tested solutions and saliva (1000X): a – apple juice, b – green tea, c – mineral water, d – lemon juice, e – Red Bull

Results and discussions

The figures 1.a-e. present the surface topography of enamel analyzed using SEM for control samples, for samples in continuous immersion in the five tested solutions and for samples in alternative immersion in saliva and beverages.

All enamel samples immersed continuously in the tested solutions presented severe erosions of enamel (pinched aspect) (fig. 1- 1a, 1b, 1c,1d). For the samples that were immersed alternatively in saliva and in tested solutions and which presented the acquired pellicle formed on the surface, it was observed a layer similar with a biofilm covering intact enamel surface, while adjacent enamel had an eroded aspect (fig.1-2a,2b,2c). The severity of demineralisations was lower for these samples comparing with samples that were continuously immersed in the tested solutions.

The qualitative chemical analysis of enamel showed high concentrations of calcium and phosphorus ions. In this context, only calcium and phosphorus ions were related to quantitative chemical analysis of enamel samples. The mean values of calcium and phosphorus ions in enamel, expressed as weight percents (wt%), are presented in table 2.

It was recorded a decreasing tendency of calcium and phosphorus ions concentrations after continuous immersion in the five acidic solutions. The highest differences of calcium ions concentration in enamel were recorded for samples immersed in lemon juice, when the mean of calcium ions concentration of 29.09% in control group, decreased to 18.67% after immersion (table 2). The solution Red Bull produced a decreasing of calcium ions concentration from 32.09 % in control group to 23.13 % after immersion (table 2). The lowest variation of calcium ions concentration was recorded for Lipton ice tea (from 31.845% in control group to 30.39% after immersion) (table 2). For samples in alternative immersion in acidic solutions and saliva, the calcium and phosphorus ions concentrations decreased comparing with control group (table 2). For all five tested acidic solutions, the mean value concentration of calcium and phosphorus ions were closer to the values of control group for samples from the study

	Apple juice		Green tea		Mineral water		Lemon juice		Red Bull	
Ions concentration (wt%)	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Control group	32.65	12.67	31.84	13.56	32.65	13.58	29.09	12.67	32.09	13.20
Study group-continuous immersion in solution	30.13	12.20	30.39	12.24	29.58	12.82	18.67	9.90	23.13	10.25
Study group-alternative immersion in solution and saliva	31.25	12.51	31.69	12.83	31.07	12.20	28.91	11.14	31.69	12.76

Table 2
MEAN VALUES OF ENAMEL CALCIUM AND PHOSPHORUS IONS CONCENTRATIONS (WT%) FOR THE CONTROL AND STUDY GROUPS

	The values p for Bonferroni test performed in order to compare the calcium ions concentrations														
	Apple juice			Green tea			Mineral water			Lemon juice			Red Bull		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Control group (1)	-	.000	.034	-	.000	.000	-	.000	.000	-	.000	.000	-	.009	.009
SG - continuous immersion in solution (2)	.000	-	.038	.000	-	.000	.000	-	.000	.000	-	.000	.009	-	.009
SG-alternative immersion in solution and saliva (3)	.034	.038	-	.000	.000	-	.000	.000	-	.000	.000	-	.009	.009	-

Table 3
RESULTS OF BONFERRONI TEST PERFORMED IN ORDER TO COMPARE THE CONCENTRATIONS OF CALCIUM IONS IN THE STUDY GROUPS (SG)

	The values p for Bonferroni test performed in order to compare the phosphorus ions concentrations														
	Apple juice			Green tea			Mineral water			Lemon juice			Red Bull		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Control group (1)	-	.000	.000	-	.000	.000	-	.000	.000	-	.000	.000	-	.009	.009
SG - continuous immersion in solution (2)	.000	-	.000	.000	-	.000	.000	-	.000	.000	-	.000	.009	-	.009
SG- alternative immersion in solution and saliva (3)	.000	.000	-	.000	.000	-	.000	.000	-	.000	.000	-	.009	.009	-

Table 4
RESULTS OF BONFERRONI TEST PERFORMED IN ORDER TO COMPARE THE CONCENTRATIONS OF PHOSPHORUS IONS IN THE STUDY GROUPS (SG)

group with alternative immersion in saliva and acidic solutions comparing with samples from the study group with continuous immersion in acidic solutions.

The data were statistically analysed using tests ANOVA and post-hoc Bonferroni, with a 95% confidence interval, and *p* value 0.05. It were recorded statistically significant differences between the two study groups and between control group and study groups, when compared the concentrations values of calcium and phosphorus ions in enamel (table 3 and 4).

The oral fluids and their components represent a relevant biological factor for dental chemical disolution. The characteristics of fluid oral and factors like dental structure and erosive agents, influence both apparition and evolution as well as the prevention and arresting of erosive lesions. The acquired pellicle can protect against demineralisation, acting like a diffusion barrier or like a membrane with selective permeability, avoiding direct contact between acidic substances and dental surfaces. In this way they intervene in the decreasing of the hydroxyapatite solubility rate.

Our study proved the existance of mineral loss (calcium and phosphorus ions) in enamel and acidic solutions, both in the presence and absence of salivary acquired pellicle. However the mineral loss was lower for samples covered with salivary acquired pellicle. The protective effect of salivary pellicle was demonstrated by Xiaojie et al. In their study, the application of artificial saliva for minimum 4 h , conducted to a partial mild recovery of local structure of hydroxyapatite cristals in the eroded enamel. However, the destructive processes were not stopped by the presence of saliva in the enamel submitted to acidic challenge of citric acid [16]. The same tendency of mineral loss was recorded by Wiegand et al., but in this study the protective effect of acquired pellicle was two times more efficient at the enamel level comparing with dentine areas [17].

In some studies, the artificial salivary acquired pellicle offered protection on short-term when citric acid 0.1% or 1% was used to induce erosive changes [18]. The authors demonstrated that salivary pellicle was able to significantly inhibit the demineralisation of enamel only if the immersion in acidic solution was limited to 1 min, or maximum five minutes. In the case of samples immersed for 10 min in citric acid the protective effect was unsignificant. This study demonstrated that chemical disolution of dental tissues can be accelerated by powerful acidic atacks or by salivary disfunctions.

The *p*H of beverages can directly influence the disollution rate of dental enamel. A low level of saturation related to enamel or dentine conducts to initial surface demineralisation, followed by a local increase of *p*H and an increase of mineral content in the surface liquid localised nearby dental surface. This surface layer will become saturated related to enamel (or dentine) and will not produce further demineralisation. In our study the highest loss of minerals (calcium and phosphorus ions) was obtained after continuous immersion in lemon juice, the acidic solution with lowest *p*H (*p*H 2.4).

The acid type contained by beverages may influence the erosive potential. The phosphoric acid and citric acid are agents frequently present in carbonated and energising beverages. Both phosphoric acid and citric acid are triprotic acids that can release up to three hydrogen ions in solution, while phosphate and citrate can sequester calcium ions [8]. The acids that can sequester calcium ions can cause dissolution even at high *p*H levels [19]. Up to 32% from salivary calcium ions can be complexed by citric acid from fruit juices, producing an increase of dental minerals disollution [20]. Some in vitro experimental studies demonstrated that citric acid can produce more pronounced erosions than phosphoric acid at similar levels of acidity [21].

The calcium and phoshporus content of food and drinks are important factors that influence concentration gradient in the local environment related to the dental surfaces [11]. The addition of calcium and phosphat salts in acidic beverages conducted to promising results. Larsen suggested that erosive potential of a beverage can be calculated accordingly to the saturation degree related to hydroxyapatite and fluoroapatite, by the determination of *p*H, calcium content, phosphat content and fluor content [22]. A minor change of saturation degree by the addition of calcium (and low quantity of phosphat), without *p*H change, can reduce the erosive potential in vitro [23]. These data can explain the reduced erosive effects of mineral water with low *p*H (*p*H 3.2) taken in our study. The minerals addition can slow down the progression of erosive processes, with important clinical implication both for patient and practitioner. Some mineral waters present a more pronounced erosive potential than pure mineral waters. This fact can have important implications because there are no written warnings related to the erosive potential of some acidic beverages presented as mineral waters. A citric acid 1% solution (*p*H 2,2) supplemented with different concentrations of calcium, phosphat and/or fluor can also reduce the erosive potential [24]. In the case of acidic beverages modified with calcium, phosphat and/or fluor, the most efficient reduction of enamel erosion was obtained for citric acid both by the addition of 1mmol/L calcium and by the addition of combination of calcium 0.05 mmol/L with phosphat 0.5 mmol/L and fluor 0.031 mmol/L [25].

Regarding the surface topography, the aspects presented by our study were similar with other studies that recorded severe erosions of enamel unprotected by salivary acquired pellicle [26, 27]. In the presence of saliva, reduced erosions

were recorded, only in the areas related to the prism sheath areas [20].

Conclusions

The calcium and phosphorus ions concentrations decreased after continuous immersion in the tested solutions. The most aggresive solutions is lemon juice, followed by Red Bull, mineral water, apple juice and green tea. The presence of salivary acquired pellicle offered enamel protection against aggresive effect of acidic beverages.

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