In vitro Evaluation of Acidic Beverages Effect in Dentine and Cement, with and without Storage in Artificial Saliva

SIMONA STOLERIU[1]*, GIANINA IOVAN[1], GALINA PANCU[1], ANDREI GEORGESCU[1], ANDREI VICTOR SANDU[2], SORIN ANDRIAN[1]

[1] „Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iaşi, Romania

[2] „Gheorghe Asachi" Technical University of Iasi, Faculty of Materials Science and Engineering, 53A D. Mangeron Blvd., 700050, Iaşi, Romania

The aim of this study was to investigate the surface topography and to compare the calcium and phosphorus ions concentration in dentine and cement following their 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 has 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 min, 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 enlarged dentinal tubules. The specimens successively stored in acidic beverages and saliva showed a slightly eroded surface of dentine and cement. The calcium and phosphorus ions concentration in dentine and cement significantly decreased following continuous storage in all the tested beverages (mean calcium ion concentration (wt%) in dentine (D)/ cement (C): 28.17/23.93 control group, 16.45/11.07 apple juice, 22.84/11.48 Lipton Green Tea, 24.01/21.46 natural carbonated mineral water, 15.06/9.22 lemon juice, 18.09/10.52 Red Bull; mean phosphorus ion concentration (wt%) in D/C: 10.80/ 9.92 control group, 7.84/6.55 apple juice, 8.70/6.98 Lipton Green Tea, 9.39/9.21 natural carbonated mineral water, 7.45/6.28 lemon juice, 7.85/6.79 Red Bull). The decrease of mineral ions concentrations in dentine and cement was significantly lower when saliva has been used as a storage medium between immersions in acidic beverages ($p < 0.05$, ANOVA and Bonferroni test). Under the conditions of this study, saliva offered to dentine and cement a protective effect against the acidic challenge of tested beverages.

Keywords: dentine, cement, acidic beverages, SEM, calcium ions, phosphorus ions

It is well known that over the last few decades the consumption of acid beverages has increased wordwide. Related to that, some studies reported an increase of dental erosion [1, 2]. Erosion is the chemical dissolution of dental hard tissues by acids that are not produced by oral bacteria [3]. Some of the chemical characteristics of the beverages can influence the potential to induce dental erosions: type of acid [4], buffer capacity [5], *p*H [6], titratable acidity [7], chelating properties [7], and concentration of calcium, phosphates and fluoride [8]. The *p*H value and the calcium, phosphate and fluoride content of the beverage influence the level of solution saturation comparing to the minerals content from enamel, dentine and cement, which represent the driving force for chemical dissolution. The solutions that are undersaturated when comparing to dental hard tissues are capable to induce an initial demineralization of the dental surface. After that, the local *p*H increases and also the mineral content in the liquid nearby the dental surface. This liquid layer will become oversaturated when comparing to enamel, dentine and cement and consequently will not induce future demineralization.

Oral fluid is an important biological factor that can influence the appearance and the progression of dental erosion [9-15]. The salivary acquired pellicle can protect the tooth structure against demineralisation, acting like a diffusion barrier or like a membrane with selective permeability, avoiding direct contact between acidic substances and dental surfaces [16-20]. In this way it is possible to decrease the solubility of hydroxyapatite crystals. Saliva also presents buffering capacity causing neutralization of dietary acids.

The aims of this study were to investigate the surface topography and to compare the calcium and phosphorus ions concentration of dentine and cement following the contact with five acidic drinks in the presence or absence of saliva.

Experimental part

25 caries free extracted teeth were used in this study. The teeth were extracted for periodontal reasons. All the teeth were selected after the examination of the cervical area using an optical microscope (Nikon Eclipse E 600, Nikon, Japan) at a 10X magnification in order to have a free dentine layer at the junction between enamel and cement. The teeth were stored in distilled water until the start of the study. The teeth were randomly divided in five study groups. All the teeth were longitudinally sectioned in three slices with diamond discs (Germany), under watercooling. All the slices were covered with an acid resistant varnish (France) except a 5mm × 5 mm area at the enamel-dentine junction. From each tooth one slice

* email: stoleriu_simona@yahoo.com

has 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, a natural carbonated mineral water (Romania), and natural lemon juice. 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 composition and the *p*H of these beverages are presented in table 1. The samples were then washed with distilled water and observed using a scanning electron microscope VEGA II LSH (Czech Republic) to analyze the surface topography and an EDX detector QUANTAX QX2 (Germany) to evaluate the quantitative chemical composition. For each sample five determinations of dentine chemical composition and five determinations of cement chemical composition were performed and the mean values of these determinations were recorded. The data were statistically analysed using ANOVA and post-hoc Bonferroni tests, with a 95% confidence interval, and *p* value 0.05.

Results and discussions

All dentine samples continuously immersed in the tested solutions showed different degrees of demineralization (fig. 1.A/a-e). Lemon juice caused a high demineralization of the inorganic part of the dentine and exposure of the collagen matrix (fig. 1.A/d). After the immersion in Red Bull, the dentine samples also showed the matrix exposure after the removal of mineral content, but the collagen network was less visible when compared to the samples immersed in lemon juice (fig. 1.A/a). Lipton Ice Green Tea and apple juice caused high enlargement of the dentinal tubules as a result of the demineralization (fig. 1.A/b and fig. 1.A/e). For the samples that were immersed alternatively in saliva and in tested solutions the severity of demineralisations was lower when compared with the samples exposed to a continuous immersion in the tested solutions (fig. 1.B/a-e).

Continuous immersion in lemon juice also caused the highest demineralization of the cement samples. After the removal of inorganic part, the collagen matrix was exposed (fig. 2.a). The cement samples after the alternative immersion in saliva and lemon juice showed only irregularities of the cement surface, without the collagen matrix exposure, as a result of a less important demineralization (fig. 2.b).

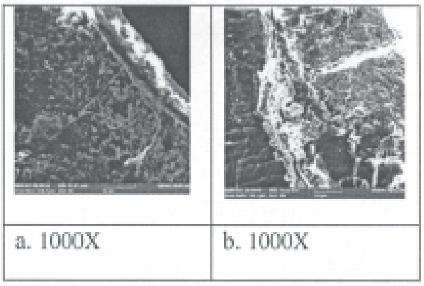

Fig. 2. SEM aspects of the cement after continuous immersion (a) and alternative immersion (b) in lemon juice

The qualitative chemical analysis of the dentine and cement samples showed the predominance of calcium and phosphorus ions. For this reason only calcium and phosphorus ions concentration was recorded for quantitative chemical analysis of the samples. The mean

Table 1
THE COMPOSITION AND pH OF THE BEVERAGES USED IN STUDY GROUPS

Beverage	Company	Composition	pH
Red Bull	Red Bull GmbH	coffeine, taurine, glucuronolactone, B vitamins, glucose, sucrose	2.8
Lipton Ice Green Tea	Lipton	coffeine, tea (green tea extract), sucrose, citric acid	3.0
Mineral water	Borsec	calcium 325,9mg/L, magnesium 113,4 mg/L, Na 72 mg/L, HCO_3^- 1634 mg/L	3.2
Apple juice	Auchan	apple concentrated juice	3.4
Natural lemon juice			2.4

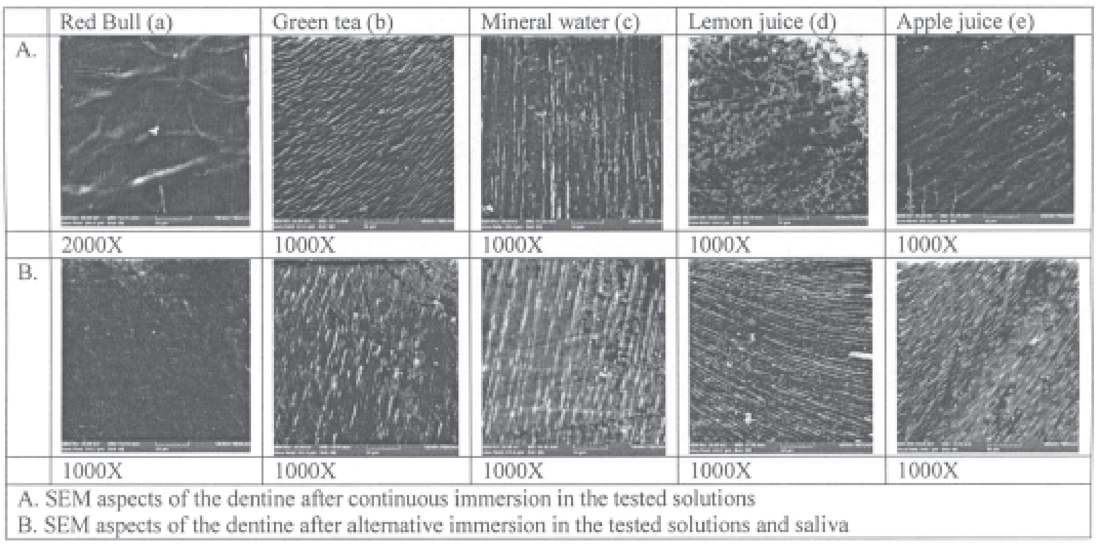

Fig. 1. SEM aspects of the dentine after treatments: A/a-e. Continuous immersion in the tested solutions; B/a-e. Alternative immersion in the tested solutions and saliva

The mean calcium (Ca) and phosphorus (P) ions concentrations in dentine and cement (wt%)										
Dentine										
	Lemon juice		Red Bull		Apple juice		Lipton Tea		Mineral water	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
(1)	28.58	10.54	27.65	10.21	28.17	10.80	28.17	10.76	28.51	11.32
(2)	15.06	7.45	18.09	7.85	16.45	7.84	22.84	8.70	24.01	9.39
(3)	27.96	10.35	26.22	9.65	27.57	10.48	28.16	10.48	27.17	10.80
Cement										
	Lemon juice		Red Bull		Lipton Tea		Apple juice		Mineral water	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
(1)	23.93	9.99	22.31	9.55	24.89	9.72	23.93	9.92	28.07	10.10
(2)	9.22	6.28	10.52	6.79	11.48	6.98	11.07	6.55	21.46	9.21
(3)	22.87	9.20	22.01	8.65	22.66	9.68	19.99	9.75	27.11	10.00
(1) Control group; (2) Group with continuous immersion in acidic beverages; (3) Group with immersion in acidic beverages followed by storage in artificial saliva										

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

values of calcium and phosphorus ions in dentine and cement, expressed as weight percents (wt%), are presented in table 2.

A decreasing tendency of the calcium and phosphorus ions concentrations was recorded after continuous immersion in the five acidic solutions. The highest decrease of calcium ions concentration in dentine was recorded for the samples immersed in lemon juice (from a mean concentration of 28.58% in control group to a mean concentration of 15.06% after immersion) (table 2). The lowest variation of calcium ions mean concentration in dentine was recorded for mineral water (from 28.51% in control group to 24.01% after immersion) (table 2). For the samples with alternative immersion in acidic solutions and saliva, the calcium and phosphorus ions concentrations also decreased when compared to control group (table 2). For all five tested acidic solutions, the mean calcium ions concentration in dentine was higher for the samples in the study group with alternative immersion in saliva and acidic solutions when compared to the samples from the study group with continuous immersion in acidic solutions (table 2).

The cement samples continuously immersed in lemon juice had the lowest values of calcium ions concentration when compared to control group (from 23.93% in control group to 9.22% after immersion) (table 2). Like in dentine samples, the lowest variation of calcium ions mean concentration in cement after continuous immersion in the five tested beverages was recorded for mineral water (from 28.07% in control group to 27.11% after immersion).

The mean phosphorus ions concentrations in dentine and cement decreased after the continuous or alternative immersion in the five acidic drinks. Lemon juice leaded to the highest decrease of the phosphorus ions mean concentration in the group with continuous immersion when comparing to control group (in dentine samples from 10.54% in control group to 7.45% in the group with continuous immersion and in cement samples from 9.99% in control group to 6.28% in the group with continuous immersion) (table 2). The same tendency of variation of the phosphorus ions concentrations in dentine and cement was recorded even in the groups with alternative immersion in saliva and acidic solutions when compared to control group (table 2). The mean values of phosphorus ions concentrations in dentine and cement were lower in the groups with a continuous immersion in the acidic drinks when compared to the groups with alternative immersion.

Statistically significant differences between the groups with continuous immersion in the tested beverages and the control group were recorded when compared the mean calcium and phosphorus ions concentrations (table 3, 4). After alternative immersion in the tested beverages and saliva, no statistical differences were recorded when compared to control group. (table 3, 4).

All the five acidic drinks tested in this study caused different degrees of dentine and cement demineralization. The SEM aspects of the dentine after a continuous or alternative action of all five solutions have shown enlargement of the dentinal tubules and in some situations exposure of dentine matrix. For lemon juice and Red Bull beverage the organic matrix of the dentine was exposed as a result of dentine erosion. These findings support the mechanisms of erosion suggested by B. Klont and J.M. Ten Cate [21]. Previous studies have shown that demineralization of dentine first occurred at the interface between inter- and peritubular dentin and resulted in an enlargement of the tubules [22]. Finally, the peritubular dentine is completely dissolved. Similar aspects of dentine after the action of acidic solutions were reported in previous studies [23]. The SEM aspect of the samples with an alternative immersion in saliva and acidic drinks showed in our study only an enlargement of the dentinal tubules, without the exposure of collagen matrix. These aspects suggest that the presence of saliva between the periods of acid challenge offered a protective effect to dentine and cement.

The erosive effect of dietary acids on tooth tissue can be influenced by many factors including titratable acidity, type of acid, concentration, chelation potential, exposure time, and presence of sugar [24]. The *p*H of beverages can directly influence the dissolution of dental hard tissues. In our study the highest loss of minerals (calcium and phosphorus ions) in dentine and cement was obtained after continuous immersion in lemon juice, which was the acidic solution with lowest *p*H = 2,4. Red Bull also had a high erosive effect on dentine and cement and that in relation

Bonferroni test results (p values) when comparing the calcium ions concentrations in dentine and cement															
	Dentine														
	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)
(1)	-	.000	.000	-	.000	.000	-	.009	.009	-	.000	.000	-	.000	.000
(2)	.000	-	.000	.000	-	.000	.009	-	.009	.000	-	.000	.000	-	.000
(3)	.000	.000	-	.000	.000	-	.009	.009	-	.000	.000	-	.000	.000	-
	Cement														
	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)
(1)	-	.000	.040	-	.000	.040	-	.000	.040	-	.000	.070	-	.000	.060
(2)	.000	-	.000	.000	-	.000	.000	-	.000	.000	-	.000	.000	-	.000
(3)	.040	.000	-	.040	.000	-	.040	.000	-	.070	.000	-	.060	.000	-
(1) Control group; (2) Group with continuous immersion in acidic beverages; (3) Group with immersion in acidic beverages followed by storage in artificial saliva															

Table 3
BONFERRONI TEST RESULTS WHEN COMPARED THE CALCIUM IONS CONCENTRATIONS IN THE STUDY GROUPS AND CONTROL GROUP

Bonferroni test results (p values) when comparing the phosphorus ions concentrations in dentine and cement															
	Dentine														
	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)
(1)	-	.000	.002	-	.000	.002	-	.000	.000	-	.000	.000	-	.009	.009
(2)	.000	-	.000	.000	-	.000	.000	-	.000	.000	-	.000	.009	-	.009
(3)	.002	.000	-	.002	.000	-	.000	.000	-	.000	.000	-	.009	.009	-
	Cement														
	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)
(1)	-	.000	.000	-	.000	.000	-	.000	.008	-	.000	.000	-	.009	.009
(2)	.000	-	.000	.000	-	.000		-	.000	.000	-	.000	.009	-	.009
(3)	.000	.000	-	.000	.000	-	.008	.000	-	.000	.000	-	.009	.009	-
(1) Control group; (2) Group with continuous immersion in acidic beverages; (3) Group with immersion in acidic beverages followed by storage in artificial saliva															

Table 4
BONFERRONI TEST RESULTS WHEN COMPARED THE PHOSPHORUS IONS CONCENTRATIONS IN THE STUDY GROUPS AND CONTROL GROUP

with a very low *p*H = 2,8. These findings are supported by the results of other studies that suggested that energy drinks are often erosive to the tooth structure [25-27]. *p*H of the erosive agent is an important factor that can affect dentinal tubules opening [24, 28]. Substances with low pH can remove the smear layer and open the dentinal tubules [29-31]. Due to the fact that all five acidic beverages tested in this study had a low *p*H (less than 3.5), SEM aspects of the dentine samples showed an enlargement of dentinal tubules as a result of dentine demineralization.

The acid type of the beverages may influence the erosive potential [25, 32]. Citric acid is a frequent agent present in the beverages and it is one of the most erosive acids due to its chelating capacity, which is responsible for calcium sequestration from saliva and teeth. For this reason beverage having low *p*H and containing citric acid are considered to have the most erosive potential [33]. This could be another reason for the highest erosive potential of lemon juice found in our study. Beside the direct demineralization of the crystals in dentine and cement, citric acid has the capability to increase the progresion of erosive process by interacting with saliva. Up to 32% of calcium ions from saliva might be sequestrated by the citric acid and that can decrease the oversaturation of saliva and increase the driving force for mineral dissolution [18-20, 34].

In some studies, the artificial salivary acquired pellicle offered a short-term protection to dentine [35]. In contrast, Hara, et al. (2006) showed that an intraoral pellicle formed for 2 h was not effective in reducing dentin softening induced by an excessive acid challenge [15]. The higher porosity and solubility of dentin led to a faster demineralization, which might prevent the pellicle from acting as a protective barrier [15]. It has been also speculated that saliva penetrates the tubule system of dentin to produce not only a pellicle layer on the surface but a meniscus of viscous liquid in the tubule orifices [18-20, 36]. In our study the presence of saliva prevented the mineral (calcium and phosphate ions) loss in dentine and cement. Saliva also provided a protective effect on enamel dissolution against the action of the same acidic solutions [20, 37].

Conclusions

The calcium and phosphorus ions concentration in dentine and cement significantly decreased following the continuous immersion in all tested acidic beverages. For the five acidic drinks used in this study, the highest erosive effects on dentine and cement were recorded for lemon juice and Red Bull and the lowest erosive effect was recorded for mineral water. Saliva provided a protective effect on dentine and cement against aggressive challenge of acidic beverages.

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