Original article

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EVALUATION OF ENAMEL SURFACE ROUGHNESS AND MORPHOLOGICAL CHANGES AFTER EXPOSURE TO COCA-COLA, ORANGE AND ARTIFICIAL GASTRIC JUICE: AN *IN VITRO* STUDY

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Dental erosion is a pathologic, non-bacterial hard dental tissue loss induced by extrinsic or intrinsic acids. This *in vitro* study aimed to evaluate and compare the morphology and surface roughness of dental enamel after erosive challenge in some extrinsic and intrinsic acidic substances, Coca-Cola, orange and gastric juice.

Enamel samples (n = 48), obtained by preparation of surgical extracted human third molars, were subjected to the erosive challenge of the artificial gastric juice and commercially-available Coca-Cola and orange juice by immersion in 50 ml of erosive solutions for 15 min, three times daily, for 10 days. Between immersions, the samples were kept in filtered saliva. Twenty-four samples were prepared for the surface morphology analysis using scanning-electron microscope, and 24 for the analysis of Ra-surface roughness parameter (using a diamond-stylus-profilometer), including the 12 control samples (which did not undergo the erosion procedure). Results of the surface roughness were analyzed by one-way ANOVA Student-Newman-Keuls post hoc test.

Ultrastructural analysis of enamel surface after immersion in Coca-Cola and gastric juice showed type 1 etching pattern with the typical honeycomb appearance. After the erosive challenge with orange juice, a nonspecific morphological model was established. Profilometric parameter Ra was significantly increased for samples immersed in gastric juice compared to samples immersed in Coca-Cola and orange juice, as well as, in samples with Coca-Cola-erosion compared with orange juice-erosion. Gastric juice had higher erosive potential in relation to Coca-Cola and orange juice, with the most intense morphological changes and the highest roughness on the enamel surface.

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Key words: *Enamel erosion, soft drinks, gastric juice, SEM, surface roughness*

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Introduction

Dental erosion has been defined as pathologic, non-bacterial dental hard tissue loss induced by extrinsic or intrinsic acids or chelators acting on plaque-free tooth surfaces (1). The most important extrinsic source of acid exposure is diet, which could include numerous components and products with complex composition and a potential for erosive damage (carbonated and acidic drinks, acidic food, citrus pastilles, various medicaments), professional exposure to corrosive agents (acid vapors from batteries and other appliances), even exposure to chlorinated water in swimming pools during water sports (2-6). In addition, behavioral factors like eating and drinking habits (holding an acid beverage in the mouth before swallowing, swishing around the mouth or sucking juice through the teeth) contribute to its development (7). Intrinsic factors are the result of endogenous acid, generally gastric acids that contact teeth especially in patients suffering from anorexia, bulimia, chronic vomiting during pregnancy and gastrointestinal disturbances (8-11).

Many laboratory studies have found carbonated drinks, especially carbonated cola drinks, to be associated with erosion, most likely due to their low pH (2, 4, 12, 13). Further, in vitro studies have shown that fruit juices may also be potentially erosive, due to their high content of titratable acid (2, 4, 12).

On the other hand, acidic stomach contents refluxed into the oral cavity can dissolve tooth structures and cause erosive tooth wear (14, 15) because contact between the hydrochloric acid from the stomach (with pH from 1.5 to 3,5) and the oral cavity occurs for a few seconds, several times a day (16).

The aims of the present in vitro study were twofold: (1) to analyze the experimental models of enamel erosion after exposure to Coca-Cola, orange juice and artificial gastric juice at the ultrastructural level, and (2) to evaluate enamel surface roughness after erosive challenge in the same acidic solutions.

Material and methods

The material for this research included 12 human impacted mandibular third molars (from patients aged 18-25 years) disinfected in 1% thymol solution and kept in 1% sodium hypochlorite for 24 h. Organic debris was removed by carefully using a dentist's set of instruments (17).

After the removal of the roots, at least 2 mm below cementoenamel junction, the crowns were cut (using a diamond saw under water irrigation) from the distal, mesial, buccal, and lingual side. Out of the total of 48 samples, 24 were used for SEM analysis and 24 were used for the analysis of enamel surface roughness (Table 1).

Table 1. Distribution of samples used in experimental protocols.

Samples	Number of samples for SEM analysis	Number of samples for surface roughness analysis	Total
Control	6	6 (3 measurements)	12
Immersed in Coca-Cola	6	6 (3 measurements)	12
Immersed in orange juice	6	6 (3 measurements)	12
Immersed in artificial gastric juice	6	6 (3 measurements)	12
Total	24	24	48

Erosion solutions and human saliva

The erosion models caused by soft drinks were obtained by immersing the samples in Coca-Cola (HBC - Serbia A.D. Zemun) and orange juice (NECTAR' D.O.O. Backa Palanka, Serbia).

In the previous study it was established that Coca-Cola had pH 2.67 ± 0.06 and TA 1.87 ± 0.09, whereas orange juice had pH 3.73 ± 0.03, requiring 5.70 ml of NaOH to reach pH 7.0 (4).

The model of enamel erosion with GERD was created using artificial gastric juice according to the methodology of Stefaniak et al. (18) and it was modified in accordance with the stablished goals of the research. Its initial pH was 2,1 (Table 2.).

Table 2. The contents of artificial gastric juice (primary electrolytes and ionic compounds)

Contents	**Concentration**
Calcium chloride dihydrate ($CaCl_2 \times 2H_2O$)	0.264 g/L
Magnesium chloride hexahydrate hexahydrat ($MgCl_2 \times 6H_2O$)	0.152 g/L
Potassium chloride (KCl)	0.864 g/L
Sodium chloride (NaCl)	2.855 g/L
Hydrochloric acid (HCl)	1.426 (3.38 ml; 36.2%)

Human saliva was collected from healthy volunteers in the morning, 2 hours after fasting. Volunteers rinsed their mouths twice with distilled water before saliva collection (19). Filtrates were obtained with Whatman filter papers grade 1: 11 μm (Sigma-Aldrich, USA).

Erosive Challenge

This study was approved by the institution's Ethics Committee. Tooth samples planned for analysis using SEM, immediately after cutting, rinsing and drying, were distributed into one of three erosive challenges, while the samples planned for analysis using profilometer, before exposure to acidic solutions, were prepared as follows: circular molds of 16 mm in diameter and 3 mm deep were filled by self-cured resin. Each sample was embedded in resin, with labial (oral) surfaces uppermost, and was cleaned with nonfluoridated pumice, rinsed with water and dried with oil-free compressed air.

All of the enamel samples were exposed to acidic solution according to the following protocol (10): immersion in 50 ml acidic solution (Coca-Cola/orange juice/gastric juice) for 15 minutes with occasional shaking, rinsing with distilled water, and immersion in human saliva.

The cycle was repeated three times a day for 10 days. During the night, the samples were placed in human saliva, including the 12 control samples (which did not undergo the demineralization procedure). The experiment was conducted at room temperature.

Preparation of samples for SEM analysis

At the end of the experimental period the samples were dried, fixed to aluminum stubs with a fixing agent (Dotite paint xc 12 Carbon JEOL, Tokyo, Japan), sputter-coated with old/palladium (in the unit JFC 1100E Ion Sputter JEOL), and examined by scanning electron microscopy (SEM) (JEOL-JSM-5300).

Preparation of samples for surface roughness analysis

Surface roughness of the enamel samples was measured using a profilometer (Mitutoyo Surftest SJ-301) (20).

Although four parameters of roughness are registered with the stylus of the Mitutoyo type profilometer, the statistical analysis took into account only one, the most frequently used parameter, Ra, which is defined as the average distance from the profile to the mean line over the length of assessment. A detailed description of the measurement method using a diamond stylus profilometer has already been published in our recent study (4). Statistical analysis was carried out using one-way ANOVA Student-Newman-Keuls post hoc test.

On enamel surfaces not exposed to the erosive challenge by Coca-Cola, orange and gastric juice (control group), the typical structures of sound enamel (grooves and perichimata lines) were apparent. Additionally, small depressions or ditches or grinding marks were observed and they were found to be indicative of the cumulative mechanical effects the teeth have experienced. (Figure. 1)

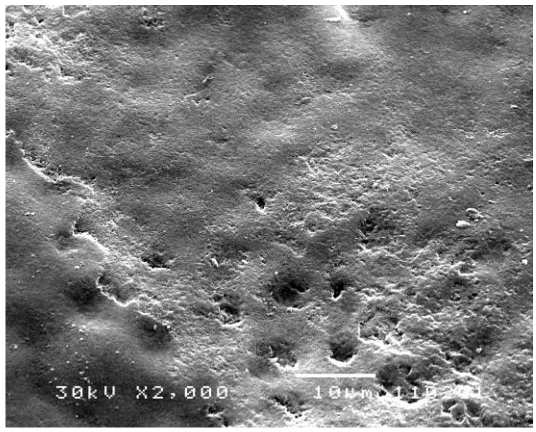

Figure 1. Control samples: the surface of untreated enamel with perikymata.

Results

SEM results are shown in micrographs 1 to 4 and the measurements of enamel surface roughness are shown in Table 3.

Table 3. The values of the enamel surface roughness parameter (Ra) in relation to the tested acid solutions

Roughness parameter	Exposure (min)	Control	I Artificial gastric juice	II Coca-Cola	III Orange juice
R_a	15	0.67 ± 0.02	1.63 ± 0.25^{a}	$1.49 \pm 0.08^{a,b}$	$1.27 \pm 0.01^{a,b,c}$

$^{a}p < 0.05$ vs control; $^{b}p < 0.05$ vs. artificial gastric juice; $^{c}p < 0.05$ vs. Coca-Cola;

The enamel surface of teeth exposed to the acidic solutions clearly demonstrated deep changes in enamel structure: scanning micrographs of enamel samples eroded by Coca-Cola and gastric juice exhibited a distinct pattern, showing hollowing of prism centers with relatively intact peripheral regions, reflecting honeycomb appearance (Figuries 2, 4).

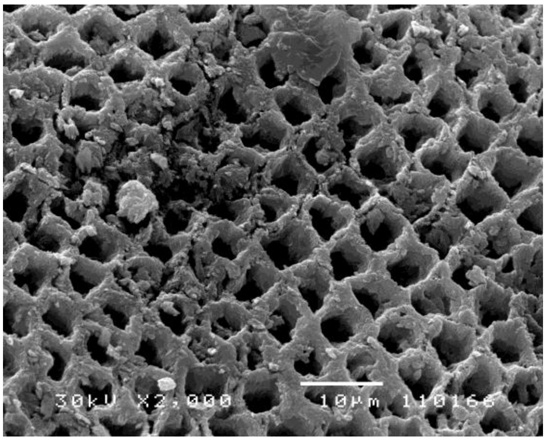

Figure 2. Erosive changes on enamel caused by **Coca Cola**: type 1 erosion, central parts of the prisms are affected and the peripheral parts are relatively preserved.

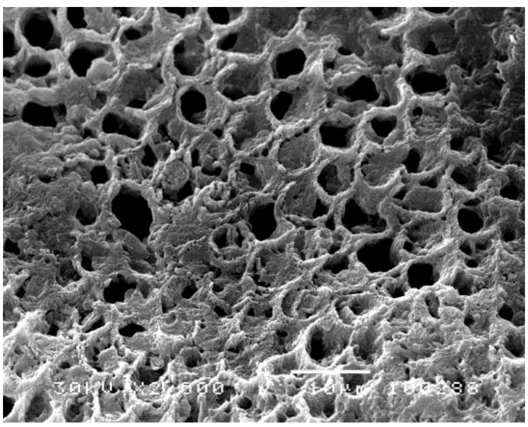

Figure 4. Erosive changes in enamel surface caused by gastric juice with significant type 1 erosion, honeycomb appearance.

In contrast, samples immerged in orange juice showed atypical etching: without prisms, with pitted enamel surfaces, as well as with structures which look like unfinished puzzles, maps, networks. (Figure 3).

The highest value of Ra roughness parameter was observed in the samples immersed in gastric juice, followed by Coca-Cola and, finally, orange juice.

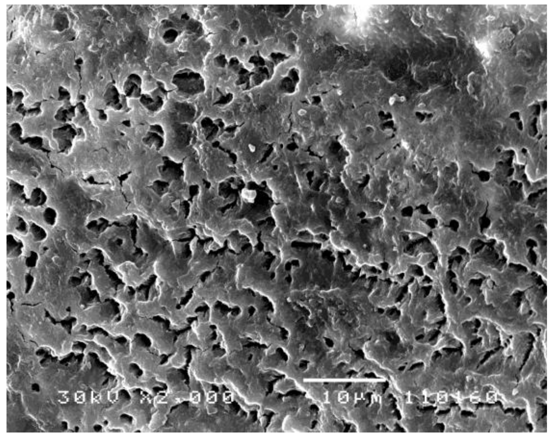

Figure 3. Erosive changes in enamel surface caused by orange juice with irregular erosion type.

There was a statistically significant difference among all of the tested roughness parameter values.

Discussion

Literature data point to an increase in dental erosions in the modern society and they represent a challenge for the researchers. Considering that there is a decreasing tendency in caries instances, erosive tooth wear is becoming a more significant element in planning a long-term model of dental health (2). An increase in the prevalence of various gastrointestinal diseases and eating disorders leads to more frequent contacts between the teeth and gastric acid. Together with increasing use of acidic beverages, these conditions are considered significant risk factors for teeth demineralization.

The goal of our research was to create the ultrastructural experimental models of enamel erosion caused by Coca-Cola, orange juice and artificial gastric acid and to determine differences and possible similarities between the erosions caused by external and internal factors on experimental model. To some extent, the findings could point to the significance of prevention of both internal and external causes of erosive tooth wear.

In order to simulate clinical conditions, the present research used gastric juice formula which, apart from HCl with 2,1 pH, contained only the primary electrolytes and ionic compounds, without organic and amino acids, carbohydrates and pepsin. Calcium, magnesium and sodium salts should act as buffer components which could probably control the erosive potential of gastric juice, similar to in vivo conditions. On the other hand, the majority of the results from laboratory studies regarding the enamel surface changes in reflux disease were obtained based on the use of pure HCl (16, 21, 22, 23, 24). Nevertheless, in studies by Barlet at al. and Braga at al. (9, 10), gastric juice which was aspirated from patients undergoing endoscopy for symptoms of reflux disease was used.

In the present study, the immersion cycles (3 times for 15 minutes) could imitate GERD symptoms for a shorter period of time (10 days). Similarly, the time of immersion of samples into Coca-Cola and orange juice can imitate the frequency of consumption of soft drinks. On the samples immersed in the Coca-Cola and gastric juice, the following was observed: diffuse demineralization involved the rod core, with decomposition of morphology of prisms: they were severely affected, and a greater prism-core dissolution compared with that in the interprismatic areas gave the enamel a "honeycomb pattern" of etching, similar to the results published by Colombo et al. (25) and Braga at al. (10). Also, Arnold et al. (22) showed that exposure to pure HCl results in four different enamel etching patterns, and the depth of the surface layer was dependent upon the etching time. Our research showed that the degree of destruction of central prism parts varied depending on location, whereby the most prominent changes were observed in the vicinity of cement-enamel junctions. In the present study, samples immerged in orange juice showed atypical etching, which is referred to as type 4 in the literature. (26).

In the current study, surface roughness was measured using a stylus profilometer that overhangs across the surface of the object, registering all of the unevenness at a certain measuring length (17, 27, 28, 29, 30). According to some literature data stylus profilometry shows some disadvantages (the risk of the diamond tip causing damage to the specimens, inability to detect valleys which are narrower than the stylus tip) but nevertheless this technique has a high degree of precision (31). Moreover, the current national standards on measuring surface texture are defined using stylus profilometry (11, 31, 32).

In a number of studies (28, 29), a difference in the surface roughness of the samples examined on various erosive challenges was determined only on the basis of the Ra parameter, where valid conclusions were drawn. The present study showed that this parameter was statistically significantly different among all the tested samples. Likewise, all samples of the experimental groups were also different from the control samples according to the SEM analysis and after analysis of the Ra parameter.

According to the latest literature data, the Ra parameter provides no information on the characteristics of surface irregularities, whereby both maximal and minimal irregularities may show the same Ra values (31, 34). Therefore, this research also included an ultramicroscopic analysis in order to obtain more precise results.

Braga et al. (10) showed that the enamel surface after orange juice had a generalized surface roughening with no apparent evidence of a prism pattern, and the surface was not completely etched. The same authors used atomic emission and FT Raman spectroscopy to analyze the mineral content of enamel after exposure to gastric and orange juice, and they determined that gastric juice has a higher erosive potential than orange juice. Our research analyzed surface roughness of enamel using stylus profilometry following the exposure to the same acidic solutions, as well as to Coca-Cola. It has been determined that gastric juice has a higher erosive effect on the enamel, which is in accordance to the results of the mentioned authors. Roughness parameter showed that, after exposure to gastric juice, the enamel surface had prominent unevenness of the surface which was statistically significant compared to the surface texture of samples exposed to Coca-Cola and orange juice.

According to information from the manufacturers, Coca-Cola contains phosphoric acid, compared to citric acid,phosphoric acid is stronger (33). The effect of phosphoric acid results in a superficial etched zone which might be permanently lost from the tooth surface (26). On the other hand, citric acid may act as a chelator capable of binding the calcium from enamel or dentine, thus increasing the degree of undersaturation and favoring demineralization (35, 36).

In our previous study the erosive potential of various soft drinks was examined by measuring initial pH and titratable acidity and enamel surface roughness using different exposure times. It was found that Coca-Cola had the highest erosive potential in the shortest time interval exposure (15 min), although it had the lowest titratable acidity (4). These results are in accordance with literature data which show that cola-based drinks have a higher erosive potential than orange juices immediately after exposure (12). Profilometric parameters have demonstrated that pure orange juice causes greater enamel erosion during longer exposures. A statistically significant lower degree of roughness compared to Coca-Cola in shorter exposure can be explained by higher initial pH in orange juice compared to Coca-Cola (3.73 vs. 2.67) (4).

In the current study, gastric juice was significantly more erosive to enamel than Coca-Cola and orange juice, and Coca-Cola is more erosive than orange juice. Other studies attest that gastric juice (aspirated from patients undergoing endoscopy) has a greater potential for erosion than orange juice (10) and carbonated drinks (Bartlett and Coward, 2001). Results by Bartlett and Coward reflect the lower pH and titratable acidity of gastric juice compared with the carbonated drink. If this result is extrapolated to the clinical situation, it confirms the suspicion that gastric juice has the potential to produce the severe pattern of erosion found in patients with eating disorders and reflux disease (9).

Conclusion

Despite the limitations characteristic of in vitro studies, it can be concluded that experimental erosion model of enamel surface exposed to Coca-Cola and artificial gastric juice shows type 1 acidic erosion (honeycomb appearance) by SEM analysis. Degree of destruction of central prism parts varied depending on location, whereby the most prominent changes were observed in the vicinity of cement-enamel junctions. Ultrastructural experimental model of enamel surface erosion after exposure to orange juice demonstrates atypical etching with no apparent evidence of a prism pattern. Profilometric parameter Ra was significantly increased for samples immersed in gastric juice compared to samples immersed in Coca-Cola and orange juice, as well as in samples with Coca-Cola-erosion compared with orange juice-erosion. The results of this study point to a higher erosive potential of gastric juice, compared to Coca-Cola and orange juice, with the most intense morphological changes and the highest roughness on the enamel surface.

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EVALUACIJA HRAPAVOSTI I MORFOLOŠKIH PROMENA NA GLEĐNOJ POVRŠINI POSLE IZLOŽENOSTI COCA-COLI, SOKU OD NARANDŽE I VEŠTAČKOM ŽELUDAČNOM SOKU: *IN VITRO* STUDIJA

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Erozija zuba je patološki gubitak tvrdih zubnih struktura izazvan spoljašnjim i unutrašnjim kiselinama, bez učešća bakterija. Ova *in vitro* studija imala je za cilj da proceni i uporedi morfologiju i površinsku hrapavost gleđi nakon erozivnog izazova nekih eksternih i internih kiselih supstanci kao što su Coca-Cola, sok od narandže i veštački želudačni sok.

Uzorci gleđi (n = 48), dobijeni ekstrakcijom humanih trećih molara, podvrgnuti su erozivnom izazovu veštačkog želudačnog soka i komercijalno dostupnih bezalkoholnih pića (Coca-Cola i sok od narandže) uranjanjem u 50 ml kiselog rastvora u trajanju od 15 min, tri puta dnevno tokom 10 dana. Između potapanja, uzorci su držani u filtriranoj pljuvački. Primljena su dvadeset i četiri uzorka za analizu površinske morfologije korišćenjem skening-elektronskog mikroskopa i 24 za analizu Ra-parametra hrapavosti (korišćenjem stylus profilometra sa dijamantskom iglom), uključujući i 12 kontrolnih uzoraka (koji nisu podvrgnuti proceduri erozije). Rezultati površinske hrapavosti analizirani su pomoću one-way ANOVA Student-Newman-Keuls post hoc testa.

Ultrastrukturna analiza površine gleđi posle potapanja u Coca-Colu i želudačni sok pokazala je tip 1 model nagrizanja sa tipičnom honeycomb strukturom. Nespecifičan morfološki model ustanovljen je nakon erozivnog izazova sokom od narandže. Profilometrijski parametar Ra je značajno povećan kod uzoraka potopljenih u želudačni sok u poređenju sa uzorcima izloženim Coca-Coli i soku od narandže, kao i u uzorcima sa Coca-Cola-erozijom u poređenju sa erozijom izazvanom sokom od narandže. Želudačni sok je pokazao veći erozivni potencijal u odnosu na Coca-Colu i sok od narandže, sa najintenzivnijim morfološkim promenama i najvećom hrapavošću na površini gleđi.

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Ključne reči: *gleđna erozija, bezalkoholna pića, želudačni sok, SEM, površinska hrapavost*