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TESTING THE EFFECT OF AGGRESSIVE BEVERAGE ON THE DAMAGE OF ENAMEL STRUCTURE

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Abstract: Examining the enamel surface with the Atomic Force Microscopy (AFM) enables more precise registering and defining of the changes of enamel surface structure and of microhardness. This method can be used to compare the efficiency of application of different preventive and therapy materials and medicaments in dentistry. Under the influence of coca cola, a change of crystal structure and nanomorphology on enamel surface occurs.

The trial was conducted on a total of 40 extracted teeth which were divided into two groups treated with the solution of coca cola during 5 minutes, and then prepared and tested with a standard AFM procedure, type SPM-5200. Quantitative analysis was performed by comparing the roughness parameters (Ra) of treated and non-treated sample.

Keywords: nanomorphology, enamel surface, AFM procedure, roughness parameters.

1. INTRODUCTION

Tooth enamel is made of a billion crystals of carbonized hydroxyapatite that are packed in individual prisms winding from enamel-dentin border toward the tooth surface. Enamel prisms are formed by complex interaction of ectodermal and ectomesenchymal tissues that coordinate the action of amelobasts (cells responsible for their synthesis). Each enamel prism is a product of a single ameloblast and stretches uninterruptedly from the enamel-dentin border to tooth surface. Enamel surface is not flat. It has a wavy structure because at places where Retzius' striae end such striae overlap in the form of steps, with the appearance of shallow grooves referred to as *perikymata*. Although enamel has a pronounced hardness, it is also fragile at the same time and similar to glass, so that for these reasons it could appear to be susceptible to breaking. Despite that, enamel can take loads higher than 1000 N several times during the day. The overall enamel microstructure is formed in such a way to adjust to such loads. This is also contributed by the support of elastic dentin and the structures such as enamel tufts at the dentin-enamel junction. Besides the fact that it represents the hardest biological tissue, tooth enamel may be quickly damaged under the influence of various factors. Enamel damage can be divided into two large groups, i.e. infective and non-infective [1,2].

Infective damage or tooth caries occurs as a consequence of demineralization caused by the bacteria organized in a special ecological formation: oral biofilm – dental plaque. In certain conditions the so-called cariogenic bacteria (specific species of streptococci) dominate on the tooth surface. They have an ability to create organic acids, but can survive in acidic conditions. They suppress neutral or useful bacteria. In order for acidogenic biofilms to form and exert cariogenic effect, the presence of sugar is necessary. Sugars originate from food and can be obvious such as those from candies, refined buns, snacks, beverages, or hidden, like for example in juices.

Beverages that decrease pH in the oral cavity and on the tooth surface, thus potentially leading to dental erosions, include fruit juices, soft drinks, sport beverages, other fizzy drinks, as well as various pickled vegetables (due to the content of acetic acid) [12,13].

In dentistry, the development of which has been greatly influenced both by the knowledge of and observing the biological and mechanical properties of hard (mineralized) tissues, the research with using, this precise technique has started only in recent years. The results of the analyses of oral cavity tissue introduced the dental science into nano-era [3−6]. Only with the development of AFM technologies it was possible to observe more subtle surface

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changes of enamel. Also, AFM studies start to be more and more used in researches in dentistry too, which observe the surface changes such as the dental plaque and mineralized and coloured deposits, surface properties of different materials and morphological and mechanical changes on mineralized tissues [7−9]. In recent years, AFM has been more and more used to investigate erosions and early stadiums of demineralization on enamel surface – the first results show that this is a very convenient tool. In order for the investigations to be comparable as much data as possible should be collected about the enamel nanomorphology in different biological and pathological conditions [10−13].

We chose to use the average roughness (Ra) of our samples as a measure, because it has been most commonly used in previous studies, so it was easier to make a proper comparison of our results [14].

By using special research nanoprobes, it is possible nowadays to measure resolutions and movements at the level of nanometers and picomolars [15]. This also enables precise registering of physical properties of healthy – unchanged enamel, dentin and cement of the tooth root, as well as carrying out of the analyses of chemical processes and biological transformations that until now were impossible to perform [3,4,15,16].

2. MATERIAL AND METHODS

The study was carried out on a total of 40 extracted teeth divided into two groups from which samples were taken (3 mm x 2 mm x 2 mm in size) and which were treated with a solution of coca cola and then prepared and tested with the standard procedure using AFM of JSPM-5200 type. One group of samples was not treated, while the other was treated for 5 minutes, with an analysis of enamel nanostructure performed after the treatment.

Every sample was fixed to the microscope holder by using cyanoacrylate adhesive.

Images were made with very slowed down scanning of the surface of every 25,0 μm^2, and with 0,1 Hz scanning frequency with 256 lines per sample, so as to avoid damaging of the probe.

Surface roughness was measured on the basis of average roughness (Ra) automatically on WinSPM software.

The average roughness is defined as an average distance of the centerline when they are observed as if they were local minimums and local maximums. This roughness is defined by the following formula :

$$R_a = \frac{1}{L}\int_0^L \left| f(s) - Z_0 \right| ds$$

where Z_0 is the centerline of the profile of the length L:

$$Z_0 = \frac{1}{L}\int_0^L f(s) ds$$

Figure 1 presents an image of the profile with absolute values in relation to the centreline. The distance of their average height from the height of the centreline profile is defined as average roughness.

Competence at the level of AFM nanometry can be observed in the same way as in conventional measurements because the average roughness (Ra) at one measurement point is actually the arithmetic mean of local maximums and minimums of enamel surface calculated for that measurement point. All our measurement points have a surface of 5 x 5 μm, i. e. 256 x 256 or 512 x 512 pixels.

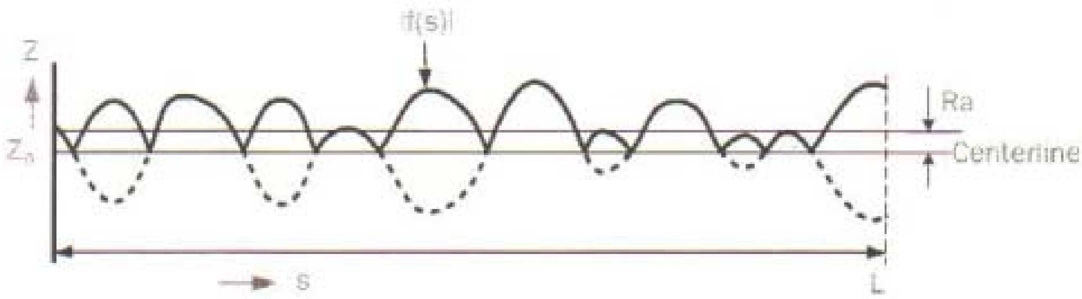

Figure 1. Average roughness

3. RESULTS

Enamel surface morphology has been described both in terms of quality and quantity. Quantitative analysis was based on comparison of Ra roughness parameter between different samples and on statistical analysis of obtained values.

In an initial assumption of the study we intended to start analyzing the effect of coca cola on the enamel surface during a 60-second interval. Preliminary tests have not identified any significant differences between the treated and non-treated samples, therefore, a study with a 5-minute treatment was undertaken.

Figures 2 and 3 present untreated samples and samples that were treated with coca cola for 5 minutes.

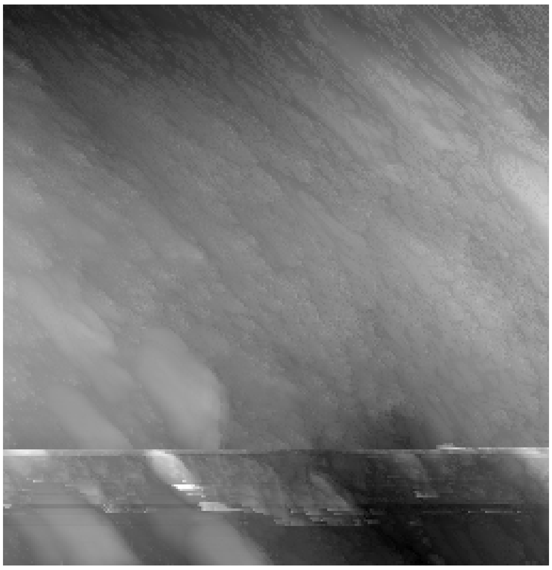

Figure 2. Untreated control surface

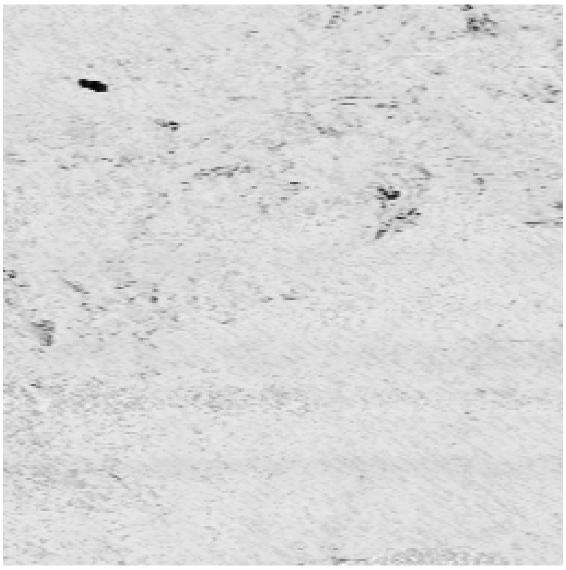

Figure 3. Surface treated with coca cola for 5 min.

On smaller magnification, the untreated surface (Figure 2) is covered with an amorphous layer which may point to aprimsatic enamel and parts of pellicle. No clear borders between prisms or existence of larger depressions can be seen. Enamel shows signs of compactness. Surface is flatter and protrusions that can be seen have uniform structure. The granules are arranged in parallel rows and no pores on enamel surface can be seen.

In contrast to that, slight porosity on the surface treated for 5 minutes with coca cola can be seen. Depressions and the grid-like structure of granules can be seen (Figure 3).

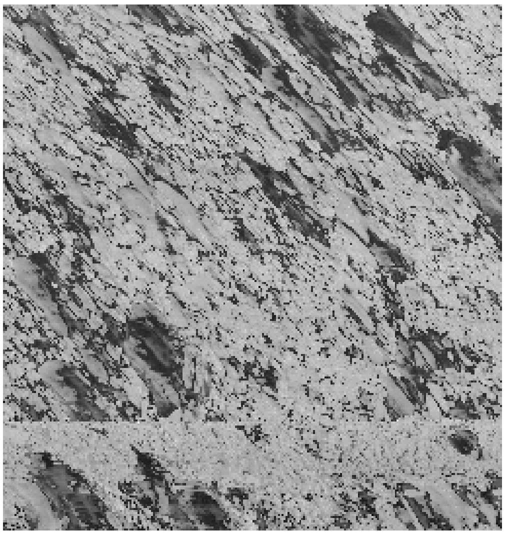

Figure 4. Untreated control surface

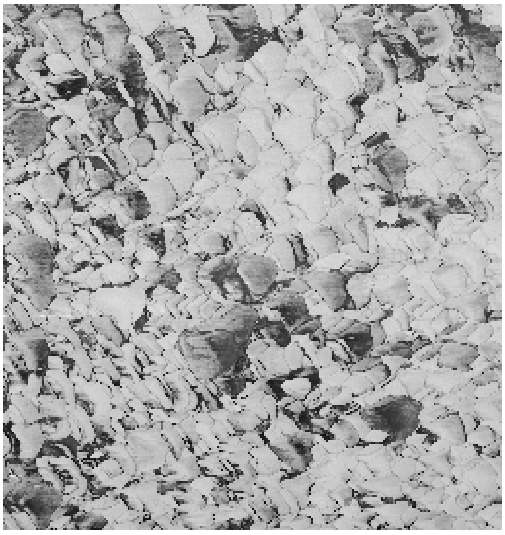

Figure 5. Surface treated with coca-cola for 5 minutes

Higher magnifications confirm the compactness of untreated surface (Figure 4). The structure of densely compacted crystals on untreated surface is more prominent, where no border can be seen between the prisms. On the treated surface, depressions between the prisms are more prominent, with protruding prism heads and depressions around them.

Figure 6. Untreated control surface

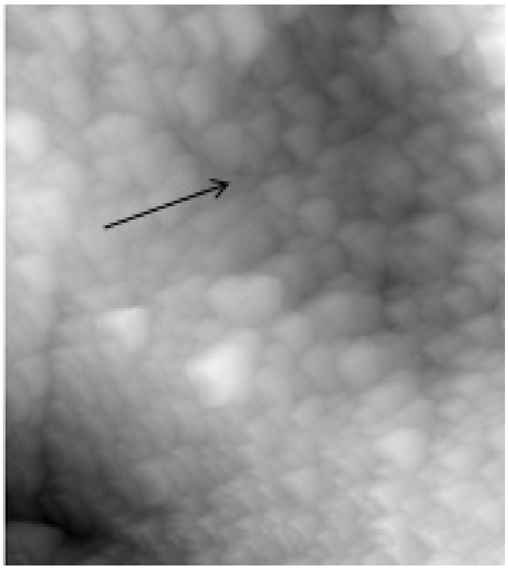

Figure 7. Surface treated with coca cola for 5 minutes

On higher magnitudes depressions can be seen (the arrow) between the „prismatic structures" in treated samples (Figure 7), while on untreated surface (Figure 6) there is a filled-in area, which would correspond to compacted crystals. The findings point to the primary action in the area of less mineralized prism rims, where dilatation of the area can be seen.

By the analysis of all measurement points, the following mean values of average roughness, expressed in Ra, were obtained:

	Untreated sample − N	Sample treated with coca cola
Arithmetic mean of Ra values of all measurement points Xsr	34,66	76,28

Variables	t_{emp}	t_{gr}	Statistical significance	p
N-Ra /CC5-Ra	−237,9093	3,291	VSZ	<0,001

Based on the test of a hypothesis of existence of differences between the treated and untreated sample, with an application of a t-test, it is shown that there are statistically highly significant differences between Ra of the treated sample with a 5-minute treatment of coca cola and Ra of the same sample without the treatment, because the absolute value t is empirically higher than t – border value $t_{emp} = |-237{,}9093| > tgr = 3{,}291$ za p = 0,001.

4. DISCUSSION

The selected studies are extremely important because non-carious damages of teeth are coming more and more in focus with a trend that exists in developed countries to place the caries under control. While a great deal of such damage would even pass unnoticed in the past, today, in developed countries, it is among the most frequent chronic diseases with the children of 5−17 years of age [2,11,17].

Carbonated beverages are dominant among soft drinks, with coca cola taking a leading place. Increased consumption is accounted for by the recession and a lower price of coca cola [18,19].

The increased consumption of carbonated beverages is correlated with an increase in erosive damages of enamel [20].

Numerous studies have shown an advantage of using AFM analyses to monitor both qualitative and quantitative changes on enamel surface [21−26].

Speaking of qualitative and quantitative monitoring of enamel dissolution, the paper [27] demonstrates correspondence of AFM examination technique with other techniques (SEM, profilometry, nanocarving), while the paper [28] confirms AFM as the most precise technique for such analyses.

The analysis of changes on our sample after five minutes of action of coca cola clearly shows that there are no densely compacted crystals which are, on the contrary, clearly seen on untreated samples; this is an indication of initial demineralization and creation of depressions, most probably at places of lower density of crystals. Other authors have also shown similar results [29].

Many other researchers, who used different techniques, also showed negative effects of carbonated beverages on both tooth enamel and composite fillings [30−33]. It was demonstrated in the paper [30] that carbonated beverages reduce the physical properties of enamel (hardness and elasticity module) as a consequence of enamel erosion. The paper also shows that such drinks have a more prominent effect compared to, for example, orange juice. Some authors have found that unpolished enamel is less susceptible to erosions compared to the polished one and that the enamel structure itself in *in vitro* conditions can significantly influence the progression of erosions. This particularly relates to the presence of aprismatic enamel, cracks and perikymata [34].

Chemical composition of acidic beverages is certainly significant in the modification of the mineral structure and thereby of the mechanical properties of enamel [34]. That the acidity of carbonated cola drinks primarily originates from phosphoric acid has been shown in the paper [32]. It has been known from earlier researches that the drinks with citric acid causes higher enamel erosion compared to the drinks that contain only phosphoric acid, such as in coca cola [34]. In addition to pH, liquid environment around the enamel and temperature are also relevant, and they all affect the physical properties such as the elasticity module, hardness and surface roughness of human enamel.

The results obtained in *in vitro* studies, such as ours, can only be partly transferred on what is going in clinical conditions, which largely depends on the dynamics of distortion of demineralization-remineralization kinetics. Remineralization periods that lead to enamel recovery, reduction of roughness and microhardness increase have not been included in our studies [21,26,35]. Likewise, certain studies have shown increased resistance with the application of remineralization pastes or alternating presence of enamel in saliva and the drink [21,27].

5. CONCLUSION

The analysis of the samples after five minutes of coca cola action shows both the qualitative and quantitative difference. On treated enamel samples, lower density of crystals with larger areas between prisms is clearly seen. Surface is more irregular with higher depressions. The analysis of Ra of treated and untreated surfaces confirms such findings as well as higher roughness of enamel surface treated with coca cola. The initial irregularity (after five minutes of treatment) is a consequence of partial destruction of aprismatic layer and the attack with the crystals of less compacted parts, such as perikymata or lamellae. The findings correlate with the other studies [29,35].

The results obtained indicate that the treated surfaces are statistically significantly rougher (higher Ra), which is also confirmed with the morphological signs of depressions and decrease of enamel crystals.

Under the influence of coca cola there is a statistically significant disruption of the integrity of the crystal grid which is observed after five minutes of action, which corresponds to the most common way of consumption of this carbonated drink. Enamel damages are primarily related to a decrease of crystal thickness and the creation of fissures which increase the overall roughness of the surface. Higher roughness leads to bigger contact with acids thus increasing the possibility of further damage.

Our AFM researches indicate irregular surface structure of enamel in physiological conditions, which has a certain degree of roughness, depending on histological properties, the presence of pellicle and compactness of crystal units in prisms.

Despite its limitations, our studies have proved that the use of AFM enables successful monitoring of changes on enamel surface as well as the interpretation of the ultrastructural configuration of the crystal stage and of the damage created under the influence of different external factors.

Likewise, we believe that coca cola, as the most common carbonated beverage, exerts aggressive influence on enamel surface thus endangering the mineral structure.

Further research should be directed toward the changes of ultrastructural enamel during a 2-h interval after the action of the examined agent, which should be correlated with the physiological conditions in oral cavity; subsequently causal relationship between them should be established.

6. REFERENCES

[1] N. Roveri, et al., *Surface enamel remineralization: Biomimetic apatite nanocrystals and fluoride ion*, Journal of Nanomaterials, Vol. 2009 (2009), pages 9.

[2] S. Chandra, et al., *Textbook of Dental and Oral Histology with Embryology and MCQS*, 2/E, Jaypee Brothers Medical Publishers (P) Ltd.; 2nd edition, 2010.

[3] A. D. L. Humphris, M. J. Miles, J. K. Hobbs, *A mechanical microscope: High-speed atomic force microscopy*, Appl Phys Lett., Vol. 86 (2005) 281−288.

[4] T. Schmid, J. Burkhard, B. S. Yeo, W. Zhang, R. Zenobi, *Towards chemical analysis of nanostructures in biofilms I: Imaging of biological nanostructures*, Anal. Bioanal. Chem., Vol. 391 (2008) 1899−1905.

[5] L. Bozec, J. de Groot, M. Odlyha, B. Nicholls, M. A. Horton, *Mineralised tissues as nanomaterials: Analysis by atomic force microscopy*, IEEE Proc. Nanobiotechnol., Vol. 152 (2005) 183−186.

[6] W. C. Oliver, G. M. Pharr, *An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments*, J Mater Res, Vol. 7 (1992) 1564–1583.

[7] S. Kimyai, et al., *Effect of three prophylaxis methods on surface roughness of giomer,* Medicina Oral, Patología Oral y Cirugía Bucal, Vol. 16–1 (2011), e110-e114.

[8] G. J. Lee, et al., *A quantitative AFM analysis of nano-scale surface roughness in various orthodontic brackets*, Micron, Vol. 41–7 (2010) 775–782.

[9] C. Poggio, et al., *Impact of two toothpastes on repairing enamel erosion produced by a soft drink: an AFM in vitro study*, Journal of Dentistry, Vol. 38–11 (2010) 868–874.

[10] J. Gimzewski, M. J. Miles, *High-speed atomic force microscopy of dental enamel dissolution in citric acid*, Arch. Histol. Cytol., Vol. 72 (2009) 328−335.

[11] U. S. Department of Health and Human Services (8 August 2007). *Preventing Chronic Diseases: Investing Wisely in Health*, National Center for Chronic Disease Prevention and Health.

[12] M. E. Barbour, M. Finke, D. M. Parker, J. A. Hughes, G. C. Allen, M. Addy, *The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures*, J Dent., Vol. 34 (2006) 207–213.

[13] P. Preethu, S. Maitreyeed, R. Dodawad, *The effect of Four Fruit Juices on the Ph of Dental Plaque - A Four Period Cross-Over Study*, Journal of clinical and diagnostic research, Vol. 4 (201) 2587–2593.

[14] J. Field, P. Waterhouse, M. Germa, *Quantifying and qualifying surface changes on dental hard tissues in vitro*, Journal of dentistry, Vol. 38 (2010) 182–19.

[15] R. A. Freitas, Jr. *Nanodentistry*, J. Am. Dent. Assoc., Vol. 131 *(*2000) 1559−1565.

[16] B. Holme, L. H. Hove, A. B. Tveit, *Using white light interferometry to measure etching of dental enamel*, Measurement, Vol. 38 (2005) 137−147.

[17] I. Al-Majed, A. Maguire, J. J. Murray, *Risk factors for dental erosion in 5–6 year old and 12–14 year old boys in Saudi Arabia*, Community Dent Oral Epidemiol, Vol. 30 (2002) 38–46.

[18] A. Smith, *The Oxford companion to American food and drink*, Oxford University Press US, 2007, p.451.

[19] T. Attin, K. Meyer, E. Hellwig et al., *Effect of mineral supplements to citric acid on enamel erosion*. Arch Oral Biol, Vol. 48 (2003) 753–759.

[20] T. Attin et al., *Impact of modified acidic soft drinks on enamel erosion*, Oral Diseases, Vol. 11 (2005) 7–12.

[21] B. Neville, D. D. Damm, C. M. Allen, J. Bouquot, *Oral and Maxillofacial Pathology*, 3rd Edition, Saunders; 2008.

[22] N. Schlueter, A. Hara, R. P. Shellis, C. Ganss, *Methods for the measurement and characterization of erosion in enamel and dentine*, Caries Res, Vol. 45–1 (2011) 13–23.

[23] M. E. Barbour, M. Finke, D. M. Parker, J. A. Hughes, G. C. Allen, M. Addy, *The relationship between softening and erosion caused by soft drinks at a range of temperatures*. J Dent, Vol. 34 (2006) 207–213.

24] M. E. Barbour, D. M. Parker, G. C. Allen, K. D. Jandt, *Human enamel erosion in constant composition citric acid solutions as a function of degree of saturation with respect to hydroxyapatite*, Journal of Oral Rehabilitation, Vol. 32 (2005) 16–21.

[25] M. Barbour, R. P. Shellis, *An investigation using atomic force microscopy nanoindentation of dental enamel demineralization as a function of undissociated acid concentration and differential buffer capacity*, Phys Med Biol, Vol. 52 (2007) 899–910.

[26] H. Devlin, M. A. Bassiouny, D. Boston, *Hardness of enamel exposed to Coca-Cola and artificial saliva*, J Oral Rehabil, Vol. 33–1 (2006) 26–30.

[27] C. Fzhen-Jiang, et al., *The enamel softening and loss during early erosion studied by AFM, SEM and nanoindentation*, Biomed. Mater., Vol. 4 (2009) 015020.

[28] B. W Sigusch, M. Beyer, E. Heurich, K. D. Jandt, *Erosion des Zahnhartgewebes - Neue*

pathogenetische und diagnostische, Aspekte durch Atom-Kraft-Mikroskopie und Nanoindentation ZWR, Vol. 117 (2008) 152–158.

[29] Q. Zeng, et al., *Comparisons of the Apatite Presentation and Demineralization in Enamel and Cementum by Atomic Force Microscopy*, Key Engineering Materials, Vol. 733 (2007) 330−332.

[30] C. Machado, W. Lacefield, A. Catledge, *Human Enamel Nanohardness, Elastic Modulus and Surface Integrity after Beverage Contact*, Braz Dent J., Vol. 19–1 (2008) 68−72.

31] A. Lussy, T. Jaeggi, S. Jaeggi-Scharer, *Prediction of the erosive potential of some beverages*. Caries Res, Vol. 29 (1995) 349–354.

[32] G. Maupome, J. Diez de Bonilla, G. Torres-Villasenor, L. C. Andrade- Delgado, V. M. Castaño, *In vitro quantitative assessment of enamel microhardness after exposure to eroding immersion in cola drink*, Caries Res,Vol. 32 (1998) 148–153.

[33] V. V. Badra, J. J. Faraoni, R. P. Ramos, R. G. Palma-Dibb, *Influence of different beverages on the microhardness and surface roughness of resin composites*, Oper Dent, Vol. 30 (2005) 213–219.

[34] J. H. Meurman, R. M. Frank, *Progression and surface ultrastructure of in vitro caused erosive lesions in human and bovineenamel*, Caries Res, Vol. 25 (1991) 81–87.

[35] E. Quartarona, et al., *Surface kinetic roughening caused by dental erosion: An atomic force microscopy study*. J. Appl. Phys., Vol. 103 (2008) 104702.

ИСПИТИВАЊЕ ДЕЈСТВА АГРЕСИВНИХ ПИЋА НА ОШТЕЋЕЊЕ СТРУКТУРЕ ГЛЕЂИ

Сажетак: Испитивање површине глеђи уз помоћ микроскопије атомском силом (АФМ) омогућава прецизније регистровање и дефинисање промјена на структури површине глеђи, као и микротврдоћу. Овај метод се може користити да би се упоредила ефикасност примјене различитих превентивних и терапеутских материјала и лијекова у стоматологији. Усљед утицаја кока-коле, долази до промјене кристалне структуре и наноморфологије на површини глеђи.

Испитивање је извршено на укупно 40 извађених зуба који су подијељени у двије групе и третирани раствором кока-коле током пет минута, а затим припремљени и тестирани уз помоћ стандардне АФМ процедуре, типа SPM-5200. Квантитативна анализа је извршена поређењем параметара храпавости (Ra) третираног и нетретираног узорка.

Кључне ријечи: наноморфологија, површина глеђи, АФМ процедура, параметри храпавости.