Original scientific papers UDK 616.314.17-085.242
doi: 10.7251/COMEN1601060M

AFM ANALYSIS OF ENAMEL DAMAGE DUE TO ETCHING WITH ORTHOPHOSPHORIC ACID

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Summary: Bearing in mind that in the field of jaw orthopedics and related scientific fields, there are no scientific studies that use the most modern technology based on the Atomic Force Microscopy (AFM) to investigate the nanostructure of tooth enamel after etching with 37% orthophosphoric acid, in this paper we will use this method, which is currently the most reliable, to determine the degree of tooth damage after its etching in the process of fixing orthodontic brackets. Considering the fact that the degree of enamel damage after etching cannot be realistically comprehended by the method called 'network', because in this way the damage cannot be seen three dimensionally (the depth of the damage), but only the damage in a single plane, a more precise analysis can be obtained with the application of the most contemporary method that we can apply in this case, and that is the application of AFM technology.

Keywords: Atomic Force Microscopy of enamel damage, etching, orthophosphoric acid.

1. INTRODUCTION

The development of the properties of adhesion of composite materials to hard tooth tissues, primarily enamel and dentin is one of the most important achievements in contemporary dentistry. The property of adhesion to enamel was found by Buonocore in 1955 [1], where he used the phosphoric acid to etch the surface layer of enamel due to the increase of retention area and the free energy of the surface. Etching enamel creates pores in which later penetrates resin or adhesive system. Different morphological forms of etched enamel were found by Silverstone. The surface of enamel after laying the phosphoric acid was demineralized in a 5−10 μm layer, which is the area of etched enamel. The pores about 20 μm thick are created beneath the surface, and these are the areas of qualitative pores, while beneath that area there is the area of quantitative pores, about 20 μm thick.

Apart from these, there are self-etching adhesives too, which can also achieve good adhesion. Low-viscosity monomers penetrate into the enamel surface and form, both within and around enamel prisms, a hybrid layer forming micromechanical retention. Having in mind that these structures are visible only under very high magnification (electronic microscope), we should speak here about nanoretention on enamel surface. And finally, we should note here that the action of self-etching adhesives on enamel surface is less aggressive than phosphoric acid, where the demineralization of surface is 1−2 μm.

Enamel prisms are formed through complex interaction of ectodermal and ectomesenchymal tissues and through the coordination of cells responsible for their ameloblasts synthesis. Ameloblasts are created from the enamel body under the inductive influence of ectomesenchymal cells that migrate into the area of stomatodeum. Ectomesenchymal cells lead to ribbon-like multiplication of ectodermal cells and the creation of a horseshoe designated as dental lamina. At ten places in lines subsequent multiplication of epithelial cells occurs in the form of the ball-like cell piles designated as dental bud. Epithelial cells exert inductive influence on ectomesenchymal cells, they multiply and deepen the ball which now assumes the shape of a hat. At this stage all the formative structures are differentiated with the bud, from which future dental tissues will arise [2−6].

Enamel surface is not flat. It has a wavy structure because at places where Retzius' striae end, these striae overlap in the form of steps, with the appearance of shallow grooves referred to as *perikymata* (Figure 1). In certain places, especially with primary (baby) teeth, there a few microns of enamel without prismatic organization on the surfaces – *aprismatic enamel* (Figure 2) [7,8].

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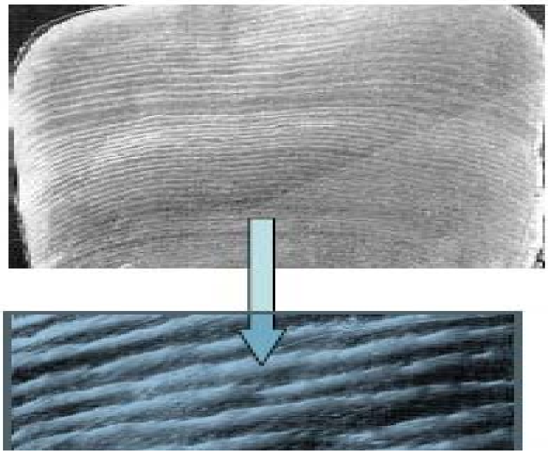

Figure 1. Perikymata

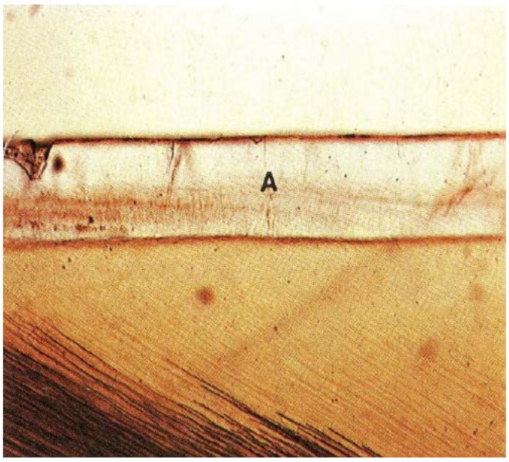

Figure 2. Aprismatic enamel

Although enamel has 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 owing to the support of elastic dentin and the structures such as enamel tufts at the dentin-enamel junction [4,6,9].

Enamel is in constant dynamic communication with the ecosystem of oral cavity. The processes of demineralization and remineralization are always present and their balance ensures the enamel integrity. If the external aggressive factors direct the balance toward the demineralization activities, the integrity of the crystal lattice weakens, reducing the hardness and resistance of enamel, which, after crossing a certain limit of mechanical resistance of enamel leads to rupture and creation of cavities, as the beginning of an irreversible damage [2,10].

Etching enamel with acid causes selective demineralization which increases the free surface energy, but at the same time porosity and the contact surface too. Bonding to enamel (adhesion) [11] depends on the capacity of the resin to penetrate into the area between the crystal prisms [12] which leads to macromechanical retention. The infiltered resin surrounds the individual crystals of hydroxyapatite forming microthorns [13] thus creating a hybrid layer which accomplishes the retention mechanism at the nanolevel between the tooth and resin [14]. These micro thorns probably contribute to adhesion more than macro thorns which entering the area between the enamel prisms [15].

The retention capacities of etched enamel depend on the chemical structure of enamel mineral phase, the type of acid and time of etching [16]. Research has proved that the variations in time of etching from 15 to 90 seconds with 35−37% phosphoric acid do not influence much the shear bond strength or orthodontic brackets [17].

With etching time damage is greater and this is primarily manifested by capturing complete enamel prisms which happens in the first 15 seconds. In the further course, between 15 and 30 seconds destruction spreads mainly in the depth covering the central prism regions [18].

Something that has not been investigated in detail so far is the surface roughness at the microscopic level [19] where the nano characterization of surface roughness could provide biophysical mechanisms on enamel surface [20]. AFM with high lateral and vertical resolution allows investigating roughness at micro and nano levels without major interference of macroscopic components such as the wavy structure [21]. AFM microprobe does not require preparation of the sample and consequently endangering of the original surface. Thus it represents a direct way to experimentally detect and quantify the surface roughness.

2. MATERIAL AND METHODS

When analyzing the teeth after etching with 37% phosphoric acid, the material of work comprises 50 teeth of permanent dentition, which are fully intact (without caries, non repaired through conservative methods and without similar intervention therapeutic dentistry procedures); the total of 256 lines were done on each of these 50 teeth (*the total 12.800 lines*).

After extraction, the teeth were immediately placed in the solution of artificial saliva, in order to preserve the physiological mineralization of enamel. After this procedure, further preparation for the study included the following: the etching material (in the concentration of 37% of orthophosphoric acid), was applied on enamel surfaces on which, in clinical orthodontic practice, brackets are bonded to teeth. This material was left to act for 30 seconds, i.e. in the way that it is done in clinical practice. After this, the surface on which the etching agent had been laid was rinsed with demineralized water and after thorough drying tooth enamel was cut to the dimensions 3mm x 2mm x 2mm, and then the surfaces to be scanned were polished. Tooth enamel samples were fixed to the AFM microscope holder. Nanotechnological device JSPM-5200 which is located in NanoLab module for biomedical engineering at the Faculty of Mechanical Engineering of Belgrade University was used to test the nanostructure of tooth enamel. This is an integrated nanosystem with multiple operating modes that enables the following functions: STM, AFM, MFM, ECSPM etc.

JSPM-5200 consists of AFM base, anti-vibration table, AFM amplifier, SPM controller, computer and optional components such as a microscopic system with CCD camera, vacuum system etc. [22].

The schematic presentation of the system with interconnected components is given in Figure 3. The following parts of the system were presented:

− SPM base: the measuring part of the microscope that consists of AFM head, table and scanner,

− anti-vibration table: its function is to prevent transfer of vibrations to the sample,

− AFM amplifier: interface between SPM base and SPM controller that contains the signal-monitor AFM and on-off switch for laser diode,

− It also includes: console (cantilever) holder, holder of the type and holder of the sample.

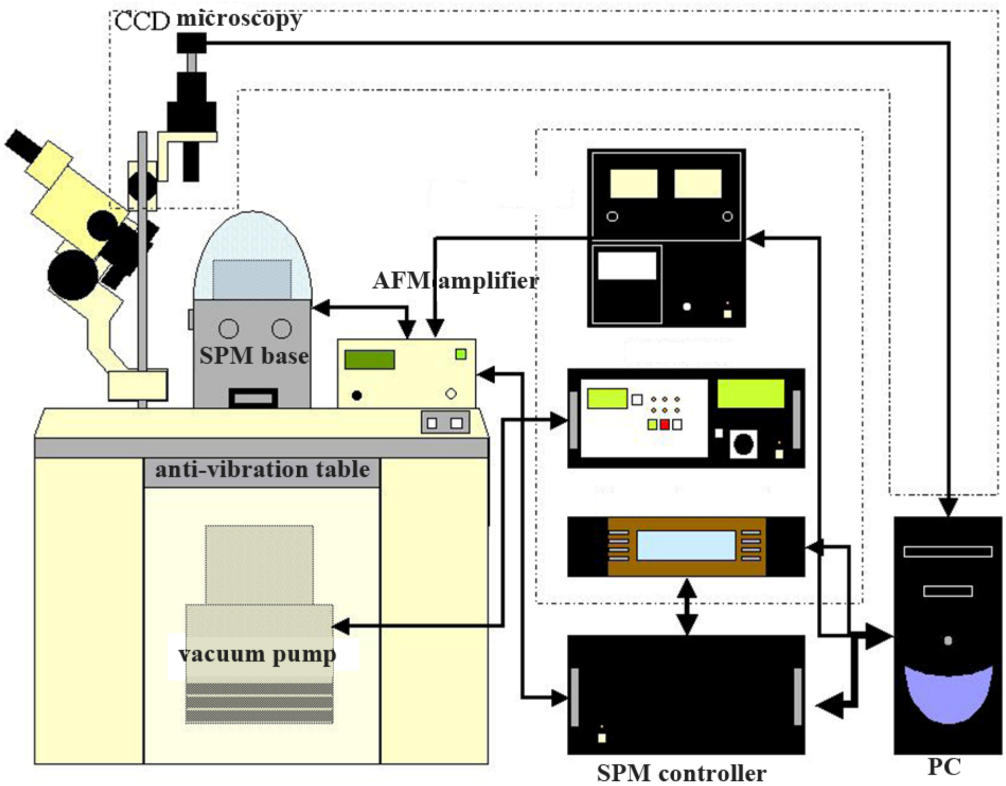

Figure 3. Schematic presentation of JSPM-5200 with interconnected components

The image was analyzed by the program WinSPM (Processing). This program packet allows the user to perform different processing functions in order to improve the quality of the image obtained by the scanning program. These functions include the following: leveling the image, adjusting brightness and contrast, applying different filters, etc. The analysis of the profile on the image of the scanned surface may be done in a number of ways: by Single, Multi, Extra and Multiple Images. With Single Analysis one production line may be placed in any direction within the image, while the distance between two points and the height difference between up to three pairs of markers are measured. With Multi Analysis up to five arbitrary lines in any direction within the image may be placed. With Extra Analysis the roughness of the scanned surface is measured within the set rectangular area, while with Multiple Images Analysis, up to three images may be placed and the profile is analyzed on the same line. We have used the Multi Analysis of Profile.

This program, WinSPM (Processing), also allows generating of three-dimensional images of scanned surface (bird's-eye view). The adjustable parameters are Position (direction of display), Zoom (height along the Z-axis) and Centering (centering the surface in relation to the screen).

We finally use the report making feature, which is used to display images, measurements, profiles and 3D images in the form of the report for printing that are presented in the results of research. It is understood that the format of the A4 page is in vertical layout. The measurement data for the selected 2D image may be displayed.

3. RESULTS

The results of research of enamel surface morphology will be presented by the regression analysis of analyzed etched-treated and non-treated teeth with roughness parameters Ra, Rq, Rzijs and Rz.

In the analysis of the results of obtained roughnesses through AFM the most used are: Roughness Average (Ra), Root Mean Square roughness (Rq), Ten Point Mean Roughness (Rzijs) and Mean Roughness Depth which is determined as the biggest height difference (Rz). Roughnesses are expressed in nanometers (nm) [23,24].

The Figures below, due to space limitation, present one AFM image (each) with appropriate displays of measurements places. Figure 4 shows the general data of AFM image of non-treated sample, while Figure 5 presents the general data of AFM image of etched-treated sample. Figure 6 gives a presentation of measurement places and arithmetic means of average roughnesses of non-treated sample, and Figure 7 presents the measurement places and arithmetic means of average roughnesses of etched-treated sample.

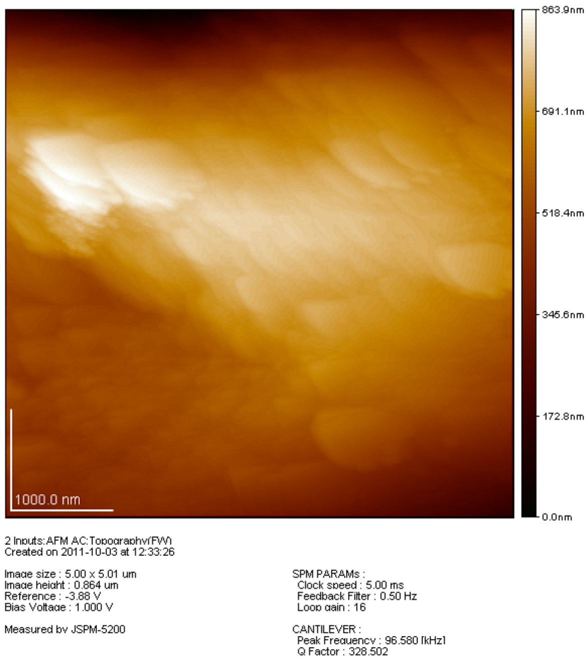

Figure 4. General data of AFM image – (Sample: 1; Non-treated, scan 1)

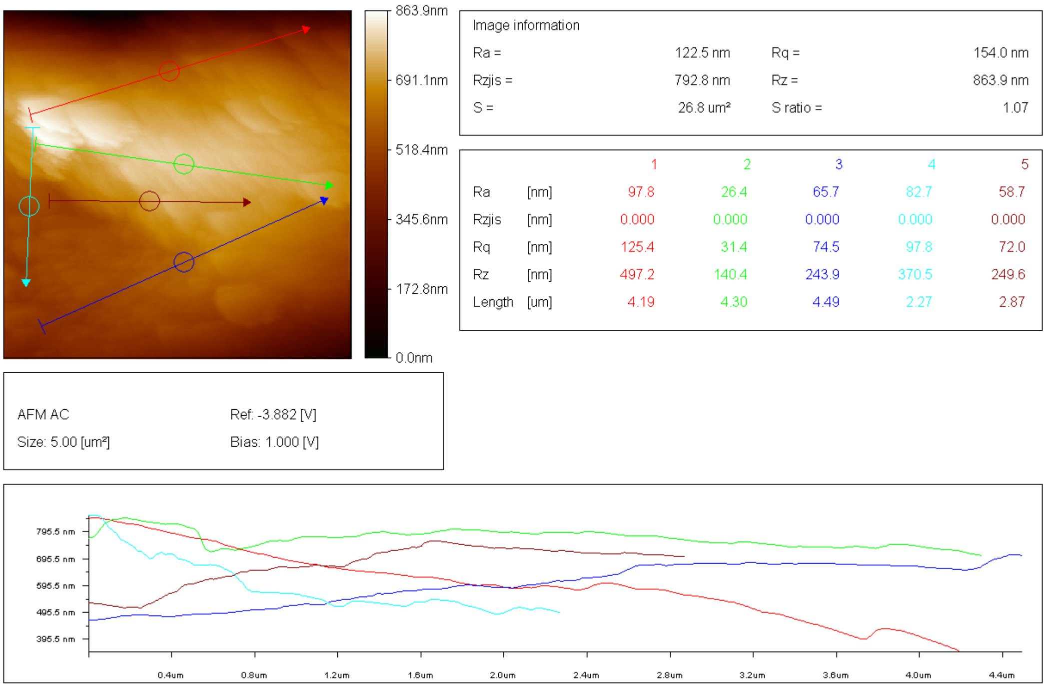

Figure 5. Presentation of measurement places and arithmetic means of average tooth roughnesses – (Sample: 1; Non-treated, image 1).

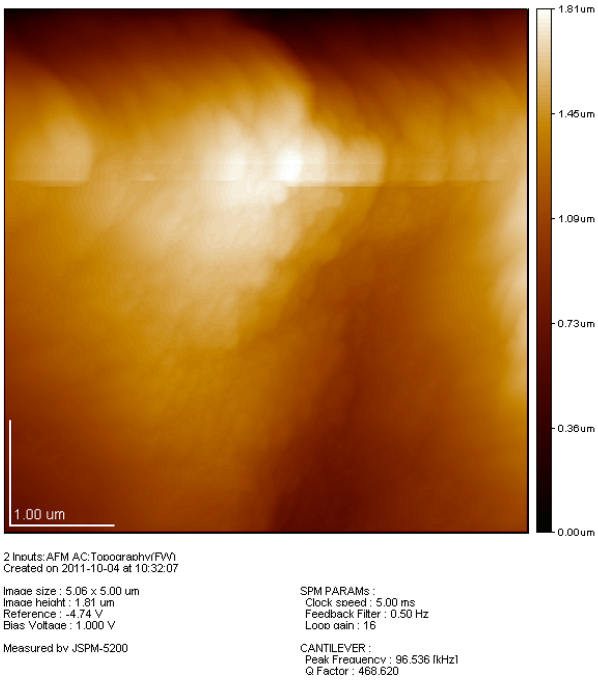

Figure 6. General data of AFM image – (Sample: 1; Etched-treated, image 1)

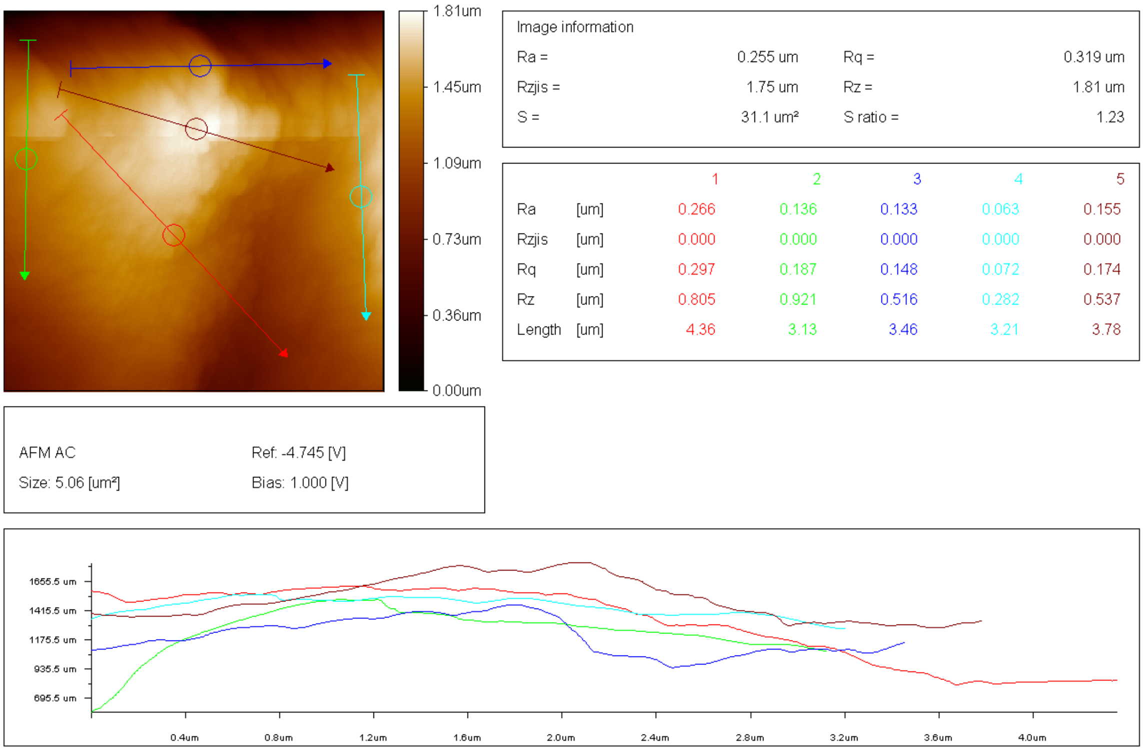

Figure 7. Presentation of the measuring places and arithmetic means of average tooth roughnesses – (Sample: 1; Etched – treated, image 1)

Due to the size of the table, we will here present only the mean values for roughness parameters. In Table 1 the mean values of parameters Ra, Rq, Rzijs and Rz for non-treated teeth are presented.

In Table 2 the mean values of the parameters Ra, Rq, Rzijs and Rz for etched-treated teeth are presented.

Table 1. Mean values of parameters Ra, Rq, Rzijs and Rz for non-treated teeth

Samples: 1-50 Non-treated				
Mean values of non-treated samples	**Ra [nm]**	**Rz[nm]**	**Rq[nm]**	**Rzisj[nm]**
Xsr-U1-NET	66.260	300.320	80.220	792.800
Xsr-U2-NET	16.240	77.360	19.860	216.300
Xsr-U3-NET	22.640	102.840	26.380	265.000
Xsr-U4-NET	55.380	316.420	71.300	461.200
Xsr-U5-NET	22.680	119.080	28.620	161.900
Xsr-U6-NET	111.640	450.100	129.580	1000.000
Xsr-U7-NET	84.380	414.440	104.020	903.600
Xsr-U8-NET	152.260	590.640	174.740	1070.000
Xsr-U9-NET	86.960	418.100	105.120	841.700
Samples: 1-50 Non-treated				
Mean values of non-treated samples	**Ra [nm]**	**Rz[nm]**	**Rq[nm]**	**Rzisj[nm]**
Xsr-U10-NET	22.200	125.760	27.180	309.700
Xsr-U11-NET	70.920	303.740	79.840	677.200
Xsr-U12-NET	38.060	121.660	42.440	246.600
Xsr-U13-NET	27.280	120.860	31.480	286.100
Xsr-U14-NET	53.820	322.140	74.760	453.800
Xsr-U15-NET	23.020	104.180	28.500	176.300
Xsr-U16-NET	117.820	432.480	127.840	913.800
Xsr-U17-NET	82.400	430.920	104.820	962.200
Xsr-U18-NET	147.820	563.560	168.580	976.900
Xsr-U19-NET	86.900	432.980	104.880	770.500
Xsr-U20-NET	33.540	115.900	33.300	355.500
Xsr-U21-NET	65.840	266.540	76.080	895.600
Xsr-U22-NET	26.740	87.780	39.720	236.700
Xsr-U23-NET	22.300	105.320	36.320	251.300
Xsr-U24-NET	55.160	333.540	84.680	467.200
Xsr-U25-NET	21.940	117.220	27.940	181.900
Xsr-U26-NET	66.560	299.380	85.320	864.900
Xsr-U27-NET	16.660	76.840	20.640	228.000
Xsr-U28-NET	24.060	104.460	25.020	293.200
Xsr-U29-NET	53.340	291.940	73.320	394.600
Xsr-U30-NET	22.920	105.020	30.780	191.400
Xsr-U31-NET	111.720	492.520	131.020	920.000
Xsr-U32-NET	82.820	454.540	100.760	836.600
Xsr-U33-NET	145.000	519.700	177.820	900.600
Xsr-U34-NET	89.140	451.180	98.220	733.700

Xsr-U35-NET	33.100	121.540	32.660	339.700
Xsr-U36-NET	63.180	266.120	70.940	1049.600
Xsr-U37-NET	28.840	99.000	44.080	210.600
Xsr-U38-NET	23.180	110.140	38.080	195.900
Xsr-U39-NET	56.860	353.940	91.480	460.100
Xsr-U40-NET	24.220	105.380	28.340	151.700
Xsr-U41-NET	111.440	479.160	132.780	884.700
Xsr-U42-NET	91.620	422.120	106.120	885.100
Xsr-U43-NET	152.140	562.140	189.600	1273.700
Xsr-U44-NET	88.080	473.120	99.420	922.300
Xsr-U45-NET	32.320	187.380	32.220	261.500
Xsr-U46-NET	74.560	329.020	86.680	556.600
Xsr-U47-NET	39.980	123.240	46.640	305.500
Xsr-U48-NET	25.180	134.200	32.660	252.500
Xsr-U49-NET	51.360	304.680	71.500	431.300
Xsr-U50-NET	18.820	113.660	27.340	224.900
Xsr-NET	**60.826**	**275.086**	**74.033**	**552.850**

Table 2. Mean values of parameters Ra, Rq, Rzijs and Rz for etched-treated teeth

Sample: 1-50 Etched - Treated				
Mean values of treated (etched) samples	**Ra [nm]**	**Rz [nm]**	**Rq [nm]**	**Rzisj [nm]**
Xsr-U1-TRE	150.600	612.800	175.600	1750.000
Xsr-U2-TRE	183.800	714.620	210.120	1040.000
Xsr-U3-TRE	107.020	417.640	123.820	568.300
Xsr-U4-TRE	69.460	256.180	74.940	507.400
Xsr-U5-TRE	72.240	290.640	82.580	762.000
Xsr-U6-TRE	43.512	135.060	67.230	304.000
Xsr-U7-TRE	40.520	217.740	50.220	366.700
Xsr-U8-TRE	35.280	170.340	43.240	278.000
Xsr-U9-TRE	34.660	162.980	41.360	325.900
Xsr-U10-TRE	38.000	167.440	45.000	277.800
Xsr-U11-TRE	147.100	612.500	169.460	1660.300
Xsr-U12-TRE	191.720	714.840	206.400	908.900
Xsr-U13-TRE	103.780	400.200	123.780	535.100
Xsr-U14-TRE	68.060	253.460	74.460	574.400
Xsr-U15-TRE	70.800	295.420	83.880	815.300
Xsr-U16-TRE	5.492	33.480	7.330	102.700
Xsr-U17-TRE	40.900	209.640	51.360	382.600
Xsr-U18-TRE	33.360	174.780	42.240	294.900

Xsr-U19-TRE	35.160	152.860	44.400	302.500
Xsr-U20-TRE	39.620	168.280	46.620	283.200
Xsr-U21-TRE	150.440	662.240	174.400	1679.000
Xsr-U22-TRE	186.700	714.260	210.880	1097.100
Xsr-U23-TRE	107.020	399.160	127.040	621.500
Xsr-U24-TRE	67.480	249.740	76.060	572.800
Xsr-U25-TRE	73.840	303.280	82.700	781.900
Xsr-U26-TRE	150.860	547.120	155.660	1534.300
Xsr-U27-TRE	192.180	797.860	205.140	1297.600
Xsr-U28-TRE	99.020	426.860	127.760	433.000
Xsr-U29-TRE	68.180	245.760	78.360	590.200
Xsr-U30-TRE	80.480	257.980	77.540	874.600
Xsr-U31-TRE	15.552	71.700	17.870	208.300
Xsr-U32-TRE	43.680	200.860	50.340	427.900
Xsr-U33-TRE	33.640	182.780	41.280	247.000
Xsr-U34-TRE	34.080	144.000	40.360	329.100
Xsr-U35-TRE	36.720	164.880	47.580	273.900
Xsr-U36-TRE	136.540	603.580	173.320	1619.900
Xsr-U37-TRE	175.620	762.060	223.460	921.800
Xsr-U38-TRE	112.920	367.480	124.160	460.400
Xsr-U39-TRE	63.780	249.100	70.740	694.300
Xsr-U40-TRE	64.940	283.520	86.740	722.800
Xsr-U41-TRE	79.552	135.720	67.430	483.500
Xsr-U42-TRE	43.380	217.580	48.600	369.800
Xsr-U43-TRE	35.800	167.880	44.500	367.300
Xsr-U44-TRE	36.440	165.900	44.540	365.200
Xsr-U45-TRE	43.380	162.740	43.980	288.800
Xsr-U46-TRE	160.820	596.220	161.340	2009.400
Xsr-U47-TRE	191.280	637.160	212.880	1148.500
Xsr-U48-TRE	114.080	390.580	115.820	500.700
Xsr-U49-TRE	75.120	248.560	81.660	654.100
Xsr-U50-TRE	71.360	298.300	78.340	620.900
Xsr-NET	**85.119**	**336.315**	**97.090**	**684.712**

The regression parameters of samples present the functional dependence of roughness in relation to the place of measurement of each specified roughness of etched-treated as opposed to non-treated samples.

Table 3 presents the regression parameters of non-treated teeth by roughnesses (Ra, Rq Rzijs, Rz) of the adhesive.

Table 4 presents the regression parameters of etched-treated teeth by roughnesses (Ra, Rq, Rzijs, Rz) of adhesives.

Table 3. Regression parameters of sample 1, 1-50, non-treated – Total data (with arithmetic means of measurement images)

Parameter designation	Regression equation (y=ax+b)	a	b	Determination coefficient (R^2)	Correlation coefficient (r)
NET-Ra	y = -0.0093x + 61.988	-0.0093	61.988	0.0002	0.0141
NET -Rz	y = 0.0121x + 273.57	0.0121	273.57	0.00002	0.0045
NET -Rzijs	y = -0.2249x + 581.08	-0.2249	581.08	0.0025	0.0500
NET -Rq	y = 0.0027x + 73.699	0.0027	73.699	0.00001	0.0032

Table 4. Regression parameter of samples 1-50, etched-treated – Total data (with arithmetic means of measuring images)

Parameter designation	Regression equation (y=ax+b)	a	b	Determination coefficient (R^2)	Correlation coefficient (r)
TRE-Ra	y = -0.0105x + 86.439	0.0105	86.439	0.0001	0.0100
TRE –Rz	y = -0.1813x + 359.07	-0.1813	359.07	0.0031	0.0557
TRE –Rzijs	y = -0.0367x + 689.32	-0.0367	689.32	0.00003	0.0055
TRE –Rq	y = -0.0398x + 102.08	-0.0398	102.08	0.0016	0.0400

4. DISCUSSION

The reason we chose the investigation by AFM is in the fact that AFM is currently the most reliable possible method for determining the degree of damage of enamel caused by its etching. There are numerous studies that showed the convenience of using AFM analyses to monitor both qualitative and quantitative changes on enamel surface [25−30].

Only with the development of AFM technology, a possibility was opened to monitor more subtle changes in enamel surface [31]. The AMF studies start to be used more and more in the researches in dentistry too, which monitor surface changes such as dental plaque and mineralized and coloured layers, surface properties of different materials and morphological and mechanical changes in mineralized tissues.

AFM technology is suitable for monitoring the enamel structure ranging from individual crystals to prisms (from nano to micro level) [32−34]. At lower magnifications it presents the enamel prisms as deep recesses. Individual crystals are arranged in parallel and present the elongated hydroxyapatite plane. The crystal surfaces are unevenly compacted in this way forming the enamel surface roughness. At higher magnifications the crystals, show characteristic hexagonal appearance, each set at an angle of 60 degrees.

AFM has a number of significant advantages when it comes to investigation of dental tissues compared to the other techniques and especially SEM (Scanning Electron Microscopy); the basic possibility is in obtaining a three-dimensional profile of the test surface.

There are numerous papers [29,30,35−37] that underline and confirm that the AFM technique is especially suitable for investigation of the erosions on enamel surface, even those at the nano level. AFM can be used to clearly monitor the enamel structures from individual crystals to prisms (from nano to micro level) [33,35].

A big number of researches have been undertaken to date in order to determine the degree of enamel damage cause by the etching procedure, with the results that were compared between certain researchers, and which were confusing, to say the least [38−43]. The explanation of these and similar findings is sought in partly different methodology, the size of the sample, insufficient precision at work, different methods of preparation-treating the preparations intended for research, etc.. However, based on a thorough and very extensive research i.e. analysis of these results (over 90% of papers that dealt with this problematic in the past 15 years) we believe that the differences in the results between individual researches and researchers, even when the identical methodological procedures were applied, are probably due to different mineralization of enamel of tested teeth. This factor – the degree of tooth mineralization is hard or almost impossible to objectivize in this type of research, and which would give a fuller picture of the state of tooth enamel. This factor is influenced by the race (it is different with the white and the black race), and is also different within the same race with different nations. Having in mind that mineralization is a dynamic process, i. e. that enamel is in constant interaction with the oral cavity ecosystem [44,45], the demineralization and remineralization processes are always present i. e.

they are carried out permanently, while their balance ensures the enamel integrity. The current state depends on the patient's age, diet, type of food and drinks (special influence is exerted by fizzy drinks [46−51]), the method and regularity of keeping oral hygiene, individual tendency to plaque creation, etc.

Based on tables 1 and 2 with the values of the parameters Ra, Rq, Rzijs and Rz, and their mean values for non-treated and etched-treated teeth we conclude that the mean values of roughnesses Ra, Rz, Rq, Rzijs of etched-treated samples are higher compared to the mean values of parameters of roughnesses of non-treated counterparts. Expressed in the percentages, the mean value of parameters Ra of etched-treated teeth is by 39.93% higher, that of Rz roughness is by 22.26% higher, while Rq and Rzijs roughnesses are by 31,14% and 23,85% higher, respectively, compared to the same roughness parameters of non-treated samples.

The obtained results of the analysis of etched enamel as a preparation for bonding brackets suggest significant distortions in the structure compared to non-treated. All the parameters for measuring roughness are significantly higher with the surfaces treated with acid. Investigating etched enamel areas show on AFM images, in addition to micro concavities, the devastation areas of prismatic structures in wider intervals. Nevertheless, it is not possible to completely compare the findings due to the different degree of enamel mineralization with different persons and teeth. Similar results were recorded in other studies too. Endo et al. reported that enamel treated with 37% phosphoric acid becomes very porous with exposure to numerous crystals and the honeycomb structure [52]. The main advantage of AFM compared to other technologies is in the fact that it provides roughness quantification [53]. The changes of enamel surface compared to the type of surface treatment were reported [54−57]. Changes were observed in the morphology, surface roughness and the profile depth of treated enamel. The erosion effects are different compared to the acidity of treating agents (acid, adhesives, etc.) and are related with clinical effects [58]. Surface hardness of enamel may be determined on AFM images based on the curve of cantilever force [59,60]. The easiest way to achieve this is to record the curves while the cantilever tip performs raster scanning through the sample [61,62]. Bayer et al. [63] investigated the creation of erosions under the influence of different acids on human enamel and discovered the existence of its loss, and also that pH is not the most significant in determining the erosive potential. Surface hardness may be determined by AFM on the basis of the curves after scanning [64, 65]. Thus, it was determined that different acids, and especially phosphoric acid decreases the surface hardness, whereby pH is not a decisive factor. A number of possible factors are mentioned, whereby the self-bonding adhesives have higher hardness without the influence of thermal changes unlike the classical etching with acid. In etching with phosphoric acid, there is penetration of water in the contact zone that makes the enamel softer due to the loss of calcium [66]. Hanning et al. [67] reported that individual hydroxyapatite crystals on enamel surface are in a way encapsulated in the self-bonding adhesive system at the nano-level.

5. CONCLUSIONS

Based on the obtained results of research, their statistical processing and detailed analyses, the following conclusions may be made:

− Based on the obtained values for the parameters Ra, Rq, Rzijs and Rz and their mean values for non-treated and etched-treated teeth, we come to a conclusion that the mean values of roughnesses of Ra, Rq, Rzijs and Rz of etched-treated samples is higher than mean values of parameters of roughnesses of their non-treated counterparts. Expressed in percentages, the mean value of parameters of roughness of Ra of etched-treated samples is higher by 39.93%, of Rz roughness by 22.26%, and of Rq and Rzijs roughnesses are by 31,14% and 23,85% higher, respectively, compared to the same roughness parameters of non-treated samples.

− The obtained results of the analysis of etched enamel as preparation for bonding brackets show significant distortions in the structure compared to the treated counterparts. All the parameters for measuring roughness are significantly higher with the surfaces treated with acid. Investigating the etched enamel areas show on AFM images, in addition to micro concavities the areas of devastation of prismatic structures at bigger intervals.

− Preparing enamel for bonding orthodontic bracket by etching with acid causes extensive changes in the nanostructure of enamel crystals that can influence its biomechanical properties and resistance toward the action of acids from oral biofilm (caries) or microenvironment (erosion).

− Enamel treated with acids may be considered risky and requires enhanced preventive care because brushing the teeth with toothpaste containing with fluoride is not sufficient.

− After debonding of orthodontic brackets bonded with composite material by enamel etching a long and complex treatment of enamel remineralization is necessary.

− Manufacturer's instruction on the time of enamel etching should be strictly adhered to, because the longer it is, the bigger is the damage of the central prism areas.

6. REFERENCES

[1] V. Jeromilov, *Osnove stomatoloških materijala*, Stomatološki fakultet, Zagreb 2005.

[2] J. Vojinović i sar., *Biologija zuba*, Naučna knjiga, Beograd 1990.

[3] O. E. Smith, A. Nanci, *Overview of morphological changes in enamel organ cells associated with major events in amelogenesis*, Int J Dev Biol., Vol. 39 (1995) 153−161.

[4] M. A. Major, S. J. Nelson, *Dental anatomy, physiology, and occlusion*, 8th ed., W. B. Saunders, Philadelphia 2003.

[5] M. Bath-Balogh, M. J. Fehrenbach, *Illustrated Dental Embryology, Histology, Anatomy*, 2nd ed., W. B. Saunders, Philadelphia 2006.

[6] R. C. Melfi, K. E. Alley, *Permar's oral embryology and microscopic anatomy: a textbook for students in dental hygiene*, Williams & Wilkins 1996.

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

[8] N. Harris, F. Garcia-Godoy, N. Christine, *Primary Preventive Dentistry*, 7th edition, Pearson 2007.

[9] M. Goldberg, P. R. Garant, S. Takuma, *Cell Biology of Tooth Enamel Formation*, Karger 1990.

[10] J. P. Simoner, J. C. Hu, *Dental enamel formation and its impact on clinical dentistry*, J Dent Educ., Vol. 65 (2001) 896−905.

[11] D. R. Beech, T. Jalaly, *Bonding of polymers to enamel: Influence of deposits formed during etching, etching time and period of water immersion*, J Dent Res., Vol. 59 (1980) 1156−1162.

[12] M. J. Shinchi, K. Soma, N. Nakabayashi, *The effect of phosphoric acid concentration on resin tag length and bond strength of a photo-cured resin to acid-etched enamel*, Dent Mater., Vol. 16 (2000) 324−329.

[13] B. Van Meerbeek, J. De Munck, Y. Yoshida, S. Inoue, M. Vargas, P. Vijay, et al., *Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges*, Oper Dent., Vol. 28 (2003) 215−235.

[14] N. Nakabayashi, D. H. Pashley, *Chapter III, Acid Conditioning and Hybridization of Substrates, Hybridization of Dental Hard Tissues*, Tokyo: Quintessence Publishing Co., Ltd., 1998, 37−39.

[15] M. Hannig, H. Bock, B. Bott, W. Hoth-Hannig, *Inter-crystallite nanoretention of self-etching adhesives at enamel imaged by transmission electron microscopy*, Eur J Oral Sci., Vol. 110 (2002) 464−470.

[16] L. R. Legler, D. H. Retief, E. L. Bradley, F. R. Denys, P. L. Sadowsky, *Effects of phosphoric acid concentration and etch duration on the shear bond strength of an orthodontic bonding resin to enamel*, *An in vitro study*, Am J Orthod Dentofacial Orthop., Vol. 96 (1989) 485−492.

[17] W. W. Barkmeier, A. J. Gwinnett, S. E. Shaffer, *Effects of reduced acid concentration and etching time on bond strength and enamel morphology*, J Clin Orthod., Vol. 21 (1987) 395−398.

[18] B. B. Cerci, L. S. Roman, O. Guariza-Filho, E. S. Camargo, O. M. Tanaka, *Dental enamel roughness with different acid etching times: Atomic force microscopy study*, Eur J Gen Dent., Vol. 1 (2012) 187−191.

[19] E. B. L. Casas, F. S. Bastos, G. C. D. Godoy, V. T. L. Buono, *Enamel wear and surface roughness characterization using 3D profilometry*, Tribol Int., Vol. 41 (2008) 1232−1236.

[20] S. Sharma, S. E. Cross, C. Hsueh, R. P. Wali, A. Z. Stieg, J. K. Gimzewski, *Nanocharacterization in Dentistry*, Int J Mol Sci., Vol. 11 (2010) 2523−2545.

[21] A. Méndez-Vilas, J. M. Bruque, M. L. González-Martín, *Sensitivity of surface roughness parameters to changes in the density of scanning points in multi-scale AFM studies*, Application to a biomaterial surface, Ultramicroscopy, Vol. 107 (2007) 617−625.

[22] L. Matija, et al., *Uvod u nanotehnologije*, Nauka, Beograd 2011.

[23] Đ. Mirjanić, V. Mirjanić, J. Vojinović, *Uticaj agresivnog napitka na nanostrukturu gleđi zuba*, Zbornik radova „Savremeni materijali", Banja Luka 2014, 575−585.

[24] Đ. Mirjanić, V. Mirjanić, J. Vojinović, *AFM analiza nanostrukture gleđi nakon djelovanja agresivnog napitka*, Zbornik radova „Savremeni materijali", Banja Luka 2015, 603−617.

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

[26] S. R. Grobler, P. J. Senekal, J. A. Laubscher, *In vitro demineralization of enamel by orange juice, apple juice, Pepsi Cola and Diet Pepsi Cola*, Clin Prev Dent., Vol. 12 (1990) 5−9.

[27] M. Eisenburger, M. Addy, *Influence of liquid temperature and flow rate on enamel erosion and surface softening*, J Oral Rehabil., Vol. 30 (2003) 1076−1080.

[28] A. J. White, C. Yorath, V. Hengel, S. D. Leary, M. C. D. N. J. M. Huysmans. M. E. Barbour, *Human and bovine enamel erosion under 'single-drink' conditions*, Eur J Oral Sci., Vol. 118 (2010) 604–609.

[29] A. Lussi, T. Jaeggi, S. Schärer, *The influence of different factors on in vitro enamel erosion*, Caries Res., Vol. 27 (1993) 387–393.

[30] 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.

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

[32] 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.

[33] P. Schaad, E. Paris, F. J. Cuisinier, J. C. Voegel, *Atomic force microscopy study of human tooth enamel surfaces*, Scanning Microscopy, Vol. 7 (1993) 1149−1152.

[34] 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.

[35] 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.

[36] 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.

[37] 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.

[38] L. Eslamian, A. Borzabadi-Farahani, N. Mosavia, A. Ghasemi, *A comparative study of shear bond strength between metal and ceramic brackets and artificially aged composite restorations using different surface treatments*, The European Journal of Orthodontics, 2011.

[39] G. V. Newman, *First direct bonding in ortodontia*, Am J. Orthod Dentofacial Orthop, Vol. 101 (1992) 190−192.

[40] D. Battes, D. H. Retief, H. C. Jamison, F. R. Denys, *Effects of acid etch parameters on enamel topography and composite resin-enamel bond strength*, Pediatric Dentistry, Vol. 4 (1982) 106−110.

[41] D. H. Retief, *A comparative study of three etching solutions; effects on enamel surface and adhesive-enamel interface*, J. Oral Rehabil., Vol. 2 (1975) 75−96.

[42] B. Wendl, H. Droschl, *A comparative in vitro study of the strength of directly bonded brackets using different curing techniques*, European Journal of Orthodontics, Vol. 26−5 (2004) 535.

[43] V. Mitić, M. Janošević, *Uticaj vremena aplikacije ortofosforne kiseline na jačinu veze ortodontskih bravica na zub*, Serbian Dental J., Vol. 55 (2008) 23−33.

[44] 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.

[45] S. Chandra, et al., *Textbook of Dental and Oral Histology with Embryology and MCQS*, 2th edition, 2/E. Jaypee Brothers Medical Publishers (P) Ltd. 2011.

[46] E. O'Sullivan, A. Milosevic, *UK National Clinical Guidelines in Paediatric Dentistry: diagnosis, prevention and management of dental erosion.* International Journal of Paediatric Dentistry, Vol. 18−1 (2008) 29–28.

[47] M. Edwards, S. L. Creanor, R. H. Foye, W. H. Gilmour, *Buffering capacities of soft drinks: the potential influence on dental erosion*, Journal of Oral Rehabilitation, Vol. 26−12 (1999) 923–927.

[48] C. R. Dugmore, W. P. Rock, *A multifactorial analysis of factors associated with dental erosion* (PDF, British Dental Journal, Vol. 196−5 (2004) 283–286; discussion: 273.

[49] S. R. Grobler, G. van der Horst, *Biochemical analysis of various cool drinks with regard to enamel erosion, de- and remineralization*, J Dent Assoc S Africa, Vol. 37 (1982) 681−684.

[50] A. J. Rugg-Gunn, J. H. Nunn, *Diet and dental erosion*, *Nutrition, diet, and oral health*, Hong Kong: Oxford University Press 1999.

[51] M. J. Larsen, B. Nyvad, *Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate*, Caries Res., Vol. 33 (1999) 81−87.

[52] T. Endo, R. Ozoe, K. Shinkai, M. Aoyagi, H. Kurokawa, Y. Katoh, S. Shimooka,

Shear bond strength of brackets rebonded with a fluoride-releasing and recharging adhesive system, Angle Orthodontist, Vol. 79 (2009) 564−570.

[53] J. P. Loyola-Rodriguez, V. Zavala-Alonso, E. Reyes-Vela, N. Patiño-Marin, F. Ruiz, K. J. Anusavice, *Atomic force microscopy observation of the enamel roughness and depth profile after phosphoric acid etching*, J Electron Microsc (Tokyo), Vol. 59 (2010) 119−125.

[54] Đ. Mirjanić, *Atomska mikroskopija nanostrukture gleđi*, magistarski rad, Medicinski fakultet Univerziteta u Banjoj Luci, Banja Luka 2012.

[55] M. Beyer, J. Reichert, J. Bossert, B. W. Sigusch, D. C. Watts, K. D. Jandt, *Acids with an equivalent taste lead to different erosion of human dental enamel*, Dent Mater., Vol. 27 (2011) 1017−1023.

[56] M. Hannig, C. Hannig, *Nanomaterials in preventive dentistry*, Nature Nanotechnol., Vol. 5 (2010) 565−569.

[57] C. R. Mattick, R. S. Hobson, *A comparative micro-topographic study of the buccal enamel of different tooth types*, J Orthod., Vol. 27 (2000) 143−148.

[58] Y. Hashimoto, Y. Hashimoto, A. Nishiura, N. Matsumoto, *Atomic force microscopy observation of enamel surfaces treated with self etching Primer*, Dent Mater J., Vol. 32−1 (2013) 181–188.

[59] K. Nakajima, H. Yamaguchi, J. C. Lee, M. Kageshima, T. Ikehara, T. Nishi, *Nanorheology of polymer blends investigated by atomic force microscopy*, Jpn J Appl Phys. Part 1: Regular Papers and Short Notes and Review Papers, Vol. 36 (1997) 3850−3854.

[60] T. Tsujioka, K. Tsuji, *Metal-vapor deposition modulation on soft polymer surfaces*, Appl Phys Express, Vol. 5 (2012) 021601−3.

[61] D. R. Baselt, J. D. Baldeschwieler, *Imaging spectroscopy with the atomic force microscope*, J Appl Phys., Vol. 76 (1994) 33−38.

[62] M. Radmacher, M. Fritz, J. P. Cleveland, D. A. Walters, P. K. Hansma, *Imaging adhesion forces and elasticity of lysozyme adsorbed on mica with the atomic force microscope*, Langmuir, Vol. 10 (1994) 3809−3814.

[63] M. Beyer, J. Reichert, J. Bossert, B. W. Sigusch, D. C. Watts, K. D. Jandt, *Acids with an equivalent taste lead to different erosion of human dental enamel*, Dent Mater., Vol. 27 (2011) 1017−1023.

[64] S. E. Bishara, V. V. Gordan, L. VonWald, J. R. Jakobsen, *Shear bond strength of composite, glass ionomer, and acidic primer adhesive systems*, Am J Orthod Dentofacial Orthop., Vol. 115 (1999) 24−28.

[65] F. L. Romano, A. B. Correr, L. Correr-Sobrinho, M. B. B. A. Magnani, A. C. O. Ruellas, *Clinical evaluation of the failure rates of metallic brackets*, J Appl Oral Sci., Vol. 20 (2012) 228−234.

[66] S. Kitayama, T. Nikaido, M. Ikeda, R. M. Foxton, J. Tagami, *Enamel bonding of self-etch and phosphoric acid-etch orthodontic adhesive systems*, Dent Mater J., Vol. 26 (2007) 135−143.

[67] M. Hannig, C. Hannig, *Nanomaterials in preventive dentistry*, Nature Nanotechnol., Vol. 5 (2010) 565−569.

АФМ АНАЛИЗА ОШТЕЋЕЊА ГЛЕЂИ УСЉЕД ЈЕТКАЊА ОРТОФОСФОРНОМ КИСЕЛИНОМ

Сажетак: Имајући у виду да у области ортопедије вилица и сродних научних области не постоје научна истраживања која користе данас најсавременију технологију засновану на атомској микроскопији (АФМ) за испитивање наноструктуре глеђи зуба након јеткања 37% ортофосфорном киселином, у раду ће се утврдити, на данас најпоузданији могући начин, АФМ (Atomic Force Microscopy) методом степен оштећења глеђи зуба након њеног нагризања (јеткања) у циљу фиксирања ортодонтских бравица. Степен оштећења глеђи након јеткања не може се реално сагледати методом тзв. „мреже", јер се оштећење, на овај начин, не може сагледати просторно (у дубини оштећења), већ се може видјети само оштећење у једној равни. Прецизнија анализа може се добити примјеном АФМ технологије, као најсавременије методе коју имамо у овом случају.

Кључне ријечи: атомска микроскопија, оштећење глеђи, јеткање, ортофосфорна киселина.