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AFM TESTING OF NANOSTRUCTURE OF RESILIENCE ORTHODONTIC BONDING SOLUTIONS ORTHODONTIC ADHESIVE

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Abstract: Nanostructure of Resilience Orthodontic bonding solutions, an orthodontic adhesive that is mostly used nowadays in the orthodontic practice, is analyzed in the paper. After determining the adhesive properties, a correlation was established between the nanostructure of tested adhesive and the tooth bracket bond strength. Based on AFM images of the analyzed adhesive, by way of correlations of arithmetic means of debonding force (I) and average adhesive roughnesses (Ra, Rq, Rzijs, Rz) we come to a conclusion that by increasing the average adhesive roughnesses, increases the debonding force too (I). After that we compared the obtained results with the other adhesives that are also most commonly used. It was observed that with all the roughness parameters (Ra, Rz, Rzijs and Rq) the strongest bond was achieved with Resilience Orthodontic bonding solutions, followed by Heliosit, Orthodontic (Ivoclar, Vivadent), GC Fuji Ortho LC, while the weakest bond was with ConTec LC − Dentarum. Higher roughness of Resilience Orthodontic bonding solutions at the nano level is most probably due to a bigger number of thorns which penetrate into micro cavities developed under the action of acids. Higher roughness is a consequence of the chemical structure itself of the composite material.
Keywords: orthodontic adhesive, nanostructure, Atomic Force Microscopy (AFM).

1. INTRODUCTION

Tooth enamel is made of a billion crystals of carbonized hydroxylapatite [1−3] that are packed in individual prisms winding from enamel-dentin border toward the tooth surface. When observed on cross-sections by electronic microscopes, the enamel prisms do not have an appearance of a prism (small stick), but are seen as structures in the form of a key hole, 6−8 microns in diameter. With such an appearance, a larger part is differentiated, designated as the „head“ while the narrower is designated as „tail“. Each head fits between two tails. The crystals are in the area of the head lined along the longitudinal axis, designated as a „C axis“, while on the periphery („tail“) they are ordered at a 30^{o} angle of [4−6].

The mineral phase with mature enamel takes up about 87% of total volume of enamel mass, and makes over 95% of the weight mass, of which only 5% belongs to organic matters and water (other biological mineralized tissues contain about 20%). 3-5% of voluminous mass is made of porosities formed from the network of channels. Through it, throughout the whole enamel cover, diffuse the fluids, ions and the small molecules. This area is located between the prisms, but also between the crystals. This network is joined by morphological structures more rich in proteins such as the above mentioned striae of Retzius, enamel tufts and spindles. The canalicular system is considered to have a protective role because 1) it enables physiological remineralization of enamel prisms throughout life and 2) space, liquids and proteins partly participate in amortization of big pressures that are released during chewing and prevent forming of fractures. At the same time, this canalicular system enables penetration of acids, and even of bacteria and helps the development of caries and erosions. [3−5,7−8].

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 , and the shallow grooves referred to as *perikymata* appear. At certain places, especially with deciduous teeth, there are a number of microns of enamel on the surface without prismatic organization – *aprismatic enamel* [3−4].

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Although enamel has pronounced hardness, it is also especially fragile at the same time and glass-like, and as such it would be prone to breaking. Despite that, enamel can withstand 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 [9−11].

Enamel is in constant dynamic communication with the oral cavity ecosystem. Demineralization and remineralization processes are always present and their balance ensures enamel integrity. If external aggressive factors direct the balance toward demineralization activities, the integrity of the crystal grid weakens, hardness and resistance of enamel reduces, which, after crossing a certain limit of mechanical resistance of enamel, leads to its cracking and formation of cavities, as a beginning of irreversible damage [7,8].

Etching the enamel with acid causes selective de-mineralization that increases the free surface energy, and at the same time the porosity as well as the contact surface too. Bonding to enamel (adhesiveness) [12] depends on the ability of resin to penetrate into the area between the crystal prisms [13] which leads to macromechanical retention. The infiltrated resin encloses covers the individual hydroxylapatite crystals forming microthorns [14] and thus creates a hybrid layer which accomplishes the retention mechanism at the nano-level between the tooth and resin [15]. These microthorns probably contribute to adhesion more than macrothorns that penetrate the space between the enamel prisms [16].

The retention abilities of etched enamel depend on the chemical structure of enamel mineral phase, the type of acid and etching time [17]. Researches have shown that the variations in the etching time from 15 to 90 seconds with 35-37% phosphoric acid do not influence the shear bond strength of orthodontic brackets much [18].

With the etching time, damage is bigger and this is primarily manifested in covering the whole enamel prisms which happens in the first 15 seconds. In further course between 15 and 30 seconds the devastation mainly spreads in the depth affecting the central prism regions [19]. Most studies that conducted the description of etched enamel used SEM technology providing the qualitative picture only [20, 21]. The state-of-the art technology will be used here based on Atomic Force Microscopy (AFM) to examine the nanostructure of orthodontic adhesives for bonding the brackets to teeth [22,23].

The AFM analysis of the profile of dental enamel treated with 35% phosphoric acid is presented in Figure 1.

Profile A-B presents the comparison between T15 (a) and T30 (b). Convexity vs. recess ratio is the biggest with T30 (red). The width of the prisms and the walls does not significantly differ between T15 and T30 (green), while the distance of the prism tip from the base is bigger with T30 (blue) which represents higher demineralization and depth of the prism base [24].

2. MATERIAL AND METHODS

Nanotechnological device JSPM-5200 which is located in NanoLab module for biomedical engineering at the Faculty of Mechanical Engineering of Belgrade University [25−27] was used to test adhesive nanostructure. This is an integrated nanosystem with a number of operating modes which enables the realization of the following functions: STM, AFM, MFM, ECSPM etc.

JSPM-5200 consists of AFM base, the anti-vibration table, AFM amplifier, SPM controller, computer and optional components such as the microscopic system with CCD camera, vacuum system, etc. [28]. Adhesive samples are fixed to the AFM microscope holder.

Testing the surface was done in „contact mode“ function, which means that the physical contact between AFM probe and the tooth surface is a constant force.

The scan was analyzed by using the program WinSPM (Processing). This program package enables the user to perform different processing functions to improve the quality of the image obtained by the scanning program. These functions include: image leveling, adjustment of the light and contrast, application of different filters, etc. The analysis of the profile in the image of the scanned surface may be done in a number of ways: Single, Multi, Extra and Multiple Images. With Single analysis, one production line may be placed in whichever direction within the image, while the distances between two points and the height difference between up to three marker pairs are measured. With Multi Analysis up to five arbitrary lines in whichever direction within the image may be placed. With Extra analysis the roughness of the scanned area is measured within the placed rectangular area, while with Multiple Images Analysis up to three images may be placed, and the profile is analyzed on the same line. Here we used the Multi analysis of the profile.

This program, WinSPM (Processing) also enables generation of three-dimensional images of the scanned area (bird-eye-view). The parameters that may be adjusted are the following: Position (direction of view), Zoom (height per Z-axis) and Centering (centering the surface in relation to the screen).

We finally use the function of making reports which is used to display images, profiles and 3D images in the form of reports for printing that are presented in research results. It is implied that the format of the page is A4, in vertical layout. The data on measuring for the selected 2D image may be presented.

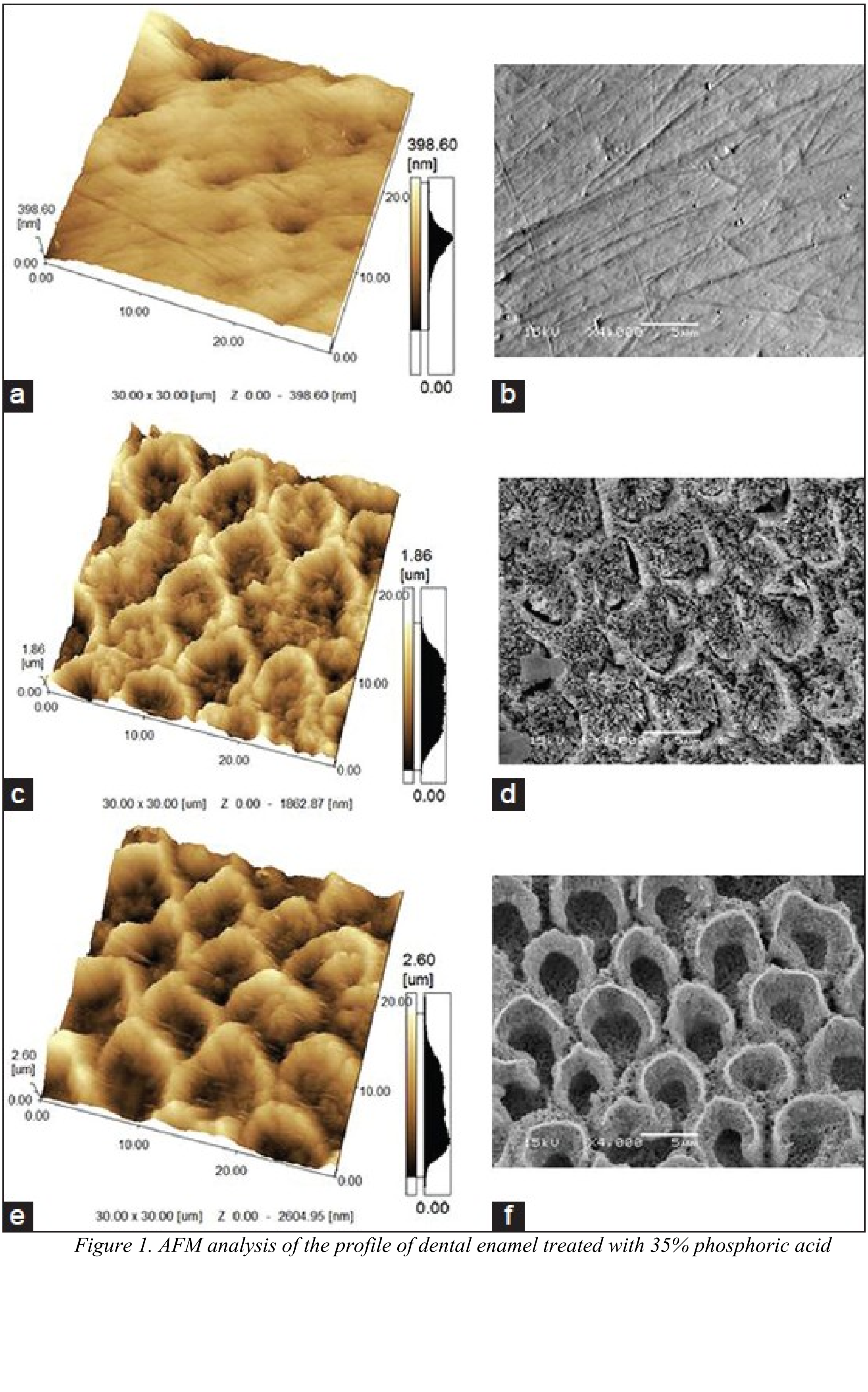

Figure 1. AFM analysis of the profile of dental enamel treated with 35% phosphoric acid

One of the orthodontic adhesives that are most commonly used nowadays in the clinical procedure, Resilience Orthodontic bonding solutions, Ortho Technology inc. Florida was analyzed in order to test the nanostructure of orthodontic adhesive for bonding brackets to teeth. The topography of nanostructure of adhesive Resilience Orthodontic bonding solutions, Ortho Technology inc. Florida will be used for statistical analysis. The topography represents the surface of adhesive nanostructure obtained by calculating the roughnesses Ra, Rq, Rzijs and Rz. Each sample will have 256 lines. The dimensions of each of the observed nanostructures are calculated for statistical analysis, while the main parameter is presented by the height of certain nanostructures, i.e. the difference between „the highest hill" and the „deepest valley" along the Z-axis.

3. THE RESULTS

The results of the study will be presented in the following manner:

– AFM images of the sample of the adhesive Resilience Orthodontic bonding solutions, Ortho Technology inc. Florida.

– Regression analysis of the analyzed adhesive with regression parameters and comparison of the samples by average roughness for the observed adhesive.

Due to the limitation of space we will present here only one image with appropriate presentations of measuring places and arithmetic means of average roughness of analyzed adhesive Resilience Orthodontic bonding solutions, Ortho Technology inc. Florida.

General data of AFM scan of Resilience Orthodontic bonding solutions are presented in Figure 2, while Figure 3 gives a presentation of measurement places and arithmetic means of average roughnesses of the adhesive Resilience Orthodontic bonding solutions.

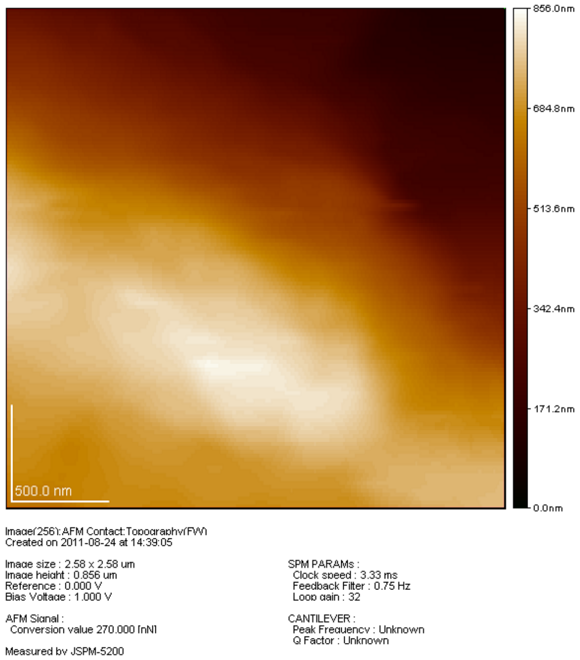

Figure 2. General data of AFM image – Resilience Orthodontic bonding solutions

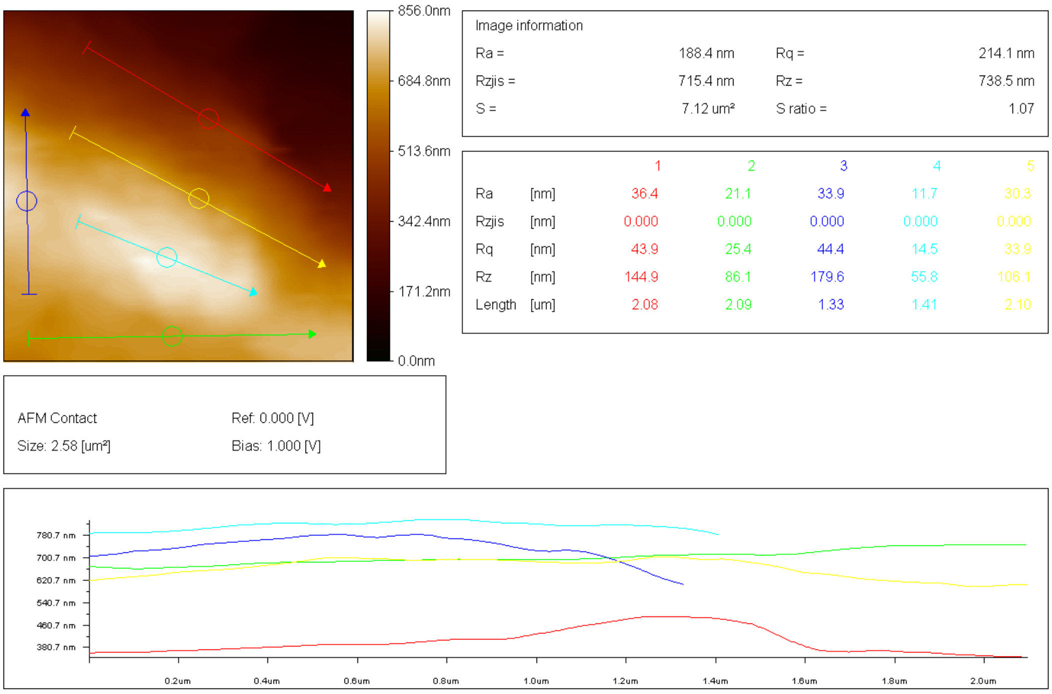

Figure 3. Presentation of the measuring places and arithmetic means of average roughnesses of the adhesive Resilience Orthodontic bonding solutions

The regression parameters of the samples show the functional dependence of average adhesive roughnesses relative to the place of measurement.

Roughness is defined as a complex set of irregularities or bulging and prongs which give appearance to the surface and affect wetting, the quality of adhesion and lightness. Although it is underlined that micromechanical roughness is a basis of a good junction between the etched enamel and resin, precise characteristics of enamel necessary to realize such a bond are not known [22].

The influence that roughness has on the bond strength is not completely understood either. [23]. Higher roughness is assumed to make a bigger contact area through which contact with resin is realized, and thereby a stronger bond too [28].

Something that has not been investigated so far in detail is the surface roughness at microscopic level [29] where nano characterization of surface roughness could provide biophysical mechanisms on enamel surface [30]. AFM with high lateral and vertical resolution enables testing the roughness at micro and nano levels without higher interference of macroscopic components such as the wavy surface [31]. AFM microprobe does not require any sample preparation and consequently jeopardizing the original surface. Thereby it represents a direct way to experimentally detect and quantify the surfac roughness.

A number of different parameters provided in Table 1 are used to interpret profilometry results of roughness.

Table 1. Different parameters for calculating the surface roughness

Parameters	Description
Ra	Arithmetic mean of all the deviations of the profile from the centerline
Rq	Geometric mean of all the deviations of the profile from the centerline
Rz	The center of five roughness recesses of five alternant profile lengths
Rmax	The biggest of five roughness recesses
Rp	The height of the highest point above the centerline within the whole profile length
Rv	The depth of the lowest point below the centerline within the whole profile length
Rpm	The mean value of five consecutive lengths of the sample
Rt	Vertical height between the highest and the lowest point of the profile within the investigated length
Rtm	Rmax mean value in five consecutive sample lengths
R-z	Similar to Rz, apart from the fact that the individual depth of roughness is the depth of the highest peak up to the third biggest recess within the sample length

With the analysis of the results of obtained roughnesses by way of AFM, the most frequently are used, as follows: mean roughness (Ra), roughness by the least square method (Rq), ten point mean roughness (Rzijs) and roughnesses that are determined as the biggest difference of heights (Rz). Roughnesses are expressed in nanometers (nm).

Mean (average) roughness (Rs) is defined as an average distance of the centerline when viewed as the local minimums were the local maximums (Figure 4). It is defined by the formula (1):

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

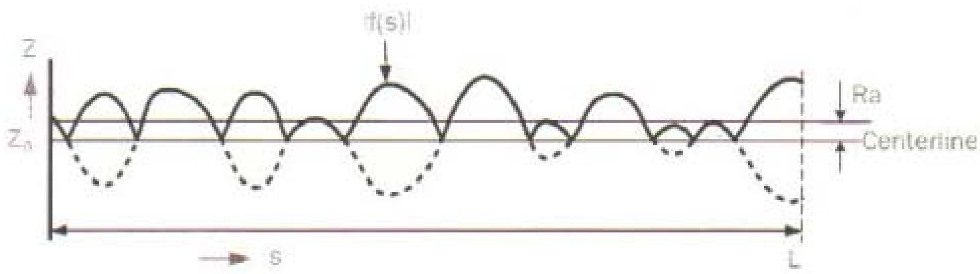

Figure 4. Profile with absolute values relative to the centerline. The distance of their average height from the height of the profile centerline is defined as Ra roughness.

Roughness with the least squares method (Rq) is defined as a deviation of the least square method relative to the centerline Z_0 (2):

$$Rq = \sqrt{\frac{1}{L}\int_0^L (f(s) - Z_0)^2 ds} \tag{2}$$

Ten point roughness (Rzijs) is defined as a sum of the mean values of absolute values of the deviations from the centerline between the biggest deviation and the fifth biggest deviation and absolute value between the smallest deviation and the fifth smallest deviation (3).

The profile of five local maximums and five local minimums is presented in Figure 5.

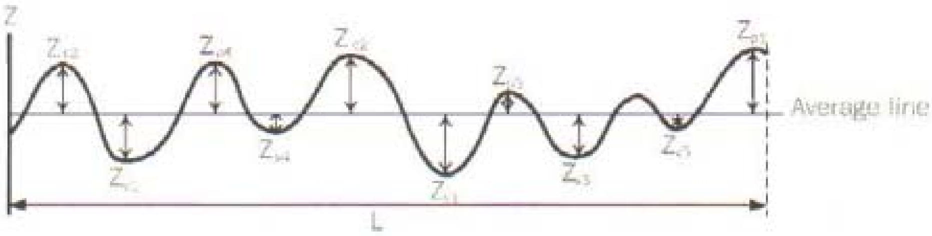

Figure 5. Ten point mean roughness

$$Rzijs = \frac{\left|z_{p1} + z_{p2} + z_{p3} + z_{p4} + z_{p5}\right| + \left|z_{v1} + z_{v2} + z_{v3} + z_{v4} + z_{v5}\right|}{10} \quad (3)$$

Rz is the biggest difference of heights and may be presented as a difference between „the highest hill“ and the „deepest valley“. This is defined as a difference between the biggest measured height Z_{max} and the least measured height Z_{min}, measured with piezo scanner along its *z* axis during the analysis of the scan (4):

$$R_z = R_{\max} - R_{\min} \quad (4)$$

Regression parameters of the analyzed sample per roughnesses (Ra, Rz, Rzijs, Rq) are presented in Table 2.

Table 2. Regression parameters of Resilience Orthodontic bonding solutions – *Total data (with arithmetic means of measuring image)*

Parameter code	Regression equation (y=ax+b)	a	b	Determination coefficient (R^2)	Correlation coefficient (r)
4– Res– Ra	y = – 0.7456x + 29.661	– 0.7456	29.661	0.0631	0.2512
4– Res– Rz	y = – 4.9704x + 133.18	– 4.9704	133.18	0.2072	0.4552
4– Res– Rzijs	y = – 18.884x + 431.94	– 18.884	431.94	0.6377	0.7986
4– Res– Rq	y = – 1.0445x + 35.75	– 1.0445	35.75	0.101	0.3178

Based on the conducted regression analysis and the obtained correlation coefficients (r) which is within the limits $0 < r < 0.25$ we can conclude that there is no good statistical relation between the adhesive roughness and the measurement places of scanned samples, which shows that the nanostructures of analyzed adhesive samples are homogenous.

Based on correlations of arithmetic means of debonding forces (I) and average adhesive roughnesses (Ra, Rz, Rzijs, Rq) for the sample of Resilience Orthodontic bonding solutions, Ortho Technology inc. Florida, we obtain the same dependence of the debonding force upon the roughness, i.e. with an increase in roughness increases the bond strength too [34−36].

If we compared the obtained roughness results for individual materials with the obtained values in literature for bond strengths [37−36] we could see that with all the roughness parameters (Ra, Rz, Rzijs and Rq) the strongest bond was made with Resilience Orthodontic bonding solutions, followed by Heliosit, Orthodontic (Ivoclar, Vivadent), GC Fuji Ortho LC, and the weakest with ConTec LC − Dentarum. The differences between the last three adhesives are not so noticeable. On the other hand, the biggest material roughness was reported with GC Fuji Ortho LC, followed by Rezilience Orthodontic bonding solutions, while with the other two, it is by far lower. If we take into account that GC Fuji Ortho LC is a glass ionomer-based material and that it does not require etching the enamel with acid, we assume that its big roughness increases the total contact surface through which the chemical bond between the hydroxyl groups of polyacrylic acid with calcium ions in hydroxylapatite is realized [47,48].

On the other hand, higher roughness of Rezilience Orthodontic bonding solutions at a nano level probably enables higher number of thorns that penetrate into micro recesses formed under the action of acids. Higher roughness is a consequence of the chemical structure itself of the composite material. [49,50].

It is quite known that the orthodontic treatment with fixed dentures increases the risk of the carious process which endangers the treatment itself and discourages the patient. The patient's risk is decisive in all that, but the additional factors such as the materials that are applied may contribute even more. The placing of the fixed device itself disturbs the ecosystem in oral cavity leading toward increasing the number of cariogenic bacteria and the development of white spots [51,52].

4. CONCLUSIONS

Based on the obtained results of the study, their statistical processing and detailed analysis, the following conclusions may be drawn:

– AFM technology is suitable for following the enamel structure from individual crystals to prisms (from nano to micro level). At lower magnifications it presents the enamel prisms as deep recesses. Individual crystals are positioned in parallel and show an elongated hydroxylapatite plane. Crystal surfaces are unevenly compacted forming in that way the roughness of enamel surface. At higher magnifications the crystals show characteristic hexagonal appearance, placed to each other at a 60° angle.

– AFM has a number of significant advantages for testing the dental tissues, compared to other techniques and especially SEM (Scanning Electrone Microscopy), and is a basic possibility for obtaining the three-dimensional profile of tested surface.

– We can see that with all the roughness parameters (Ra, Rz, Rzijs and Rq), the strongest bond was with Resilience Orthodontic bonding solutions, and that this is followed by Heliosit, Orthodontic (Ivoclar, Vivadent), GC Fuji Ortho LC, while the weakest bond was with ConTec LC – Dentarum adhesive.

– Higher roughness of Rezilience Orthodontic bonding solutions at a nano level is probably enabled by a bigger number of thorns penetrating into micro cavities formed under the action of acids. Bigger roughness is a consequence of the structure itself of composite material.

– After debonding the orthodontic brackets fixed with composite material by way of enamel etching, a long and complex treatment of enamel remineralization is necessary.

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АФМ ИСПИТИВАЊЕ НАНОСТРУКТУРЕ *RESILIENCE ORTHODONTIC BONDING SOLUTIONS* ОРТОДОНТСКОГ АДХЕЗИВА

Сажетак: У раду је помоћу атомске микроскопије (АФМ) анализирана наноструктура Resilience Orthodontic bonding solutions ортодонтског адхезива који се данас најчешће користи у ортодонтској пракси. Након одређивања својства адхезива успостављена је корелација између наноструктуре испитиваног адхезива и јачине везе бравице за зуб. На основу АФМ слика анализираног адхезива, помоћу корелација аритметичких средина јачине везе дебондирања (I) и просјечних храпавости адхезива (Ra, Rq, Rzijs, Rz) долазимо до закључка да са повећањем просјечних храпавости адхезива расте и јачина везе дебондирања (I). Затим је извршено поређење добијених резултата са другим адхезивима који се такође најчешће користе. Уочено је да је код свих параметара храпавости (Ra, Rz, Rzijs и Rq) најјача веза остварена са Resilience Orthodontic bonding solutions, па затим Heliosit, Orthodontic (Ivoclar, Vivadent), GC Fuji Ortho LC, а најмање са ConTec LC − Dentarum. Већа храпавост Resilience Orthodontic bonding solutions на нано нивоу вјероватно омогућава већи број трнова који задиру у микроудубљења настала под дејством киселина. Већа храпавост је посљедица саме хемијске структуре композитног материјала.

Кључне ријечи: ортодонтски адхезив, наноструктура, атомска микроскопија (АФМ).