IJIRR

International Journal of Information Research and Review
Vol. 1, Issue, 12, pp. 239-245 December, 2014

Review Article

CHARACTERISTICS OF DENTIN SURFACES AT IN VITRO EXCAVATION WITH DIFFERENT METHODS FOR MINIMAL INTERVENTION BY ATOMIC FORCE MICROSCOPY (AFM) AND SCANNING ELECTRON MICROSCOPY (SEM)

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ARTICLE INFO

Article History:
Received 05th Octember, 2014
Received in revised form
13th November, 2014
Accepted 28th December, 2014

Keywords:
Dentin,
Excavation,
Minimal Intervention,
Er: YAG laser,
Carisolv.

ABSTRACT

The micro-invasive method applied to dentine, requires selective controlled excavation. New and various alternative methods for selective excavation and high-tech equipment for excavation control are being created. Modern science is concerned with researching these methods in regards to the control during excavation as well as the choice of method for the excavation itself. The aim of this study was characterization of dentinal surfaces during in vitro excavation with different methods for minimal intervention by atomic force microscopy (AFM) and scanning electron microscopy (SEM). The presented SEM micrographs showed that the obtained dentinal surfaces after excavation by different methods next to healthy dentin differed among themselves. Relatively preserved tubular structure was observed only in the laser processing, while in mechanical treatment the structure was masked by coating “smear layer”. In the resulting dentinal surfaces after excavation to healthy dentin with Carisolv, no visible structure of dentin was observed. The surface was very rough with clearly distinguishable particles of dentine that were flaking and remained bound to it.

INTRODUCTION

Micro invasive (MI) approach to dentin requires selective, controlled excavation. Conventional mechanical rotary instruments are not most suiTable for this type of excavation. New and different alternative techniques for selective excavation and high-tech means to control the excavation are being invented (Banerjee *et al*., 2000; Banerjee and Boyde, 1997, Bjørndal *et al* 2000). On one hand, classical rotary instruments cannot act selectively, on the other, they are potentially responsible for the pain and discomfort when working on dental tissues due to the sensitivity of the vital pulp, the pressure on the tooth, bone transmitted pressure and vibration, development of high temperature and noise of the high speed handpiece. All these effects make them difficulty accepted by children. However, preserving round burs (soft alloy) with slow motion in combination with hand excavators can be used from conventional techniques (Splieth, 2011). Chemically-mechanical method of excavation is performed when hand excavators are combined with the chemical action of Carisolv.

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The sodium hypochlorite in Carisolv reacts with amino acids and forms chloramine, at high pH (alkalinity). Chloramine dissolves denatured collagen and degraded carious dentin is removed in 30 sec with special patented excavators. The remaining collagen fibrils do stabilize and remain as cores for mineral nucleation. The high pH maintains sTable energy barrier and prevents the dissolution of the mineral ions (Flückiger *et al*., 2005, Pitts, 2009).

Our previous studies pointed that this method applied in clinical conditions showed excellent effects among negative and very negative children on the scale of Frankl. Patented excavators of Carisolv allow the excavation of hard to reach enamel-dentin border and the pulp base to be controlled while processing (Rashkova *et al*., 2011). Our studies on the use of Er: YAG laser technology in pediatric dental practice demonstrated that and it was completely suiTable for dental caries treatment with minimal intervention. It is very important the operator to select the suiTable laser parameters for minimal impact, which can obtain the maximum effective ablation with minimal side effects (Zhegova and Rashkova, 2012 a, Zhegova and Rashkova, 2012 b).

Er: YAG laser technology is MI technology. SEM analysis showed the ablated enamel surfaces with cut enamel prisms, preserved enamel structure, no smear layer, no carbonization, dentin surfaces with open dentinal tubules, protruding peritubular dentin and no smear layer (Borsatto 2009, Delme 2007) [10-11)]. The selective effect of the laser on the carious dentine is determined by the fact that the ablation rate depends on the structure water content and degree of mineralization. Substantial evidences are protruding dentinal tubules due to varying degrees of mineralization of peritubular and intertubular dentin that reflect the degree of ablation-less mineralized dentin is ablated faster (Kornblit *et al*., 2009, Lizarelli *et al*., 2006). Laser cavity preparation may be combined with hand excavators and Carisolv, with additional acid etching, both the enamel and dentin, in order to smooth the resulting surface, additional treatment with NaOCl for removal of denatured collagen and thus a suiTable surface for optimum adhesion or stimulated internal remineralization to be prepared (Lahmouzi *et al*., 2012; Olivi *et al*., 2011). The Er: YAG laser as a new technology shows many advantages in modern MI cariesology. It acts selectively according to the degree of mineralization, does not create a "smear layer", opens dentinal tubules, reduces bacterial colonization, creating rough surfaces suiTable for adhesion, it is accepted perfectly by children.

Purpose – Characterization of dentinal surfaces during in vitro excavation with different methods for minimum intervention by atomic force microscopy (AFM) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

The objects of our experiment were 30 extracted teeth with similar sized occlusal or proximal carious dentinal lesions (D3). The teeth were divided into 6 groups 5 teeth each, depending on the method and extent of excavation. Each method of excavation was implemented in two versions: next to dentin with characteristics of healthy dentin and affected dentin (partially demineralized dentin with the possibility of re-mineralization). Distribution of extracted teeth included in our experiment is shown in Table 1.

When using fluorescent control during excavation, fluorescence color: red, dark red, light red is evaluated. The extracted teeth used in this study were stored in a solution of distilled water with thymol. At least 24 hours prior to the excavation they are left in pure distilled water. Mechanical excavation was carried out using conventional techniques (slow speed and high speed handpiece).

Laser excavation was carried out with Er: YAG laser Lite Touch Syneron with a wavelength of 2940 nm, pulse duration of 50 µsec, energy / pulse rate when opening the carious lesions for enamel processing 300 mJ / 20Hz (energy density 22.61 J / cm²) and for carious dentin excavation 200 mJ / 20Hz (energy density 15.08 J / cm²), non-contact method (working distance 0.5-1.0 mm), with a water-air cooling 39 ml / min.

Excavation with Carisolv was performed by a combination of hand excavators with the chemical action of the product. The methodology followed the manufacturer's recommendation.
Criteria for evaluation of dentin during excavation

Healthy dentin - excavation was performed until yellow or light yellow dentin; hard, slightly creaking and resistance during probing.

Affected dentin - The visual-tactile criteria of Bjiornal was applied and Thylstrup,1998 for excavated dentin: after excavation in the enamel/dentin junction, excavation continued until light brown dentin at the bottom of the cavity with medium firm texture, mild resistance on probing and leaving a white trail. For greater accuracy and objectivity excavation was controlled by fluorescent carious dentin detector Proface: until the fluorescence signal disappears in healthy dentin and preserves pale pink fluorescence in the bottom of the cavity in affected dentin. After the excavation was completed, the roots were separated from the clinical crown. Then the samples were dried in alcoholic solutions with increasing concentrations. Cavities were isolated by obturation with temporary filling material. Then they were packed with epoxy resin in plastic cylinders with a diameter of 1.5 cm and height 3.5 cm.

Table 1. Grouping of extracted teeth included in the experiment

Method of excavation					
Mechanical		Laser		Chemically-mechanical	
To healthy dentin	To affected dentin	To healthy dentin	To affected dentin	To healthy dentin	To affected dentin
1 group	2 group	3 group	4 group	5 group	6 group

Table 2. Criteria for samples‘ evaluation

Visual criteria – color of the dentin	Tactile criteria (with probe) - Consistency of dentin
black	Very soft dentin- probe enters and easily flakes off pieces of dentin;
dark brown	Soft dentin - the probe is sinking in and out of the dentine without resistance;
light brown	Average hard dentin - a slight resistance when probing;
yellow	Hard dentin - when driving on dentin with little resistance remains white trail;
light yellow	Hard non-carious dentin - slightly creaking and resistance on probing.

During the excavation, the samples were evaluated clinically by a visual tactile method (Bjorndal and Thylstrup, 1998). For greater accuracy and objectivity excavation was controlled by fluorescence with caries detector - Pro Face. It is an innovative method for detection of infected dentin, which is seen in red and healthy structures are colored in green.

After polymerization, the specimens were bisected at the axial axis of the tooth in the medio-distal direction. The temporary restoration was removed from both halves and then 1 sample from each group was subjected to histological analysis for descriptive characterization of residual dentin by atomic power (AFM) and scanning electron microscopy (SEM).

The study was conducted in a laboratory for computer analysis of surfaces at the Faculty of Chemistry and Pharmacy at the University "St. Kl. Ohridski".

Atomic force microscopy (AFM) – this is a modern method for testing and characterization of surfaces. It allows access to the topography of the surface in three dimensions on a submicron level, from which the basic statistical characteristics of the tested surfaces are obtained. AFM- device scans with two independent scanners - in the XY plane and the direction Z (vertical displacement). The mode in which images were made out of the 6 samples is tapping mode, or halted contact mode - the console at the end of which is the blade, is forced to oscillate at a frequency close to its resonant frequency of oscillation, and the blade contacts with the surface only at the lower end of the oscillating movement of the bracket. The quality of the image depends on the radius of curvature of the blade as well as the number of scanning lines. All photos were taken with a resolution of 512 x 512. For 15 µm side, this is about 29, 3 nm between any two points. Blades that were used, have radius of curvature of about 10 nm (manufacturer's information), which means about 20 nm diameter.

Points subjected to AFM - analysis were pre-marked on the samples. These sections were placed in the bottom of the cavity immediately above the pulp wall. *Scanning electron microscopy (SEM)* - The study was conducted on the same samples immediately after the AFM-analysis. The same points, marked prior to AFM-study were analyzed. SEM-device used for the purposes of our study has the following characteristics: Lyra (Tescan) 30 kV with Back Scattering Electron (BSE) detector and EDX detector Quantax 200 (Bruker) with resolution of 126 eV. Specimens were prepared for examination with the scanning electron microscope (SEM) according to the following protocol: immersion in 2.5% glutaraldehyde (Merck KGaA, Darmstadt, Germany) in 0.1 M sodium cacodylate buffer at pH 7.4 (Merck KGaA) for 12 h at 4°C. After fixation, the samples were rinsed with 0.1 M sodium cacodylate buffer several times, and sequentially dehydrated in an ascending ethanol series (25% for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min, and 100% for 60 min), and then immersed in hexamethyldisilizane (Merck KGaA), for 10 min, and placed on absorbent paper on glass plates and left to dry in an exhaust hood. The specimens were mounted on stubs with their lased surfaces face-up, sputter-coated with gold, and examined.

RESULTS

AFM analysis allows creating of images of the tested surfaces that are a reflection of the object's surface such as it was "crawled" with the edge of the microscope needle.

Regarding the realism of the image on the surface, the advantage of AFM is that much smaller surfaces can be scanned and that quantitative data for statistical parameters that characterize the roughness can be obtained. The results obtained by AFM analysis of the 6 dentin samples of our study are shown in the following Figure 1.

The data from the histogram of the analyzed samples are reported in the following Table 3.

The Table presents the main parameters used in the analysis of AFM images:

Average roughness - represents the arithmetic average of the heights of each point on the surface. Higher value means a rough surface.

Root mean square (RMS) roughness - similar in meaning to arithmetic average roughness, it always has bigger value from it, but is more sensitive to the presence of more extreme points.

Peak amplitude (Peak to peak) - Distance between the highest and lowest point on the surface.

Asymmetry (Skewness) - A measure of the asymmetry of the probability distribution of the values in the histogram around the average value - if there are more convex zones (positive), concave (negative value) or equally (zero or close to zero). A negative value is for the expectation of surfaces which have been subjected to some type of friction. A positive value in the sample with a laser excavation until affected dentin may be due to an artifact – a significant wearing of the blade, because of which it cannot penetrate sufficiently into the recesses, and they become more invisible to it.

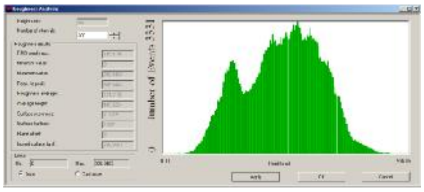
Excess (Kurtosis) - A measure of the probability distribution of the histogram - if there are more small bumps or depressions (negative values) or fewer, but larger in size (positive values). In a normal distribution the value is 0.

The surfaces obtained by treatment with Carisolv and Er: YAG laser after excavation to affected dentin showed the most significant roughness in comparison to all other samples. Surfaces obtained with Carisolv after excavation to healthy dentin showed roughness similar to those obtained in treatment with mechanical excavation until affected dentin, and the differences in roughness between mechanical and laser treatment next to healthy dentin are minimal. Immediately after the AFM analysis, the same samples were subjected to SEM analysis. Images were made in two magnifications for each sample. The results are presented in the following Figure 2.

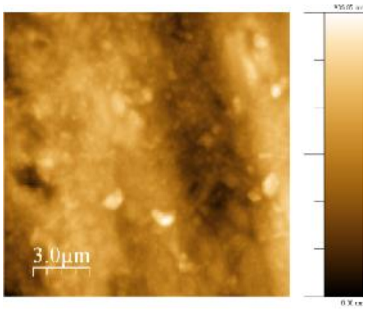
Table 3. Data from AFM-histograms of dentin surfaces - healthy and affected dentin, obtained by 3 different methods of excavation

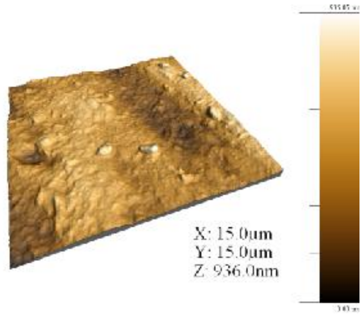
Sample	Average roughness, µm	RMS roughness, µm	Peak amplitude, µm	Asymmetry	Excess
1 group	0,146	0,121	0,936	-0,12	2,31
2 group	0,242	0,195	1,97	-0,38	3,26
3 group	0,171	0,138	1,33	-0,41	3,35
4 group	0,306	0,224	2,12	0,02	4,40
5 group	0,232	0,176	1,97	-0,95	5,31
6 group	0,377	0,288	3,17	-0,55	3,79

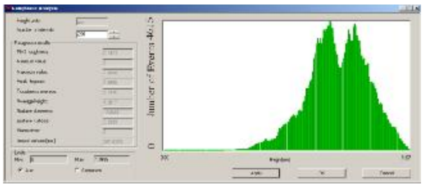
Histogram of the corresponding specimen	2 D image of the specimen	3 D image of the specimen



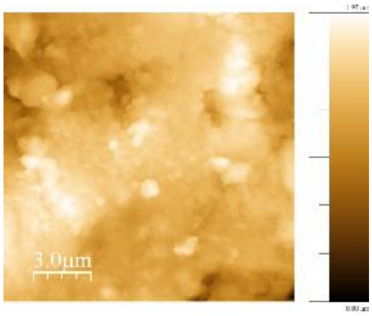
Mechanical excavation to healthy dentin

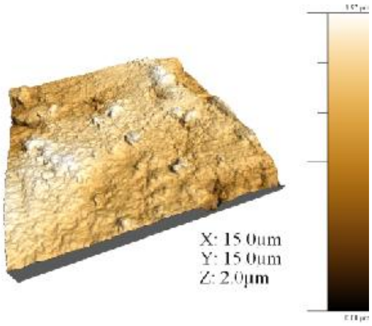


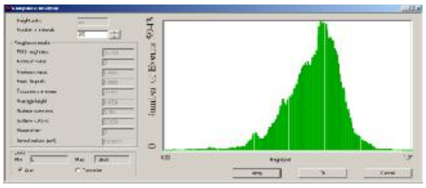




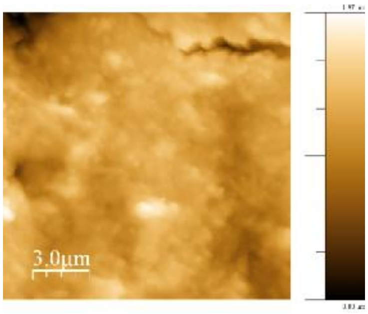
Mechanical excavation to affected dentin

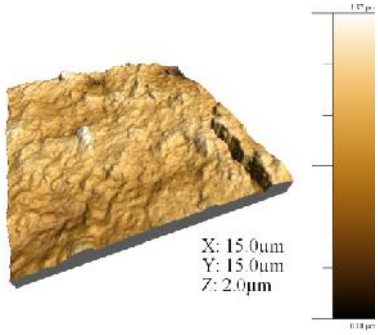


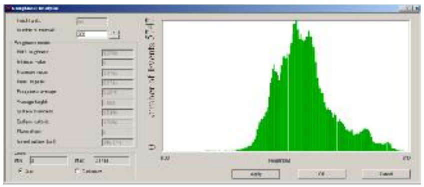




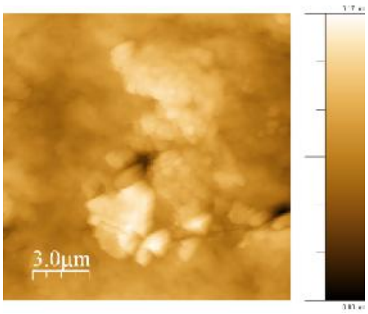
Er:YAG laser excavation to healthy dentin

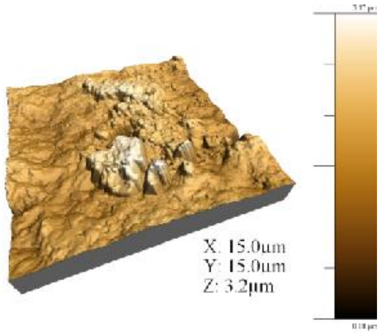


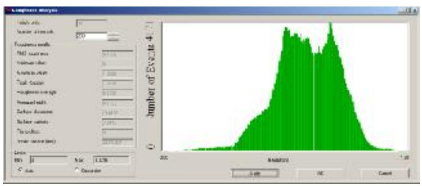




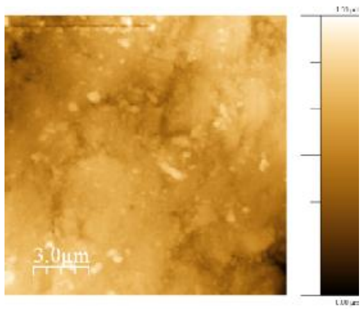
Er:YAG laser excavation to affected dentin

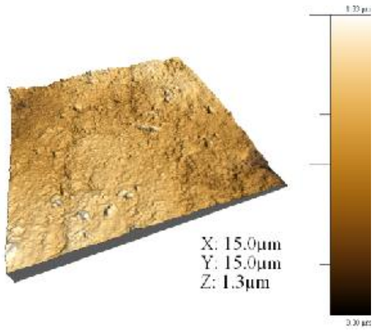






Carisolv excavation to healthy dentin





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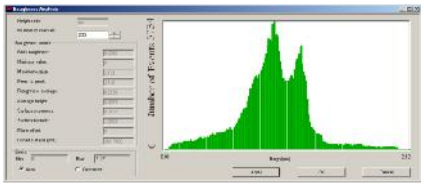
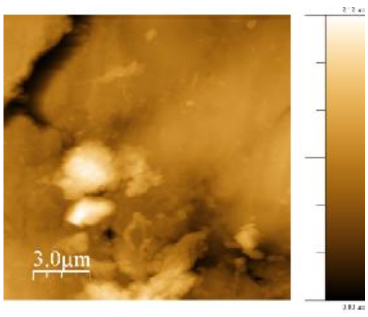


Carisolv excavation to affected dentin



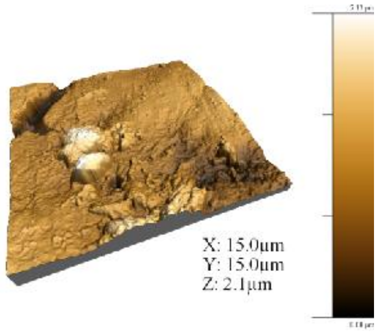
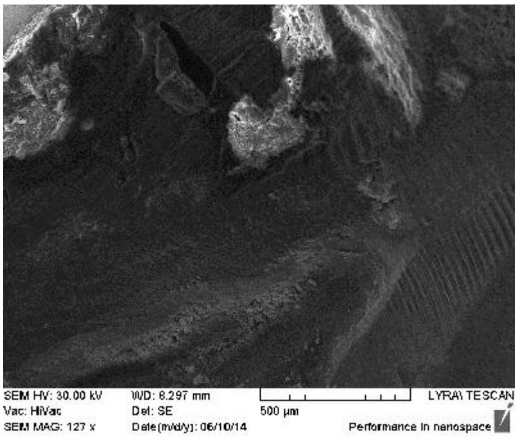
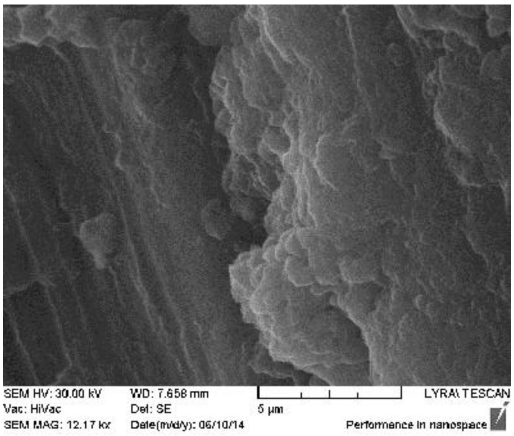


Figure 1. AFM images of samples of dentin surfaces - healthy and affected dentin obtained after three different methods of excavation

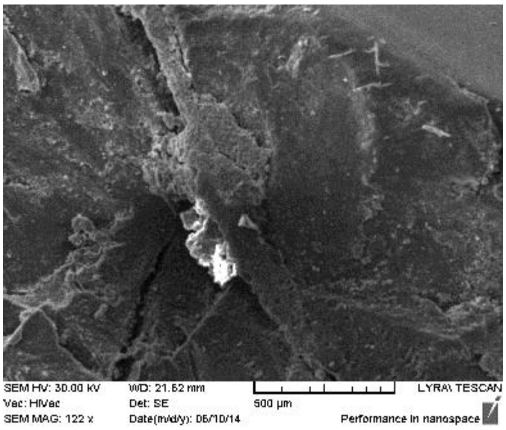
Sample | Magnification 120÷130 X | Magnification 12 кX

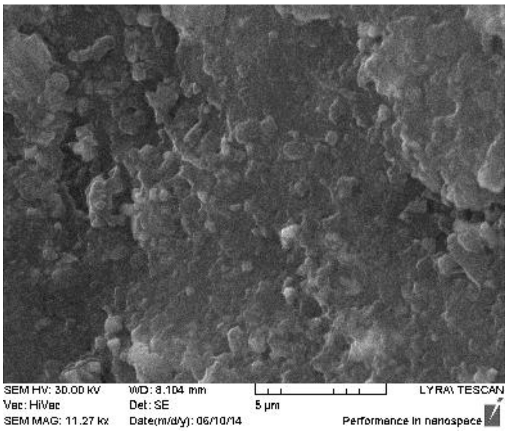
Mechanical excavation to healthy dentin



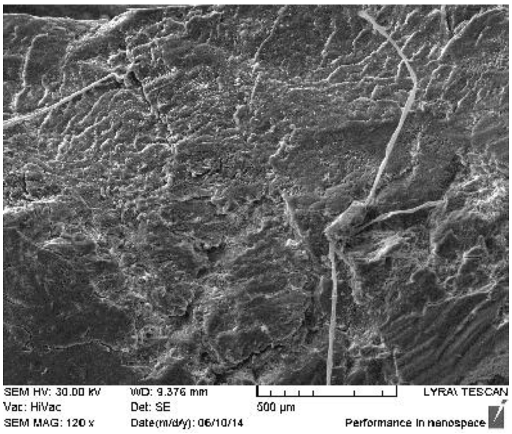


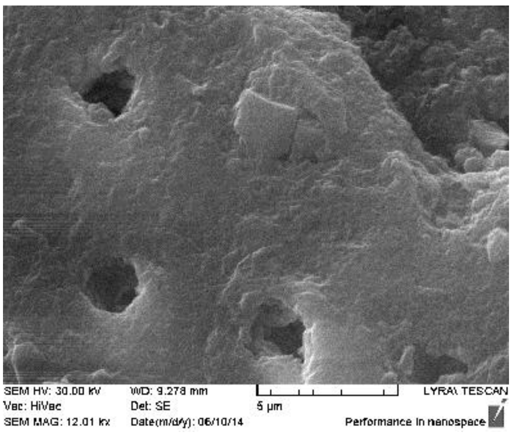
Mechanical excavation to affected dentin





Er:YAG laser excavation to healthy dentin





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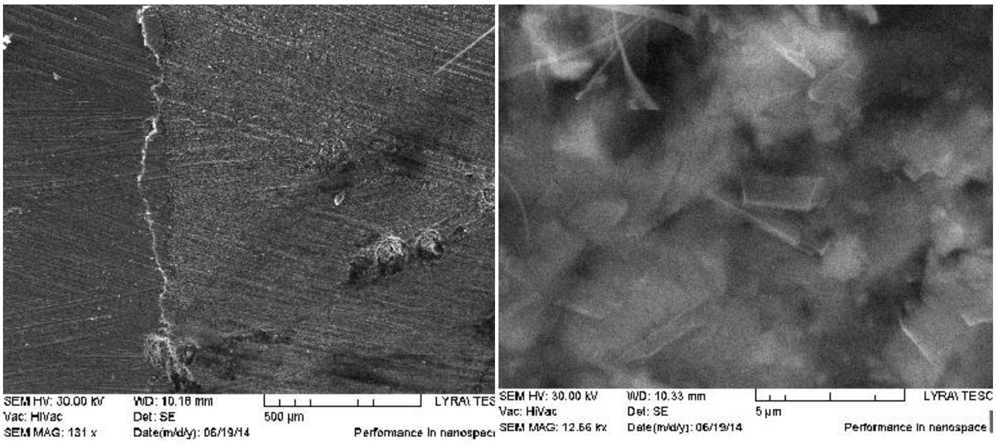
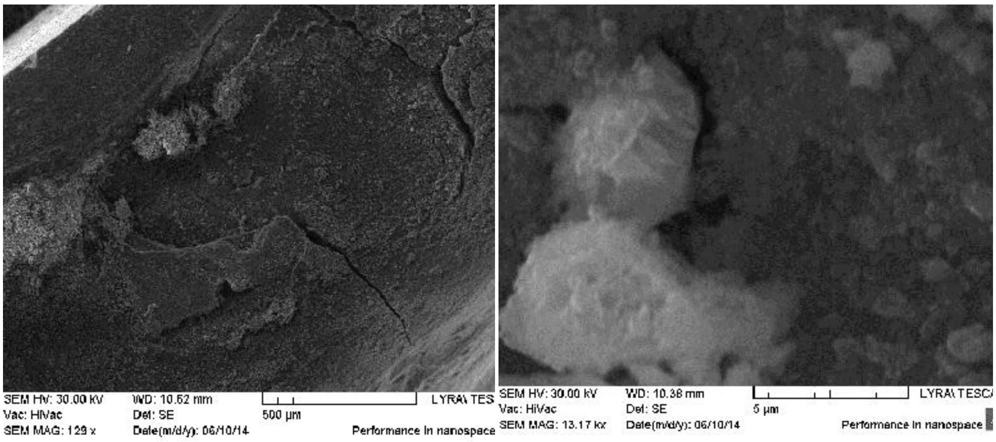
Er:YAG laser excavation to affected dentin



Carisolv excavation to healthy dentin



Carisolv excavation to affected dentin

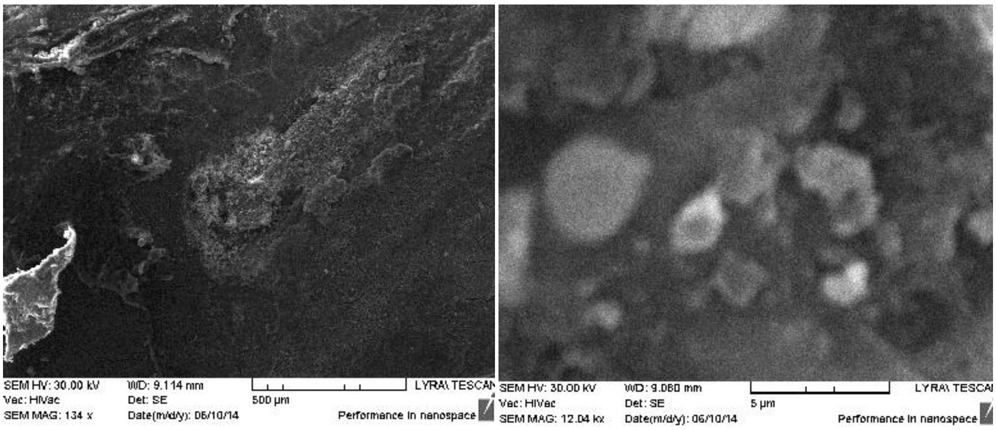


Figure 2. SEM images of dentin surfaces – healthy and affected dentin, obtained after three different methods of excavation

The SEM micrographs showed that the dentinal surfaces after excavation by different methods next to healthy dentin differed among themselves. Only Er: YAG laser treatment resulted in visible relatively preserved tubular structure, while in mechanical treatment the structure was covered by smear layer, which is to a subsequent acid removal (etching). The excavation to healthy dentin with Carisolv prevented observing of dentinal structure. The surface was very rough with clearly distinguishable particles of dentine that are flaked and remained bound to it. In the studied methods of excavation, the dentin surfaces of affected dentin also varied in the way of processing as well as in comparison to the surfaces with healthy dentin.

In all of the three methods of excavation, the dentin has rough uneven surface, no visible dentinal structure, with the presence of many particles of different sizes that are scattered on the surface.

DISCUSSION

The recent *in vitro* study revealed different characteristics of dentin surfaces after excavation with the 3 different methods for conventional treatment and minimal intervention. The observed characteristics were influenced, on one hand by the method of excavation, on the other hand by the rate of excavation.

AFM analysis conducted in our study showed the most significant roughness in Carisolv followed by Er: YAG laser treated samples after excavation to affected dentin. Only minimal differences with regard to the roughness between mechanical and laser treatment were detected when excavation was to sound dentin. SEM micrographs revealed rough surfaces with small particles removed from the treated structures and bound on surfaces. Mechanical and Carisolv treatment resulted in smear layer, in contrast with Er: YAG laser treatment where preserved tubule dentinal structure was observed.

Characteristics of the treated dentinal surfaces can be explained with the action-mechanisms of the used methods. Er: YAG laser irradiation acts on hard dental structures by mechanism of thermo-mechanical ablation. The Er: YAG laser wavelength (2940 nm) matches the absorption peak of water in the process of this wavelength- hard dental structures' interaction. The energy is converted into heat, leading to water vapor formation, which expands and produces high pressure inside the target structure and induces instantaneous micro-explosions and ejection of particles of irradiated tissue (Hibst and Keller, 1989). Chemo-mechanical method involving Carisolv and hand excavators acts on denatured collagen, dissolving it as well as degraded carious dentin followed by mechanical removal with special patented excavators (Flückiger *et al*., 2005). Differences in the texture of dentin surfaces obtained after different types and levels of excavation are a prerequisite for a different type of adhesive interface. Evidently, the obtained in the microinvasive cavity preparation with different methods for excavation, especially when removing to affected dentin, dentinal surfaces showed features that do not involve good adhesion and bonding of the filling material.Further studies are needed in order to determine the best method for excavation that is a combination of biological microinvasive approach and an additional treatment resulting in favorable for adhesion dentin surfaces.

Conclusion

Characteristics of dentin surfaces obtained during excavation by different methods for minimal intervention seem to be not favorable for a good adhesion and bonding of restorative composite materials.

Acknowledgements

The publication is a result of research under Contract № 43/ 2013, the project № 20 / 2013, funded by the Council of Medical Science at Sofia University.

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