Ultrastructural Analysis by Scanning Electron Microscopy of Dental Structures Conditioning with Ortophosphoric Acid and ER.CR: YSGG Laser Irradiation

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This study evaluated the ultra-morphological effects of ortophosforic acid and Er.Cr: YSGGLASER on human dentin by means of a field emission in-lens scanning electron microscope. The study was conducted in vitro on human teeth extracted for orthodontic or periodontal reasons, after obtaining informed consent of patients. The samples were sectioned mesial-distal in the longitudinal direction (diamond discs) finished 400, 600, 1200 and 2400 grit SiC paper, polished with gums and abrasive paste 6, 3, 1 and 0,25 μm under continuous irrigation. The samples were divided in five groups depending on the type of conditioning as follows: GR.1= 5 s; GR.2= 10 s; GR.3= 15 s; GR.4= 20 s; GR.5= Er,Cr: YSGGlaser irradiating at 30% water and 60% air at 3W/15 Hz. The materials used for conditioning the teeth were SE™35% (Scotchbond™ Etchant Phosphoric Acid -3M ESPE-Seefeld, Germany) and MG6-MZ6 tipswith Er, Cr: YSGGlaser (Biolase Waterlase-MD), according to manufacturer's instructions. The specimens were prepared for observation under SEM with ×1,000 and ×4,000 magnifications. The acid type, concentration and time of action determines dentin demineralization and modifying or removing smear layer. Er.Cr: YSGG laser treatment of non-carious dentin, a laser power of 3 W is found to be the optimal to improve the micromorphology of dentin.

Keywords: scanning electron microscopy, Er, Cr: YSGGlaser, phosphoric acid, dentin, ultra-structure, collagen fibriles

The dentin structure is unfavorable for adhesion of the dental material because it has heterogeneous composition (collagen, hydroxyapatite), a high proportion of organic matter with different free energy surface, hydrophilic structure, 25-30 mmHg pulp pressure[1, 2].There is a constant pulp pressure of 3.3 - 4.0. KPa, leading to a permanent dentinal flow [3]; content of dentinal tubules; the presence of the smear-layer; dentin permeability in the pulp horns occlusal law [4]; approximale and root area [5]; the layer of pigmented dentine (thick 0.5-5μm); the impossibility of perfect cleaning and drying dentine. The dentin conditioning acid is a preliminary step required to remove the smear layer dentin that creates porosity needed its impregnation with an adhesive monomer, which is mainly aimed upward removal of debris to allow adhesion to the underlying dentin matrix. The improved adhesion of dental nanomaterials at dental structure aims modification or removal of the smear layer to obtain a higher cohesive strength, and a better adhesion. Also there is many pursuits for investigation the interfaces between the dentin biostructure and composite [6-8]. Conditioning of the dentin substrate with citric acid, hydrogen peroxide ensures the debris removal, disinfection and release active site dentin (calcium and collagen) but do not realize the etching of the dentin [9,10]. Dental substrate conditioning with 40% phosphoric acid was expected to Fusayama in 1940 [11] but most practitioners did not accept this method, using lower concentrations of it. Further controversy in the literature are in terms of conditioning during dentin meaning its protection against disintegration of dentin structure. Currently it is recommended to use a weak organic acid polyacrylic acid type, itaconic, maleic that produce only a slight modification or removing the smear layer, material adhesion sometimes limited to smear layer adhesion to dentin substrate. Also these weak acids does not remove the smear plugs in the dentinal tubules, the material having a lower power accession.On the other hand the use of acids stronger than ortho-phosphoric acid can cause demineralization too strong and even dissolution of dental substrate with exposure of collagen network. Clinic appears postoperative sensitivity. Adhesive techniques are in continuous improvement, total-etching by conditioning both the enamel and dentin for 20 s is one of these. Because any side effects of ortho-phosphoric acid dentin we desired to follow the structure of dentin after different time intervals dictated by etching work up to 20 s in total-etching technique. Also there are concerns regard to the dentinal structure laser treatment in order to improve the adhesion [12]. The laser with Er: YAG is an active environment of erbium-yttrium aluminum garnet (*Er: Y3Al5O12*) with a wavelength of 2,940nm (infrared light). Dentin surface morphology irradiated with an *Er: YAG* laser is characterized by clean surfaces, in which the layer is removed smear layer of dentinal tubules remain open and clean, favorable for adhesion process. Studies have shown that the *Er, Cr: YSGG*laser can effectively improve the bonding property between non-carious sclerotic dentin and resin composites by increasing the roughness and mean percentage area of open tubules [13] So the aim of this study is to evaluate the ultra-morphological effects of ortophosforic acid and LASER on human dentin by scanning electron microscope.

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Experimental part

The analysis of the dentin structure by SEM

The dentin structure was analyzed by SEM (Scanning Electron Microscopy) and EDS– (Energy-dispersive X-ray spectroscopy)by JEOLJSM 6390S Japan. The study was conducted in vitro on human premolar and molars extracted for orthodontic or periodontal reasons, after obtaining informed consent of patients. The samples were stored in a physiological serum, sectioned mesial-distal in the longitudinal direction (diamond discs) finished 400, 600, 1200 and 2400 grit SiC paper, polished with gums and abrasive paste 6, 3, 1 and 0.25µm under continuous irrigation. The samples were divided in five groups depending on the type of conditioning. The materials used for conditioning the teethwere SE™35% (Scotchbond™ Etchant Phosphoric Acid-3MESPE-Seefeld, Germany) and MG6-MZ6 tipswith *Er, Cr: YSGG*laser (Biolase Waterlase-MD), according to manufacturer's instructions, as for dentin 30% water and 60% air at 3W/15 Hz. The samples were etched with H_3PO_4 35% according to protocol and washing for 10 s with distilled water after the Bates et al. [14].

The samples were conditioned as follows: GR.1= SE™35% - 5 s; GR.2= SE™35% - 10 s; GR.3= SE™35% - 15 s; GR.4= SE™35% - 20 s; GR.5= Er.Cr: YSGGlaser irradiating at30% water and 60% air at 3W/15 Hz;

The teeth were then stored in saline solution 48 h. SEM and EDS observation was done with JEOLJSM 6390[a] Japan.

Results and discussions

EDS analysis revealed the structure of dentin is according to the table 1 and figure1.

After preparing organic substructure using rotary and manual instruments on hard tissues appears amorphous layer organo-mineral, *called smear layer, dirty layer* or the debris dentinaire remaining.Also the appearance of dentin structure conditioning with phosphoric acid 35% after 5 s can track figure 2-A and B, after 10 s in figure 3-A and Bafter 15 s in figure 4-A and B, after 20 s in figure 5-A and B and after conditioning of the substrate with *Er, Cr: YSGG*laser - Biolase Waterlaser figure 6-A and B.

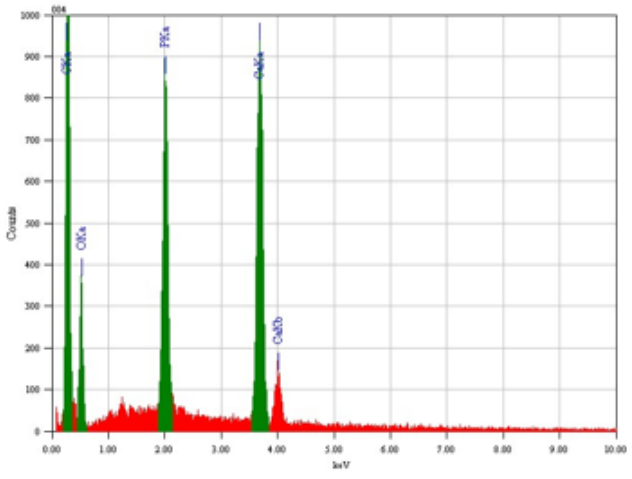

Fig. 1.The EDS analysis of the dentin

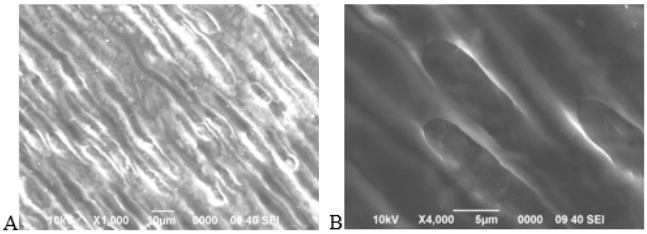

Fig. 2. Top-view SEM photomicrographs of the dentinal tubules in the dentine midshaft - longitudinal section after etching with 35% orthophosphoric acid 5 s;A– SEMX1000; B – SEMX4000

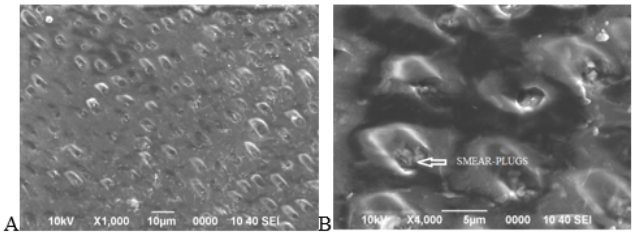

Fig. 3. Top-view SEM photomicrographs of the dentinal tubules in the dentine midshaft - longitudinal section after etching with 35% orthophosphoric acid 10 s A – SEMX1000 B– SEMX4000. Can be seen smear layer of the dentin surface and also smear plugs

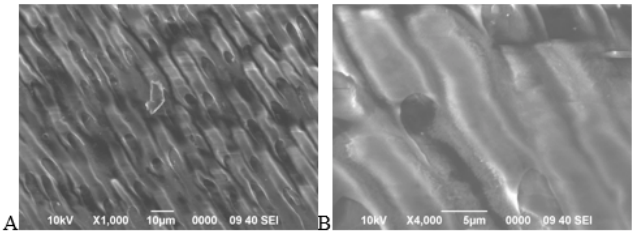

Fig. 4. Top-view SEM photomicrographs of the dentinal tubules in the dentine midshaft - longitudinal section after etching with 35% orthophosphoric acid 15 s A – SEMX1000 B– SEMX4000. Peritubular dentin is observed, affected by the attack mode of acid, dentinal and intertubular dentinal surface.

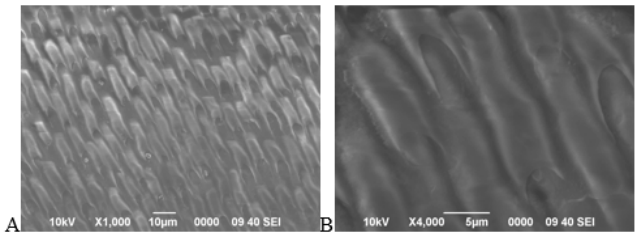

Fig. 5. Top-view SEM photomicrographs of the dentinal tubules in the dentine midshaft - longitudinal section after etching with 35% orthophosphoric acid 20 x A – SEMX1000 B– SEMX4000. It is observed that the dentine support is destroyed

Element(keV)		Mass%	Error%	Atom%	Compound	Mass%	Cation	K
C K	0.277	56.76	0.07	69.50			42.7513	
O K	0.525	25.18	0.37	23.15			15.5680	
P K	2.013	6.76	0.07	3.21			15.8764	
Ca K	3.690	11.29	0.11	4.14			25.8043	
Total		100.00		100.00				

Table 1
MINERALE COMPOUND OF THE DENTINE

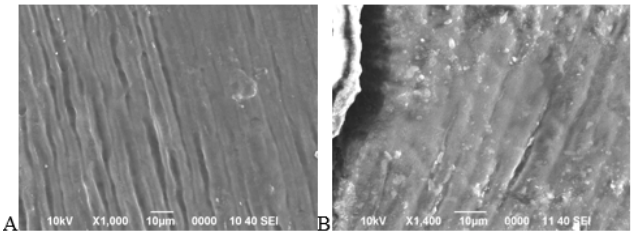

Fig. 6. Top-view SEM photomicrographs of the dentinal tubules in the dentine midshaft - longitudinal section after *Er, Cr: YSGG* LASER E- Biolase WaterlaserA – SEMX1000 B– SEMX4000.It is observed densification of the dentin

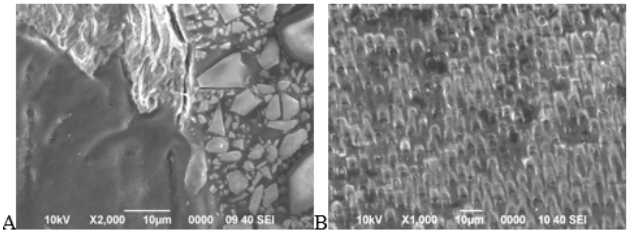

Fig. 7. Top-view SEM photomicrographs of the dentin tubules A-SEMX2,000

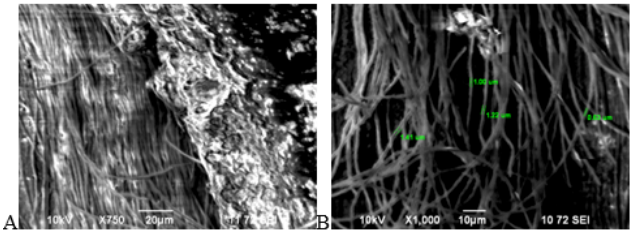

Fig. 8. Top-view SEM photomicrographs of the collagen fibers after exposure to sodium hypochlorite solution during preparation of the samples. Porous zone with demineralized residual collagen particles with dentin demineralization products in acid globules, and dissolved peritubular dentin cuff A - SEM X 750, B - SEMX1,000

Analysis of the dentin structure depending on the depth of the cavity shows a very low density of dentinal tubules - figure 7 in the surface layers and a very high density of dentinal tubules/mm^2 on surface dentin in the deeper layers of the dentin figure 7B.

Section near the enamel-dentine junction - there is an obstruction of tubules after the etching with phosphoric acid 37% for 5 s. B - SEMX1,000 Longitudinal section near the pulp chamber - is observed density dentinal tubules.

Also in the case of some samples conditioned with ortho-phosphoric acid was obtained collagen network exposure, as shown in figure 8-A,B.

Removing the smear layer only superficial, eliminates the possibility of penetration of resin into dentinal tubules filaments, situation in which it would greatly increase the adhesion. On the other hand the dissolution of *plugs* for smear layer would greatly increase the dentin permeability, so in the absence of very tight restorations, the micro-percolation of bacterial products and saliva would lead to a pulp damage. The mineral components of the smear layer can be dissolved by acid conditioning, this depending on the degree of ionization, *p*H, chemical concentration and viscosity of the solution. In our case, the action of acid in the structure of non vital dentin, with the lack of pulp pressure and dentin hydrophilicity, has led to a stronger demineralization of the substrate, sometimes even with the exposure of collagen network.The acid type, concentration and time of action determines dentin demineralization and modifying or removing smear layer. Effective concentrations of aqueous solutions of phosphoric acid, which removes the smear layer and provides a corresponding demineralization in dentin thickness varies between 10-45%. We tested a 35% concentration miming the situation that would achieve total etching of tooth enamel and dentin for 20 s. Higher concentrations slows the diffusion of monomer in depth due to precipitation of calcium salts in the pores of the dentin matrix. Moreover, in order to avoid blocking of the pores of the debris remaining and solubilized salts, as well as to obtain a maximum potential of the dentin plagues, wetting agents, acid etching must be followed by a rigorously wash with water. The peritubular and intertubular dentin are dissolved by the acid action than the primer and adhesive will fix the exposed collagen network. Free diffusion of the acid slows as the product viscosity increases as the molecular weight of the acid is higher. The time of acid action for obtaining a favorable demineralization process adhesion is 5 - 10 s. A longer time of action cause the disintegration of the dentin matrix undermining its resistance. Our study indicates that, for some samples was had produced a very high exposure of the collagen network.Also to achieve the best possible adherence should consider the following factors: the degree of mineralization of the dentine; environmental conditions - the presence of water and oxygen in the dentinal fluid; the collagen reactivity and chemical aggression (to be effective in the short time conditioning agents are hypertonic and monomers have a high osmotic concentration, leading to outward movement of dentinal fluid, causing some degree of sensitivity) [4,5]. One factor that may influence the dentin substrate modification after acid conditioning is given by the porosity of natural dentin [4,5]. It depends on the depth of the cavity thus the total area of shallow cavities sectioned tubules is 0.44% - 1.26% of the wound surface dentin (20,000 tubules /mm^2 at the junction enamel - dentin - (fig. 7-A)[4,5]. In medium deep cavities the proportion is increased (fig. 7-B) from 1.27 to 2.38% because in the deepest to go down from 5.34 to 3.37% [(45000 dentinal tubules/mm^2 surface pulp) [4,5]. Near the pulp the dentinal tubules occupies 22% of the cross-sectional area, while near the enamel occupies only 1%[4,5]. The question is whether to try a chemical adhesion to dentin or one type micromechanical the ducts, similar to that achieved to enamel. The adhesion of restorative materials to dentine is made especially of inorganic phase of dentin (45%) which at this level is less represented than the enamel (92%) and is arranged irregularly in a matrix composed mainly of collagen (fig. 3, fig. 3 b). At this stage, it is possible that chemical bond with the organic phase of the dentin is mostly made up of collagen [6,17]. Another study shows different etching times using the same phosphoric acid concentration result in different morphological changes in demineralized dentin surface. Moreover, based on a comparison with current studies, prolonged etching time causes morphological changes to dentin surface. Such changes have, in turn, negative effects on the dentin hybridization process [15-17]. Montes et colab. when they used 37% orthophosphoric acid for 15 seconds concluded that the dentin surface alterations produced by orto-phosphoric acid appeared to be a very severe demineralization pattern, quite irregular and less permeable to monomer infiltration, while the surface provided by the self-etching primer appeared to be a more uniform, less porous surface, and the association with simultaneous monomer infiltration may reduce the occurrence of mistakes in clinical bonding procedures [18]. The teeth surfaces prepared with Er: YAG laser Lite Touch (Syneron) remained without smear layer and clearly exposed dentinal tubules orifices. The surfaces were highly retentive [19]. Other studies have indicated that laser irradiation influences the bond of dental resin to enamel and dentin, and the most important parameter controlling

the effect of laser irradiation is the output power of the laser. Lower power laser irradiations determine a less change to the tooth surface. Visuri et al showed that application of excessively high laser power can result in charring and cracking in the tooth surface, decreasing the strength of the dentin [20]. Another study demonstrated that on the surface of non-carious sclerotic dentin treated by the Er, Cr: YSGGlaser with various powers, no smear layer was not formed, and the surfaces were clean and rough and also recommend the increase of laser power because the percentages of open tubule areas of the sclerotic dentin were apparently larger than those obtained in the lower laser power groups [21]. In our study we used the strength recommended in the instructions and considering the fact that less power gives an efficient sterilization does not cause a wound dentin and not remove the smear layer and more power would produce a higher densification of dentinal tubules, leading to melting of dentin tissue. Other studies that have compared the conventional treatment methods used for non-carious sclerotic dentin with Er, Cr: YSGGlaser irradiation concluded that, can more effectively decrease the blockage of dentinal tubules by mineralized crystals and surfaces treated with 5 and 6 W laser power showed cracks in FESEM images [13, 21]. The effect of applying Er: YAG laser is the growth of dentin roughness. It is supposed that increasing roughness and increase power dentin adhesion by increasing surface contact. The structure of densification tooth that we have obtained of a power of 3W determines subsequent adhesion prevention of dental materials, especially those with mechanical adhesion mechanism. Interestingly, that another study demonstrates that (0–4 W), surface roughness increased with the increase of laser power (0–4 W) but however, in the higher output power range (>4 W), the surface roughness decreased with the increase of laser power [13, 20]. Clinical dentin surfaces not subject to the same configurations adhesion dentinal tubules so the orientation of dentinal tubules has a significant effect on the adhesion of nanomaterials in dentin structure with the formation of a hybrid layer as good.

Conclusions

With the limitations of the present in vitro study, it may be concluded that for dentin surface alterations produced by orto-phosphoric acid more than 10 seconds appeared to be a very severe demineralization, but depend by characteristics of dentine. *Er, Cr: YSGG* laser treatment of non-carious dentin, a laser power of 3 W is found to be the optimal power to improve the micromorphology of dentin to the dentin materials.

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