DE GRUYTER OPEN

ACTA FACULTATIS MEDICAE NAISSENSIS

UDC: 616.314-74:615.46

DOI: 10.2478/afmnai-2014-0007

Scientific Journal of the Faculty of Medicine in Niš 2014;31(1):67-73

Original article ■

Ultrastructure of Adhesive Bond of Composite to Dentin

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SUMMARY

Ultrastructure of adhesive bond is a significant indicator of the efficiency of the bond between material and dental tissue. Bonding between composite and hard dental tissue is also most commonly assessed by measuring bonding strength or by absence of marginal gap along restoration interface.

The aim of this investigation was to estimate ultrastructural features of the bond between composite materials and dentin after using two adhesive techniques.

Twenty Class V cavities on extracted teeth were prepared and restored for scanning electron microscope (SEM) analysis of composite bonding to dentin. Adhesion was achieved by *Adper Single Bond 2*-ASB/3M ESPE or by *Adper Easy One*-AEO/3M ESPE. Photopolymerization of adhesives and composite material *Filtek Ultimate* FU/3M ESPE was performed using halogen light (*Elipar Highlight 3M ESPE).* After vertical sections through crown of teeth and composite restorations, surfaces of sections were treated with 37% phosphoric acid-60s and 2% NaOH-60s to remove the smear layer and obtain a better view of adhesive bond.

SEM analysis of adhesive bond showed better micromorphological bonding of composite to dentin after *etch and rinse* adhesive technique (ASB/FU matherials) than after *self-etch* technique (AEO/FU materials). In *etch and rinse* technique, the ultrastructure of adhesive bond to dentin was composed of adhesive and hybrid layers, with resin processes in dentinal canals. In *self-etch* technique, apart from adhesive and hybrid layers, resin processes were rare or were not observed at all.

Better ultastructural bonding of composite restoration to dentin was obtained with *etch and rinse* adhesive technique.

Key words: **dental composite, composite adhesives, dentin**

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INTRODUCTION

The bonding of composite to enamel was clinically successful whereas the bonding to dentin was unpredictable in terms of duration and integrity of the bond. Dentin has a complex biological structure. New adhesive systems form relatively strong bond to dry or moist dentin with complete or partial removal of the smear layer. The strength and ultrastrucutre of the bond may vary depending on the internal moisture, dentin region and adhesive technique (1).

The basic principle of adhesion of composite resins to dental substrate is based on exchange processes in which inorganic dental material is replaced by synthetic resin. This process has two phases. One phase consist of removing calcium phosphate whereby microporosity appears on enamel and dentin area of the tooth (2). The second phase, so called hybridization phase, includes infiltration and polymerization of resin within the formed microporosities. This leads to micromechanical bonding which is based on diffusion mechanisms. While micromechanical bonding is a basis for forming a good bond in clinical conditions, chemical interaction between the functional monomers and dental substrate components is further improved (3, 4).

Nowadays, two adhesive techniques are used to set up fillings with composite resin: total etch and self-etch (2). Total etch includes the application of strong phosphorous acid for conditioning of both the enamel and dentin whereby the acid is rinsed with water and the procedure is called etch and rinse. After rinsing the conditioned surfaces, hydrophilic adhesive is applied and polymerized by light. The self-etch adhesive procedure does not include phosphorous acid. In this procedure, the conditioning of cavity walls is done by self-etching primer which is not rinsed (4). In newer systems of this type, the primer is combined with adhesive and the adhesive solution is called 'all in one'. Literature data show a high degree of retention and ideal marginal bonding in all clinical procedures which include conditioning by phosphorous acid, namely the etch and rinse technique. These procedures include: filling in of fissures and cavities, direct and indirect composite restorations, cementing the ceramic inlays, cementing the metal and non-metal posts (5).

However, compared to total etch adhesives, the self etch adhesives have numerous advantages. They improve the efficiency of clinical procedures because there is no rinsing phase which is mandatory with total etch adhesives. Also, the amount of time patients spend at the dentist's is reduced. Conditioning, rinsing and drying phases can be critical and difficult to standardize in clinical conditions and therefore they have been eliminated with self-etch adhesives. In that way, technical sensitivity associated with the bonding of adhesive to dehydrated, demineralized dentin is avoided since the rinsing and drying phases are no longer needed, the collapse of collagenic network is prevented and monomers can infiltrate demineralized dentin (4, 6). Self-etch adhesives are easy to use and there is less possibility of error and this may increase the clinical application of self-etch adhesives in the future. The question that arises is whether adhesive bond of composite to dentin is also better when self-etch adhesives are used.

The aim of this study was to examine the quality, i.e., the ultrastructure of adhesive bond of composite restorations to dentin after application of total etch and self-etch technique using a scanning electron microscope (SEM).

METHODS

Twenty extracted human molars were cleaned from concrements and soft tissue remnants. Class V cavities were prepared on rotating instruments. For cavity standardization, a round diamond drill was always used (107-126 μm, No 806314-001544016, Meisinger, Germany) which was used in five preparations. Cavity dimensions were 2.5-3.0 mm horizontally and 2 mm vertically, maximum depth was up to 3mm which was controlled by a graded probe. All cavity margins were above the enamel-cement junction. Enamel margins were slanted 0.5-1 mm by a fine flame diamond drill (40 μm ISO 806204 lot 540570) using small speed rotation.

Ten teeth were restored by composite system *Adper Single Bond 2+Filtek Ultimate – ASB/FU* (two-phase adhesive, etch and rinse technique and hybrid nanocomposite) and they formed the first group of samples, whereas the remaining ten teeth were restored using *Adper Easy One+Filtek Ultimate* AEO/FU (one-phase adhesive. Self-etch technique and hybrid nanocomposite) and they formed the second group of samples.

Etch and rinse adhesive procedure. *Adper Single Bond 2* adhesive was applied by etching enamel margins and the whole cavity surface by *Scotchbond Etchant* (3M ESPE) acid for 15 seconds and then rinsed for 10 seconds and dried with a cotton ball. The adhesive was applied in two consecutive layers by rubbing in for 15 seconds. A mild air current for 5 seconds thinned the adhesive layer and standards light polymerization was done for 10 seconds.

Self-etching adhesive procedure. *Adper Easy One* adhesive was rubbed in the enamel and dentin surface was done for 30 seconds. The surplus of adhesive was removed by air current in order to obtain a shiny liquid film of the cavity surface. The adhesive was then polymerized by standard light for 10 seconds. After the adhesive technique, nanohybrid composite *Filtek Ultimate* was applied in one layer. Adhesive materials and composite materials were polymerized by a halogen lamp *Elipar Highlight 3M ESPE* (series no. 938020000257) following the manufacturer's directions. The surplus of composite was removed by polishing the restorations with *Soflex* discs 3M ESPE. After the restorative procedure, first the teeth roots were cut off and then crowns were cut off sagittally through the composite restorations in order to expose the juncture between the material and hard dental tissues. The sections were then polished with *Soflex* discs, conditioned with 37% solution of phosphorous acid (for 60 seconds) and rinsed with a spray of water with air pressure. After that, the surface was immersed into 2% solution of sodium hypochlorite for 6 seconds in order to dissolve the organic debris (7).

The samples prepared in this way were fixated in cylindrical carriers (*Dotite point xc 12 carbon* JEOL) and a thin layer of gold was applied on the surface (*JFC 1100E JonSputter JEOL*). After identifying the adhesive juncture of material to dentin with SEM, the analysis of hybrid layer and resin tags was performed. The depth of adhesive and hybrid layer was estimated with a scale on the monitor of a SEM microscope. The samples were observed at 200x, 1000x and 1500x magnification.

The ultrastructure of adhesive bond of composite restorations to dentin was analyzed with a scanning electron microscope - SEM (*JSM-5300, JEOL*).

RESULTS

The restoration sections showed a better marginal bond and a more complex ultrastructure of the composite bond to dentin when total etch adhesive procedure was applied (*etch and rinse*) compared to the self-etch technique. Better ultrastructure bonding between composite and dentin was established after the use of *Adper Single Bond2-Filtek Ultimate (ASB-FU)* materials, compared to *Adper Easy One-Filtek Ultimate (AEO-FU)* materials. In total etch technique, the ultrastructure of adhesive bond was composed of adhesive and hybrid layer as well as resin micro tags in dentin canals. The etching of dentin with phosphorous acid enabled the formation of a 5 μm thick hybrid layer while the adhesive layer was 10-20 μm thick (Figure 1).

In contrast to these results, apart from adhesive and hybrid layer, the self-etch technique rarely showed or did not show any resin processes in the dentin canals. The bonding of self-etch adhesive, i.e. *Adper Easy One-Filtek Ultimate (AEO-FU)* material caused the formation of a thin (1-2 μm) hybrid layer or its absence on intertubular dentin (Figure 2).

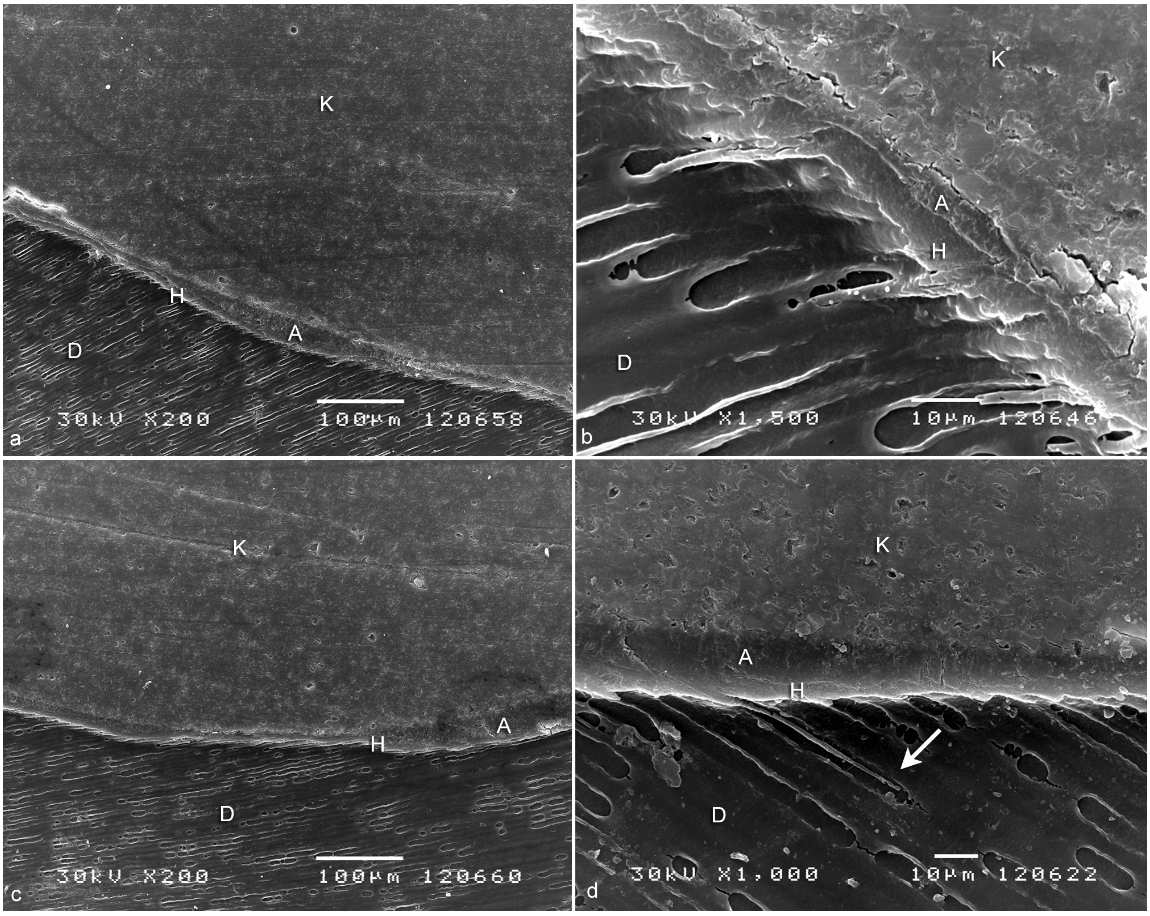

Figure 1. *SEM micrographies represent adequate adhesive bond of Adper Single Bond 2-Filtek Ultimate material to dentin on cross-sections through restorations (etch and rinse technique)*

D- dentin, C- composite, A- adhesive layer, H- hybrid layer. a) Adhesive and hybrid layers are clearly observed on dentin tissue and there is no microgap on junction (enlargement 200x, bar 100 μm). b) Resin penetration in dentin canals provides unique adhesion of composites and dentin (enlargement 1500x, bar 5 μm). c) Adhesive and hybrid layer on dentin form a unique layer (enlargement 200x, bar 100 μm). d) Long elongation of resin in dentin canal is observed in the centre, arrow. (enlargement 1000x, bar 10 μm).

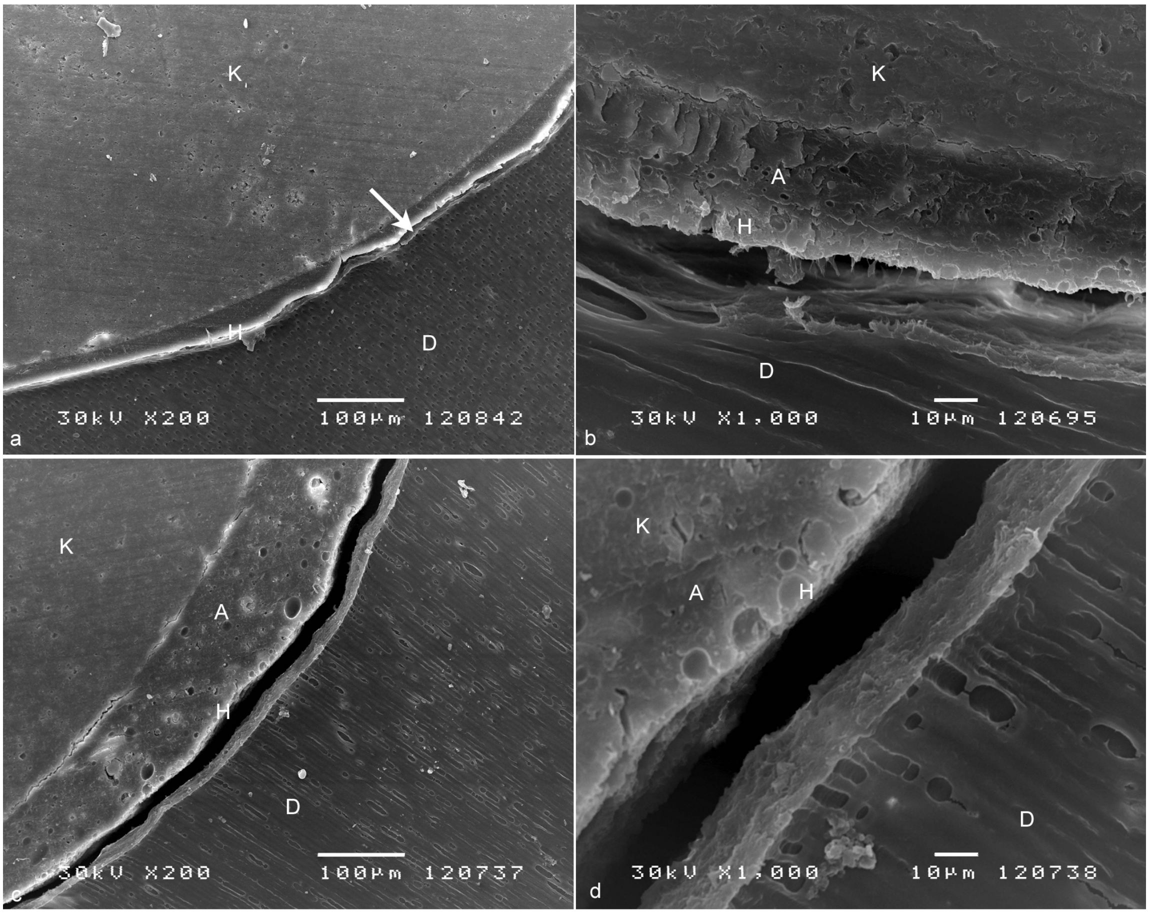

Figure 2. *SEM micrographies represent inadequate adhesive bond of Adper Easy One-Filtek Ultimate material to dentin on cross-sections through restorations (self-etch technique)*

E- enamel, C- composite, A- adhesive layer, H- hybrid layer. a) Adhesive and hybrid layer are separated from dentin by a visible microgap (arrow) (enlargement 200x, bar 100 μm). b) Thickness of adhesive layer varies along composite to dentin bond. Hybrid layer is separated from dentin, torn elongations of adhesive resin are observed in the space between material and dentin (enlargement 1000x, bar 10 μm). c) Thick adhesive layer and thin hybrid layer next to it are observed, both of them are separated from dentin (enlargement 100x, bar 10 μm). d) The same sample with image of microgap between composite and dentin (enlargement 1000x, bar 5 μm).

DISCUSSION

Bonding to dentin, among other things, depends on regional variations in the structure of this tissue, depth and position of cavities, tissue age, carries changes in dentin and a number of factors related to the oral cavity and chewing.

Compromised bonding to dentin results from a complex histological structure and different compositions of the dentin itself. Inorganic hydroxylapatite makes up 45-65% of dentin volume and it is included in organic matrix which is mostly composed of collagen. Regional variations of dentin are also expressed in different dentin permeability which varies in different locations of the teeth. The bonding to dentin is also difficult because of the presence of the smear layer which is a consequence of heating the dentin surface when dentin is cut. The smear layer is 0.5-5μm deep and it covers the openings of dentin canals. Its depth, appearance and contents vary depending on the instruments used to cut the hard dental tissue. Although it works as a 'diffuse barrier' which reduces dentin permeability, it is considered unwanted and is therefore removed so that composite adhesive (adhesive resin) could form a bond to the dentin which is below it (5).

The improved strength of dentin bond with total etch adhesive technique was first demonstrated by Fusayama et al. in 1979 (8). This adhesive procedure was thus introduced into the clinical dental practice in Japan and America. Total etch procedure includes a specific dentin preparation. After rinsing the conditioner (acid), a primer which contains one or more hydrophilic resin monomers is applied on dentin. The primer molecules such as HEMA (hydroxyethylmethacrylte), BPDM (biphenyl dimethacrylate) and 4 META (4-methacryloxyethyl anhydride) contain two functional groups - hydrophilic and hydrophobic group. Hydrophilic group has affinity for moist dentin surface while hydrophobic group has affinity for resin. The primer moisturizes and permeates the collagen dentin network by rising to the original level. In addition to moisturizing the dentin, the primer also increases the dentin surface energy. Liquid resin from adhesive permeates the dentin prepared by primer and forms collagen and resin layer termed 'resin fortified zone', 'resin infiltrated layer' or 'hybrid layer'. The formation of hybrid layer was first described by Nakabayashi et al. in 1982 and this layer is considered basic bonding mechanism of many modern adhesive systems (8).

In this study, a better quality of ultrastructure of adhesive bond between composite resin and dentin was obtained with total etch adhesive technique. In this technique (preparation of dentin with phosphorous acid), adhesive and hybrid layers were observed on dentin surface which was in contact with the material, as well as shorter and longer resin microtags in dentin canals. Contrary to these results, in self-etch technique, resin processes in canals were rarely found.

Frankenberger and Tay proved that the etching of denting with phosphorous acid enables the formation of a 5-6 μm deep hybrid layer with two-phase etch and rinse adhesives (9). According to their findings, after the application of self-etching primers, i.e. self-etch adhesives, the depth of the hybrid layer was smaller and it amounted to 2 μm.

In this study, the results of SEM analysis of the ultrastructure of adhesive bond between composite and dentin also showed a significantly better marginal adaptation of composite restorations when etch and rinse technique was used along with ASB/FU materials, compared to self-etch adhesive technique and AEO/FU material. The width of the hybrid layer on dentin was bigger with etch and rinse adhesive technique than with self-etch adhesive technique.

According to Van Meerbeek, the strength of the bond between composite and dentin (μTBS) is greater with three-phase etch and rinse adhesive and it amounts to 50MPa, compared to two-phase adhesives of the same group which amount to 39 MPa (2). The strength of the bond between composite and dentin in self-etch adhesives is weaker and it amounts to 40 MPa in two-phase adhesive and as much as 20 MPa in one-phase self-etch adhesives. However, the strength of composite to dentin bond does not need to be correlated to the thickness of the hybrid layer (10). In our study, we used two-phase etch and rinse (*Adper Single Bond 2*) adhesive and one-phase self etch (*Adper Easy One*) adhesive and a better bond ultrastructure was obtained with the first adhesive.

In this study, the sagittal sections of restorations were prepared using a diamond disc with water cooling. Even the studies that used replica technique for SEM analysis of sections of teeth with restorations could not evade artifacts (material or juncture fractures) on samples stress upon cutting. The cutting technique used in this study could cause iatrogenic formation of fractures and this affected the bond between composite and dentin. In addition, the application of high vacuum upon gold steaming used for SEM preparation of samples, could affect the quality of bond between composite and dentin. However, there were obvious differences in the bonds among the two sample groups (etch and rinse and self-etch technique) whereby a better ultrastructure of composite to dentin bond was obtained with etch and rinse technique.

Numerous *in vitro* studied tested the properties of adhesives by estimating marginal closure (2, 11-14). SEM was used to monitor the marginal gap around restorations on extracted teeth (1, 2, 15-18). It was thus confirmed that the forces developed during restorative material contraction or thermodynamic tooth load with restoration damage the strength of the enamel to dentin bond which leads to the formation of a gap around restoration margins. It was concluded that these semi-quantitative analyses of marginal gap are clinically relevant, particularly when measurements are repeated after thermocycling. Teeth dehydration, during SEM preparation, can also be a way of thermal-physical load of adhesive bond. The sections through restorations usually show adhesive and cohesive fractures of material along dentin junction. They are mainly a consequence of polymerization contraction of composite and dehydration of samples during SEM preparation. Although there is a tendency to simplify the bonding procedure, conventional three-phase etch/rinse adhesives still provide the most adequate and the most reliable bonding over a longer period of time. Self-etch approach may have a better perspective in the future. Clinically, when adhesives do not require etching and rinsing phases, application time and technique sensitivity are significantly reduced. Mild self-etch adhesive which form bonds by combined micro-mechanical and chemical interactions with the dental tissue approach the bonding quality of three-phase etch/rinse systems (3).

The persistence of the adhesive bond between composite resins and dentin is one of the most important factors concerning the durability of composite restorations (17, 18). A complete infiltration of demineralized dentin by resin is necessary. This space is filled by the water phase from dentin canals. The movement of water between hybrid layer and dentin causes hydrolysis. The process of hydrolysis disrupts the covalent bonds between collagen fibers and resin. It is believed that collagen degradation is caused by the effect of matrix metalloproteinase enzymes. These enzymes can come from bacteria and from dentin matrix and they are slowly released. This causes degradation of adhesive bond even in cases where cavities are closed without microleakage. The presence of bacteria is not necessary for dentin collagen degradation. Cholhexidine acts as a matrix metalloproteinase inhibitor and it stops the degradation of the hybrid layer. Inclusion of chlorhexidine in some future adhesive systems could provide a longer lasting quality and preservation of the ultrastructure of composite to dentin adhesive bond (19, 20).

CONCLUSION

SEM analysis of adhesive bond between composite resin and dentin showed a better ultrastructure with the use of etch and rinse technique and ASB/FU materials. When this technique was used, adhesive and hybrid layers were observed on dentin surface, as well as resin processes in dentin canal sections.

With self-etch technique and AEO/FU materials, adhesive and hybrid layers were often separated from dentin and disrupted resin processes could be observed between the material and dentin.

Acknowledgement

We would like to express our gratitude to electronics engineer Ljubiša Miljković for his technical assistance with SEM and to the staff of Biomedical Research Institute of the Faculty of Medicine, University of Niš. Restorative materials used in this study were donated by 3M ESPE from Belgrade and their associate Dr Milan Vladisavljević.

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ULTRASTRUKTURA ADHEZIVNE VEZE KOMPOZITA ZA DENTIN

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Sažetak

Ultrastruktura adhezivne veze je značajan pokazatelj efikasnosti pripoja materijala za zubna tkiva. Kvalitet veze kompozita za tvrda zubna tkiva takođe se procenjuje jačinom vezivanja ili odsustvom marginalne pukotine po obodu restauracija.

Cilj ovog rada bio je da se proceni ultrastruktura veze kompozitnih materijala za dentin nakon primene dve adhezivne tehnike.

Na ekstrahovanim zubima restaurisano je 20 kaviteta V klase za skenirajuću elektronsku mikroskopsku (SEM) analizu pripoja kompozita za dentin. Adhezija je obezbeđivana primenom *Adper Single Bond2*-ASB/3M ESPE, odnosno primenom *Adper Easy One*-AEO/3M ESPE. Svetlosna polimerizacija adheziva i kompozitnog materijala *Filtek Ultimate*-FU-/3M ESPE vršena je halogenim svetlom *(Elipar Highlight 3M ESPE)*. Nakon vertikalnog presecanja krunica zuba kroz kompozitne restauracije i pripreme površine preseka (30% fosforna kiselina-30s i 2% NaOH-30s), vršena je SEM analiza adhezivne veze.

Bolje mikromorfološko vezivanje kompozita za dentin SEM analiza adhezivne veze pokazala je kod adhezivne tehnike sa nagrizanjem i ispiranjem zubnih tkiva (ASB/FU materijali), u odnosu na tehniku samonagrizanja tkiva (AEO/FU materijali). Kod tehnike nagrizanja i ispiranja, ultrastruktura adhezivne veze za dentin sastojala se od hibridnog i adhezivnog sloja, kao i produžetaka smole u dentinskim kanalićima. Kod samonagrizajuće tehnike, pored adhezivnog i hibridnog sloja, retko su viđani ili uopšte nisu postojali produžeci smole u kanalićima.

Bolju ultrastrukturu veze kompozita za dentin obezbedila je primena adhezivne tehnike sa nagrizanjem i ispiranjem tkiva.

Ključne reči: **zubni kompoziti, kompozitni adhezivi, dentin**