Qualitative Evaluation by Means of SEM on the Qualities of the Hybrid Layer Formed by an Experimental Self Etching Adhesive

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The presence of the hybrid layer proves the existence of a micro mechanical bonding between dentine and resin. The aim of this study was to observe the aspects of an experimental adhesive at SEM, compared to two commercial adhesives. 30 molars extracted for orthodontic reasons were used as basis for preparing 60 cavities under a specific method. The adhesive systems under study were applied light-cured and the cavities were filled with composite material. The teeth were sectioned and the resulted surfaces were examined at SEM. The presence of the hybrid layer is well noticeable at all three adhesive systems studied but its characteristics differ from one material to the other. The chemical composition of the adhesives is reflected in the structural modifications, which are probably basis for the adhesion. The clinical significance of the morphological evidence is still unknown. According to the SEM analysis one can see that there is no standard hybridisation for the adhesive systems. The experimental adhesive system evaluated under SEM showed potential to form a hybrid layer and an adhesive layer with morphological aspects comparable with the commercial ones taken into study.

Keywords: adhesive system, hybrid layer, SEM

The adhesion to tooth tissues is the main factor for obtaining a durable and good restoration. During the restoration processes, some materials used for replacing tooth tissues proved in time to have a weak adhesion in the geometric spaces created inside the tooth (the bounding was performed through mechanical retention). Due to the fast development of science and technology nowadays solutions were sought to obtain new generations of dental adhesives with standardized properties [1]. The ultra morphological studies and the interface characterization of tooth/composite are absolutely necessary for the evaluation of the efficiency of the elaborated adhesive systems. Some of the newer bonding systems on the market suggest a new chemical bounding to dentine, but few are those which provide experimental proof. A successful treatment depends on both the properties of the adhesive material and on the correct practice of the used techniques. The evaluation of the adhesive systems is very complex, the structural aspects of the adhesive systems being part of it. While some of the authors are not interested at all in the noxious substance content in adhesives others bring to light their toxic action, e.g. HEMA and BisGMA [1]. Hanks *and co.* [3] referred to the molecular weight of BisGMA, TEGMA, HEMA and UDMA (512,65, 286,36, 130,14 respectively 496,69), suggesting, that, at the moment the adhesives containing these monomers are applied on dentine, HEMA produces the strongest cytotoxic effect because of the small molecular weight which allows a more rapid interdiffusion through dentine, as compared to the bigger molecules insoluble in water. As a result, a higher concentration of substance, can affect the pulp tissue of the tooth. Thus, it is correct to think, that the irritative potential of the adhesive resins applied directly on dentin depends not only on the cytotoxic effect of the components, but also on their capacity of interdiffusion through the dentinal tubule, because it was demonstrated that the monomers turn aggressive only when coming into contact with the dental pulp or the conjunctive tissues [4,5].

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Experimental part

The efforts to develop better biocompatible dental adhesives should imply a more simple bounding process, with reduced number of steps, and the realization of adhesives better tolerated by patients providing reliable quality and excellent fatigue endurance. However, there still remain some aspects of sensitive issues regarding the use of adhesives to be explored. The hybrid layer formation is called hybridisation and takes place after the acid etching of dentine, and the collagen exposure and low viscosity monomer impregnation. In order to obtain an efficient hybrid layer, it is suggested to make a correlation between type and concentration of monomers and solvents, the viscosity and temperature of the solution diffusion and affinities for the substrate and to combine these factors with the time of action of the adhesive system [6,7].

The presence of the hybrid layer demonstrates the existence of a micro mechanical bonding between dentine and resin.

Our aim was to observe at SEM the aspects of an experimental adhesive compared to two other commercial dental adhesives.

On 30 freshly extracted teeth (inferior molars) kept in artificial saliva solution ARISTAL (France), we prepared standard class V cavities, the preparation method was similar to the one presented in the specialized literature [8].

Table1
COMPOSITION OF THE ADHESIVES IN STUDY

Adhesives	Composition	Photo initiator
Single Bond 2	Ethyl alcohol, Bis-GMA*, HEMA*, glycerol 1,3-dimethacrylate, copolymers of the acrylic and itaconic acids, water, diurethan dimethacrylate	Camphorquinone *2-etilhexyl-4-dimethylaminobenzoate*
Adper Prompt L-Pop	Water, HEMA, di-HEMA phosphate, Bis-GMA	Camphorquinone *2-etilhexyl-4-dimethylaminobenzoate*
Experimental adhesive 2 step	HEMA, urethane phosphoric monomers, ethyl alcohol, water, Bis-GMA, TEGDMA*, HEMA,	Camphorquinone with DMAEMA*

*Bis-GMA- *2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane;* ***HEMA – 2-hidroxietil metacrilat*** *TEGDMA – triethylene glycol dimethacrylat; DMAEMA- N,N dimethylamino methylmethacrylate;*

The cavities were prepared with medium grit diamond burs at high speed and fine grit diamond burs were used for the finishing. The cavities were made at the level of the amelo cemental junction so that the incisal edge was placed 1 mm above in enamel and the cervical margin 1mm below the amelo cemental junction. The cavities were performed on the vestibular and oral facets of the teeth, resulting in a total number of 60 cavities. The length of the cavities was of 3 mm, a width of 2mm, and a depth of 2mm.

The application of the adhesive and the polymerization were performed in conformity with the producer instructions.

The cavities were then filled with Filtek Supreme XT composite material, colour A1E, polymerized according to the producer instructions. The teeth were sectioned using a microtome with water cooling device, Buechler LTD, with a saw blade of 0.6 mm. The slice thickness was of 1.5 mm, the sectioning was performed towards the tooth axis and in vestibular-oral direction. The slices were studied using an electronic microscope FEI-system.

Results and discussion

The examined samples showed subtle differences between the different adhesive resins, regarding the interdiffusion of resin into the demineralised dentine. The hybrid layer is clearly distinguishable in all three adhesive systems studied, but its characteristics differ from one material to the other.

Single Bond 2: The contact with the dentine is continuous, without interruptions, the filaments of resin from radicular canals have a close contact with the resin, their length exceeding 10 micrometers. The discontinuity which one can observe between the hybrid layer and the adhesive surface may be due to the preparation and examination or due to the bulk application of composite material. The hybrid layer efficiently seals the dentine. Even if the Single Bond 2 adhesive has fill material, it is not able to penetrate the collagen fibres and this is why the dark surface appears in the area of the hybrid layer when the adhesive is in contact with the dentine.

Experimental adhesive: The adhesive cured with LED has formed a clearly visible hybrid layer, which penetrates the dentinal canaliculi. The hybrid layer covers equally the dentine surface, leaving no leaks on its surface. The examination is more difficult because the material of the adhesive coating is very fine grained and its brightness is similar to the brightness of dentine.

Adper Prompt: One can clearly observe how the resin penetrates into the dental canaliculi and forms a hybrid layer and resin filaments. The fracture line crosses the interface adhezive-composite material.

At the maximum zoom level, the hybrid layer shows its qualities

The line along the junction between the dentine and the adhesive proves to be a very well formed hybrid layer, in close contact with the dentin which sends ramifications to the dental canaliculi.

One can observe the hybrid layer and the resin filaments. The fracture line crosses the interface of the adhesive-composite material. In (1995) [9] it was evaluated the bond strength and morphology of resins on caries affected dentine. After polishing the bonded interfaces and after milling with argon ions to obtain a higher contrast for the SEM observations, they observed that some samples have a large depression within the hybrid layer. By means of SEM with high resolution camp/profilometry, it was confirmed the lack of substance in the middle of the hybrid layer, even in normal dentine regardless of the bonding agent [11].

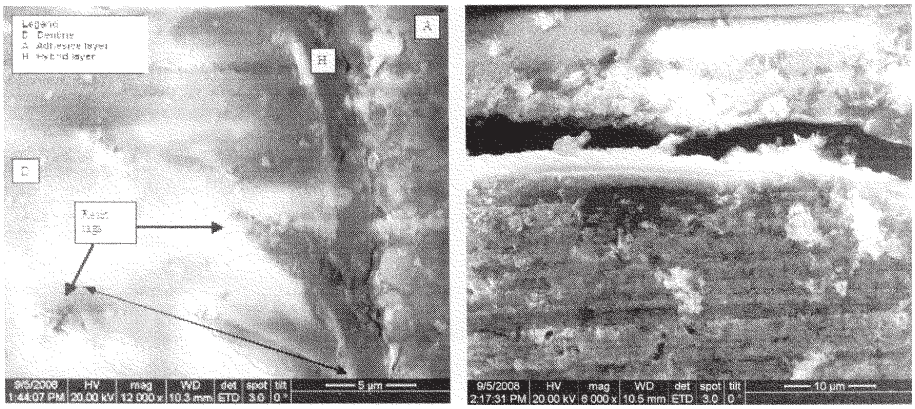

Fig. 1. Micrographs Single Bond 2 adhesive

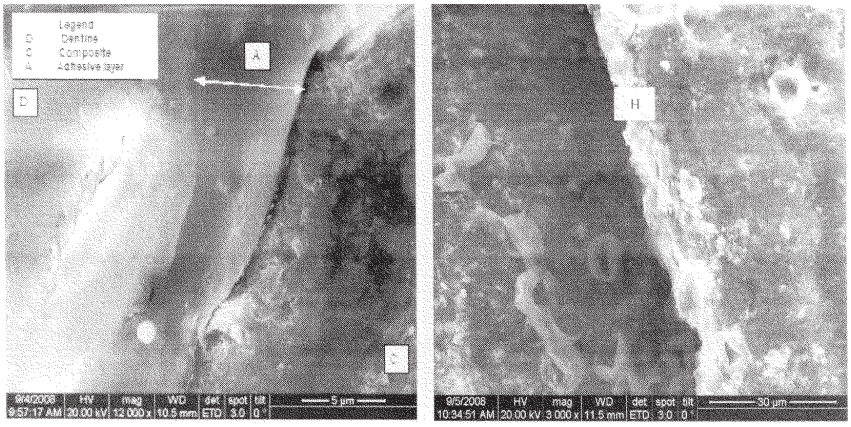

Fig. 2. Micrographs experimental adhesives system

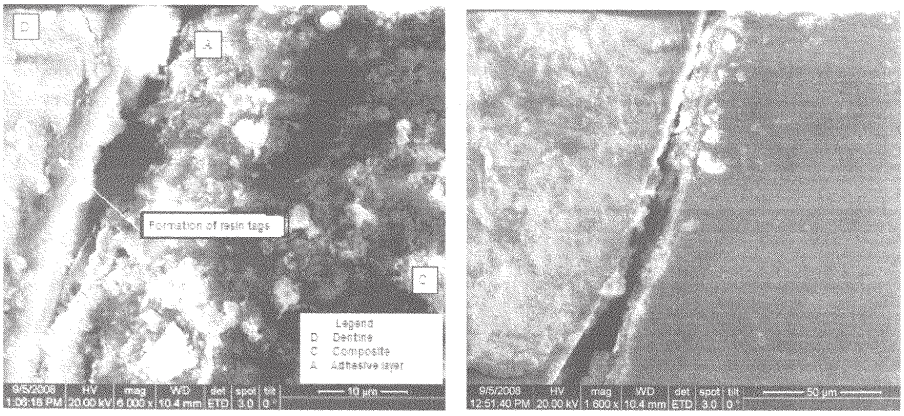

Fig. 3. Micrographs Adper Prompt adhesive

The main objective of the dental adhesives is to ensure retention of composite restoration or composite bonding. In addition to the resistance to mechanical stress, especially to the stress generated by the polymerization contraction of the overlying composite, a good adhesive must protect against micro infiltrations along the restoration margins.

Self etch adhesive systems combine the two steps, acid attack and prime phase: conditioning of the dental surface (acid attack and simultaneous priming of dentine) by means of acid monomers (without the rinsing phase) and application of adhesive resin.

There are two types of self etch adhesives: strong and weak. The strong self etch adhesives have a *p*H <1 and a bonding mechanism and interfacial morphology in dentin similar to the one produced by the acid rinse process systems. The weak self etch systems with a pH of approx 2 can only partially dissolve the dentine surface, thus a rather high number of hydroxyapatite crystals remain in the hybrid layer. Due to this fact, the carboxylic and phosphoric groups of the functional monomers can interact chemically with the residual hydroxyapatite [10]. This micromechanical and chemical bonding mechanism is believed to be more advantageous in terms of a durable restoration work. The chemical interaction creates more powerful bonds against the hydrolithic attack which leads to long lasting marginal sealing.

This study uses both self-etch and total etch adhesives, this is why the characteristics of both adhesive groups must be considered. The SEM images representing the adhesive/ dentine interface (A / D) (fig. 1-3) showed that it occurs a variation in the thickness of the layers during the interaction between the tested adhesive systems.

For the total-etch adhesives alcohol plays an important role, because it facilitates the penetration of the hydrophobic monomers into the demineralised dental structure. Unfortunately, the presence of the alcohol increases the sensitivity of the adhesive to water at the level of the dental plague. The probability of error is very small because a very dry substrate determines a decrease in adhesion to collagen and a too wet substrate leads to the separation of the hydrophilic/hydrophobic constituents of the adhesive [12-14].

The correct acid treatment is very important for the total-etch adhesive systems; a prolong acid etching determines the formation of a hybrid layer which leads to a decrease of the adhesion strength [15]. Furthermore, areas of demineralised dentine unimpregnated in adhesive resin occur between the hybrid layer and the dentine. The hybrid layer is susceptible to hydrolysis by the endogenous and exogenous enzymes. The hydrolysis reaction of the hybrid layer affects the sealing capability of the dental canaliculi and triggers a decrease in adhesion strength.

In the case of the total etch adhesives, as our study revealed, the area which is predisposed to fractures is the one between the adhesive and the hybrid layer, which allows at least temporary to preserve the canal seal and to protect de dental pulp from chemical and biochemical irritating agents (bacterial toxins) [16]. This bioprotecting effect of the hybrid layer represents one of the main desiderata in the treatment of dental wounds and as a sequence, the correct application of the total etch adhesives forming a well defined hybrid layer has a positive effect on the vitality of the dental pulp.

The self etch adhesives demonstrated the existence of an association between the structural morphology and their *p*H [17]. Unlike the total etch adhesive systems, the self-etch adhesive systems must cross the smear layer, which cannot be removed by acid etching, thus the penetration into dentine is significantly reduced.

The regular morphology achieved with this type of adhesives is due to the fact, that the demineralisation and the infiltration take place simultaneously [18].

The difference in aggressiveness of adhesives influences the thickness and the quality of the hybrid layer, and can be considered a critical factor in adhesion. The adhesives with

a high level of acidity reveal a complex hybrid layer at the interface [19, 21].

In this study, the experimental adhesive has a ph of 2,5, while the *p*H of the Adper Prompt L-Pop is of 1 [22-24]. The adhesive interface observed in this study has been continuous, with a uniform thickness, with the infiltration of the smear-layer and with intracanicular extensions.

The chemical composition of the adhesives is reflected in the structural modifications, which probably lay at the basis of the adhesion. The clinical significance of the morphological evidences is still unknown. The long lastingness can be correlated with the adhesion strength and with the morphological modifications [24,26,30].

The hybrid layer is better defined at the total-etch and self-etch adhesives, when dentine is intact and not affected by carries [27, 31].

The SEM studies reveal that there is no standard hybridisation for the adhesive systems. The presence of resin tags and their length changes according to the location in dentine and to the type of material used [28,30]. If compared to the total etch adhesives, the hybrid layer formed by the self etch adhesives is thinner, more uniform, and the resin is better infiltrated, but the number of resin tags is higher [29,31].

A comparison of the contemporary adhesives reveals the fact that the adhesives which are applied with the etch rinse technique in three steps, based on ethanol-water remain the "goal standard" regarding the adhesion duration. With each simplification of the clinical application procedure the adhesive efficiency decreases. Only the two steps self etch adhesives come nearest to this standard, with the additional benefit provided by an easy manipulation and a less sensitive technique.

Conclusions

The results revealed differences between the various adhesive systems but also defects which cannot be observed by naked eye, but which represent the main cause of failure of coronary reconstructions with composite material in the clinical practice.

There is no standard hybridisation of the adhesive systems. The analyzed samples reveal subtle differences between the various systems of adhesive resins, regarding the way of interdiffusion of resin in the demineralized dentine.

The experimental adhesive system observed at SEM proved its capacity to form a hybrid layer and an adhesive layer with morphological aspects similar to the commercial ones taken into the study.

The two-step experimental adhesives create a good interface and infiltration of the smear-layer and are examined inside the dental canaliculi.

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