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Comparison of Dentine-Resin Interface in Total-Etch and Self-Etching Adhesives Using Electron Microscopy

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Abstract: The important condition for the adhesive bond between dentine and composite filling is the change of dentine property from hydrophilic to hydrophobic. Substances able to accomplish this change, dentinal adhesives, have undergone complex development. Recently, the so-called self-etching adhesives have been formulated. The aim of the present paper was to compare the structural pattern of dentin/resin interface after the application of dentinal adhesives used in total- and self-etching technique. Dentinal adhesives Single bond, Prime bond, Prompt-L-pop Adper, and Xeno III were used. The former two belong to the total etch technique, the latter two to the self-etching technique. The dentin/resin interface was studied in transmission (TEM) and scanning (SEM) electron microscope. In TEM sixteen teeth were investigated, each adhesive was applied in four teeth. Under SEM forty teeth were studied, each adhesive was applied in ten teeth. The obtained results demonstrated that all the dentine adhesives studied were able to penetrate into dentinal tubules and to form a hybrid layer with the demineralized intertubular and peritubular dentine. There was no substantial difference in morphology of the dentine/resin interface between the techniques studied. Our results suggest that the hybrid layer function of all four adhesives might be of the same quality.

Introduction

The importance of esthetic reconstructing of the coronal part of teeth not only in the frontal, but also in the distal parts of dentition has been steadily rising. While the anchorage of a composite filling material in the enamel was solved in an acceptable way by acid etching technique, the optimal interaction between dentine and filling material is apparently more difficult to achieve because of differences in morphological structure of both hard tissues. High number of microscopic spaces can be formed in enamel rods or in the inter-prismatic substance by acid etching of enamel which enables the penetration of a low-viscosity resin (bond) into the enamel surface thus increasing substantially the micromechanical retention of the filling. The main condition for the adhesive connection between dentine and a composite material is the change of dentine properties from hydrophilic to hydrophobic. The application of a suitable intermediate layer seems to be a solution. After selective demineralization of the dentine, these materials, called dentine adhesives, are chemically bound both to inorganic and organic components of the dentine. The adhesive should infiltrate the exposed collagen fibre network, thus forming a hybrid layer, and penetrate into dentinal tubules. The hybrid layer is about 5-10 μm thick. After polymerization, the resin impregnated collagen; together with the resin in dentinal tubules constitute the adhesion between dentin and the resin. The dentine hybridization is a problematical and time-consuming method; that is

why a reduction of some steps in the procedure has a great clinical significance. In recent years, self-etching technique using single step adhesives has been introduced into clinical practice.

The aim of the present paper was to study the character of the interaction between dentine adhesives and dentine, and to compare the structural pattern of selected adhesions in total etch technique and self-etching technique.

Material and Method

TEM

The adhesives used were Single bond, a total-etch one bottle adhesive, Prime bond, a total-etch one bottle adhesive, Adper Prompt self-etch adhesive, and Xeno III self-etch adhesive. Sixteen human premolars extracted for orthodontic reasons were used. The cavities of class I or V were prepared immediately after the extraction. All adhesives were applied into prepared cavities according to the manufacturers´ instructions. The walls of the prepared cavities were covered only by dentine adhesives, four teeth were used in each group. The tooth roots were cut off and the coronal part of teeth was fixed in 3% glutaraldehyde in 0.1M phosphate buffer. After fixation the samples were demineralized in 1M EDTA, chopped into small pieces, post-osmified and embedded into Durcupan ACM. Ultrathin sections cut with LKB ultramicrotome were stained and photographed in Morgagni 268 D transmission electron microscope.

SEM

The adhesives used were Single bond, a total-etch one bottle adhesive, Prime bond, a total-etch one bottle adhesive, Adper Prompt self-etch adhesive, and Xeno III self-etch adhesive. Forty human premolars extracted for orthodontic reasons were used for the study. The teeth were stored in 10% neutral formalin. The cavities of class I or V were prepared and the adhesives were applied according to the producers' recommendation. Eight teeth were used in each group. After adhesive polymerization, the cavities were restored with composite resin filling. In each type of adhesive two teeth were restored with Spectrum and another two teeth with QuiXfil. The walls of the prepared cavities in 8 teeth were covered only by dentine adhesives, two teeth were used in each group. The tooth roots were cut off by a diamond bur in a high speed hand piece. Struers Accutom 50 was used to halve the teeth, and the obtained samples were embedded into methacrylate resin. The cut surfaces were polished with the Struers Tegra system. The polished surfaces were etched for 20 seconds with 37% phosphoric acid to remove the debris. A part of the teeth was fractured in a specially adapted bench vice without methacrylate embedding. The samples were prepared for investigation in Tescan Vega TS 5136 XM scanning electron microscope under both high and low vacuum.

Results

TEM

Transmission electron microscopy revealed the dentine surface covered by a layer of adhesive. Both Single bond and Prime bond covered continuously the etched dentine surface and entered the funnel-shaped exposed dentinal tubules (Figs. 1–4). The resin tags were in contact with dentinal tubule walls and also penetrated into branches and demineralized small openings. The intertubular matrix was also demineralized and the developing spaces were infiltrated by dentine adhesives thus forming the hybrid layer. Exposed collagen fibres were in the direct contact with the adhesive material.

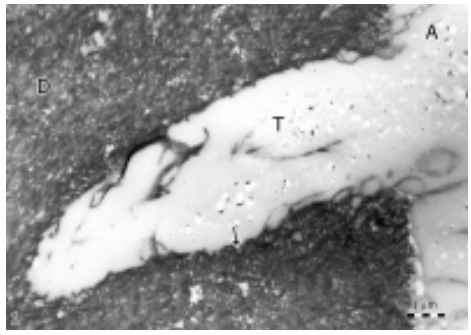

Figure 1 – TEM, total-etch technique, Single bond. Dentine adhesive (A) penetrates into a dentine tubule in the form of a tag (T). Along the dentine tubule wall a minute hybridization of dentine is visible (arrow). Dentine (D).

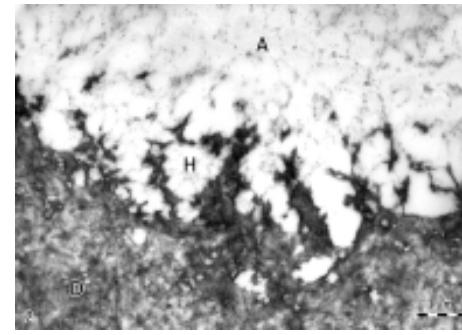

Figure 2 – TEM, total-etch technique, Single bond. Hybrid layer (H) formation, Dentine adhesive (A) penetrates into demineralized areas between collagen fibres. Dentine (D).

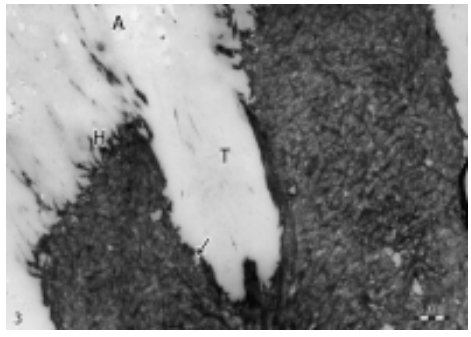

Figure 3 – TEM, total-etch technique, Prime bond. Dentine adhesive (A) penetrates into a dentine tubule in the form of a tag (T). Along the dentine tubule wall a penetration of adhesive into anastomosing tubules (arrow) is visible. Dentine (D), hybrid layer (H).

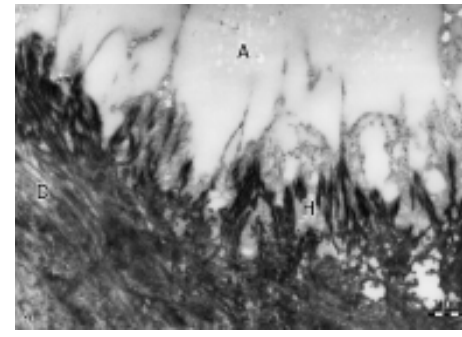

Figure 4 – TEM, total-etch technique, Prime bond. Hybrid layer (H) at higher magnification. Dentine adhesive penetrates into the demineralized dentine, exposed collagen fibres are clearly visible. Dentine (D).

The self-etching adhesives Xeno III and Prompt-L-pop, were found to form the hybrid layer both in the intertubular matrix and in dentinal tubules (Figs. 5–8). Resin tags penetrated into dentinal tubules and obturated them totally. The part of dentinal tubules in the surface area was not funnel-shaped arranged towards the surface. The dentinal tubule walls were parallel or slightly converged towards the surface. The penetration of adhesives into lateral branches and small openings was regularly found as well as demineralization of the intertubular dentine and exposure of collagen fibres. The resin filled these demineralized spaces thus forming the hybrid layer.

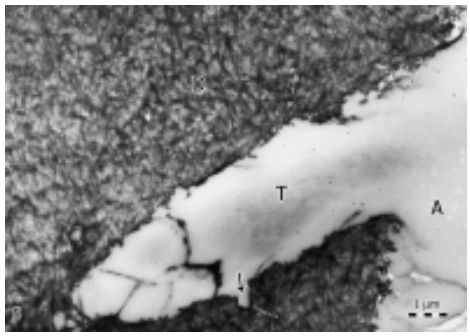

Figure 5 – TEM, self-etching technique, Prompt-L-Pop. Dentine adhesive (A) in the form of a tag (T), hybridization of the dentine tubule wall (arrow). Dentine (D).

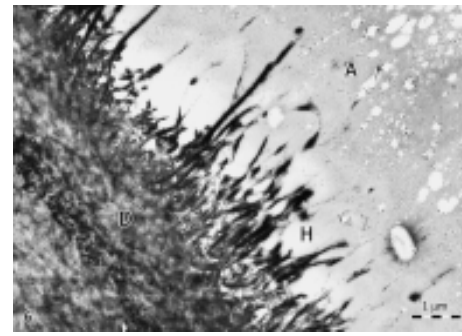

Figure 6 – TEM, self-etching technique, Prompt-L-Pop. Hybrid layer (H) at higher magnification. Dentinal adhesive penetrates into the demineralized dentine, isolated collagen fibres are surrounded by the adhesive (A). Dentine (D).

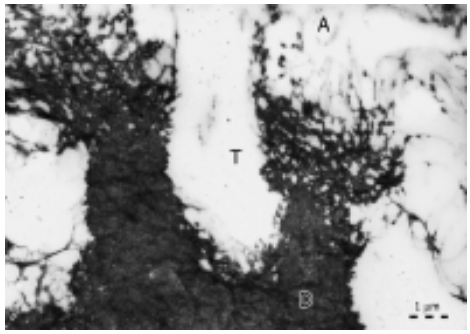

Figure 7 – TEM, self-etching technique, Xeno III. Well developed hybrid layer , the resin tag (T) penetrates into the dentinal tubules, hybridization of the dentinal tubule wall, adhesive (A), dentine (D).

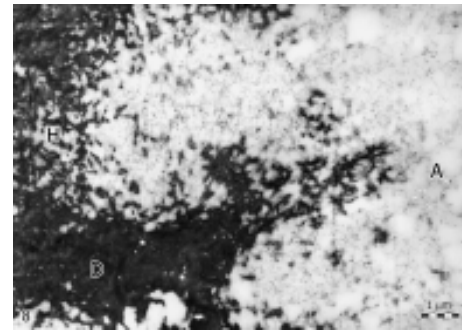

Figure 8 – TEM, self-etching technique, Xeno III. Hybrid layer at higher magnification. Demineralized areas filled with the dentine adhesive (A), the material penetrates into spaces between isolated collagen fibres. Dentine (D).

SEM

The acid-etched dentine surface was covered continuously by dentine adhesives Single bond and Prime bond in both samples treated only by adhesives and in composite resin filled samples. Dentinal adhesives penetrated into dentinal tubules in the form of tags (Figs. 9, 10) and formed the hybrid layer in the demineralized surface dentine. Individual tags were of different length ranging within 10–60 μm, in some cases even the length of 100 μm was recorded. The resin tags obturated totally dentinal tubules and sent very fine processes into anastomosing tubules (Fig. 9, inset). In a deeper part, however, the resin tags did not reach to the dentinal tubule walls. A well developed hybrid layer with resin tags penetrating into dentine tubules was also observed along the cavity walls. The high vacuum technique provided a sharper and more distinct picture of the situation and enabled morphological studies in greater details. The differences between the materials used, quality of co-polymerization, and character of filler particles were better demonstrated by the low vacuum technique (Fig. 9). Co-polymerization of dentinal adhesives with the composite resin material was found to be good and without voids.

Both self-etching adhesives Xeno III and Prompt-L-Pop Adper (Figs. 11, 12) covered continuously the surface of the exposed dentine, formed the hybrid layer and penetrated into dentinal tubules obturing them in the same way as the

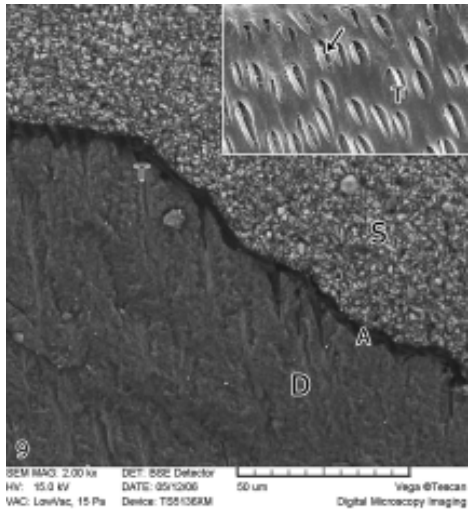

Figure 9 – SEM, total-etch technique, Single bond/ Spectrum (S). Adhesive (A) penetrates in the form of tags (T) into dentinal tubules. Dentine (D). Low vacuum. Inset: Higher magnification of resin tags (T) with resin penetrating into anastomosing tubules (arrow). High vacuum.

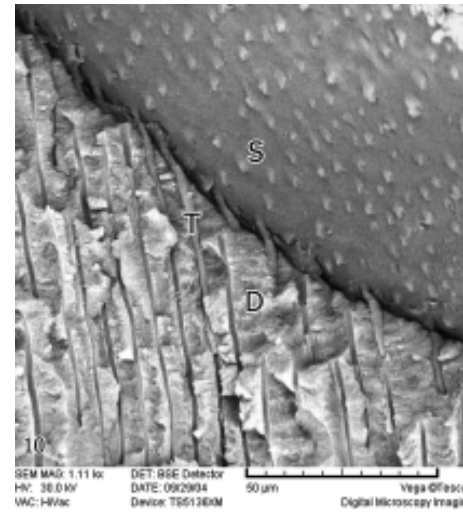

Figure 10 – SEM, total-etch technique, Prime bond/ Spectrum (S). Resin tags (T) penetrate into dentinal tubules, dentin (D). High vacuum.

adhesives Single bond and Prime bond. The length of resin tags seemed to be shorter than that of adhesives for the total-etch technique. Similarly, a well developed hybrid layer with resin tags in dentinal tubules was found along the cavity wall (Fig. 12). No substantial difference in the appearance of dentine-resin interface between the dentine adhesives studied was demonstrated under scanning electron microscope.

Discussion

The basic step in formation of a firm adhesive filling is the preparation of the enamel surface structure with many microretentions which will increase the strength of enamel-composite connection up to 20–25 MPa [1]. The same procedure in dentine, i.e. acid etching, failed to achieve sufficiently strong and sufficiently long-lasting dentin-resin interface. Majority of the bonds withstand strengths about 5 MPa, and the development of dentine bonding has stagnated for many years. The progress in the dentine bonding technique was done in 1982 when Nakabayashi et al. [2] reported the technique of dentine hybridization. The principle of the technique is the change in the dentine structure. The dentine surface can be changed by acid etching from crystalline,

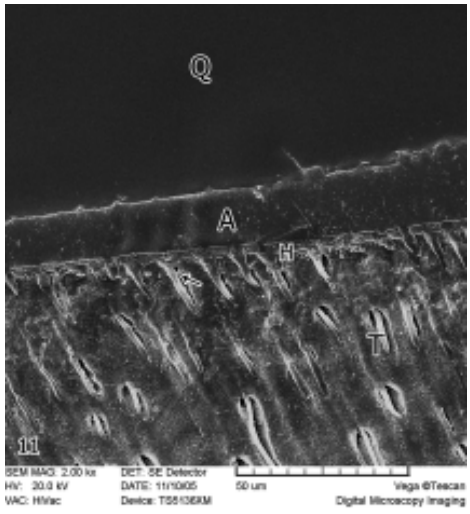

Figure 11 – SEM, self-etching technique, Prompt-L-Pop/QuiXfil (Q). Continuous layer of adhesive (A), hybrid layer (H), resin tags (T) in dentinal tubules, fine projections (arrow) direct to anastomosing tubules. High vacuum.

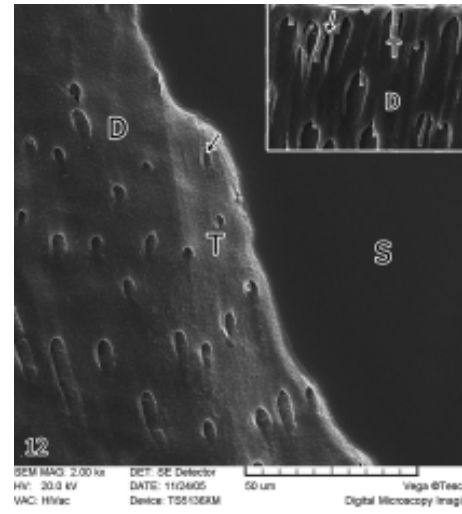

Figure 12 – SEM, self-etching technique, Xeno III/ Spectrum (S). Connection of adhesive to dentine (D) along the cavity wall, resin tags in dentinal tubules (T), fine projections direct towards anastomosing tubules (arrow). High vacuum. Inset: Area from the cavity floor, resin tags (T) in dentinal tubules, fine projections directing towards anastomosing tubules (arrow). High vacuum.

mineral, acid sensitive and relatively hydrophilic structure to organic, acid resistant and hydrophobic layer. They demonstrated that acid etching and rinsing with water was removing mineral components of the dentine leaving the organic matrix formed by collagen fibres in the surface area. Resin can penetrate between isolated collagen fibres thus forming the so-called hybrid layer. The hybrid layer is very firm and enables good micromechanical retention of resin in dentine. Moreover, resin penetrates into dentinal tubules in the form of tags increasing the strength of resin connection. Dentine bonding underwent a complicated development and many techniques of dentine bonding can be found at present [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. In addition to the total-etch technique, the so-called self-etching primer systems are used and "all in one" bonding techniques were introduced recently into dental adhesive technology with the aim to reduce the number of working steps which make the work in the oral cavity faster and simpler [13]. Self-etching techniques are reported to create the hybrid layer of high quality enabling good retention of composite filling in the dentine [7, 14, 15, 16].

Our study compared the quality of the dentine/adhesives interface of total-etch techniques with self etching systems of "all in one" type. The difference between the two methods is not only in materials used but also in the number of working steps. The total-etch method is composed of dentine etching, rinsing off with water, drying and primer/adhesives application, subsequent curing and application of a filling material. Phosphoric acid etching removes the smear layer and demineralises the dentine. This demineralised dentine becomes an important part of the adhesive system, the so-called hybrid layer. To achieve the optimal bonding to the dentine, adhesives must penetrate the demineralised dentine; enter the exposed dentine tubules and their branches. The "all in one" bonding systems are the most recent development in dental adhesive technology. They are formulated to etch the dentine, to allow infiltration of the collagen and the tubules, and to supply the bonding resin with the application of one solution so that the procedure is completed in one working step which makes the technique substantially shorter. The material is applied directly on the smear layer which is dissolved and dispersed in situ and becomes part of the bonding substrate [17, 18].

The obtained results demonstrated that all dentinal adhesives studied were able to penetrate into dentinal tubules and to form a layer with demineralised intertubular and peritubular dentine. Resin tags in the self-etching technique seemed to be shorter than in the total-etch technique. Similar findings were reported by Ramos et al [19]. The present morphological study has not revealed any substantial difference between the dentinal adhesives used. The examined teeth were not exposed to any mechanical load, conditions simulating the oral cavity environment and the adhesive forces were not studied so that the mechanical resistance of both types of adhesive techniques cannot be evaluated. Recent investigation, however, [14, 20] has advocated that, in spite of forming thinner hybrid layers than those formed by total etch systems, the self etching

primers may provide bond strengths to dentine comparable or even superior to those obtained with adhesive systems that advice the acid etching as a separate step of the bonding protocol [14, 17, 20]. A number of studies have been performed in animals to evaluate the reaction of dental pulp to acid etching [2]) and the results are promising, if the restorations are sealed to prevent bacterial leakage. Successful results are reported also on indirect and direct dental pulp capping [6, 21], when no danger for the dental pulp vitality was found.

Even though the "all in one" technique was found to form the hybrid layer of the same quality as the total etch technique, recent studies on continuing etching of "all in one" adhesives have pointed out on the danger of uncontrolled demineralization of dentine as a result of incomplete polymerization of acidic monomers [17, 22] More attention should be paid to the aforementioned findings. In spite of remaining problems, the dentine hybridization can be considered as a good and successful example of bio-engineering approach in restorative dentistry which enables forming a connection between hard dental tissues and the restorative material utilizing the microscopical structure of the dentine without damaging its vitality and integrity. The fact that there is no morphological difference in the dentine hybridization between total-etch and self-etching adhesives may be of importance in the restoration of primary teeth with composite resin fillings because the number of steps and the procedure duration is significantly reduced. In future studies our attention will be paid to the dentine hybridization in primary teeth.

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