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Scanning Electron Microscopy Evaluation of the Interface of Three Adhesive Systems

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The objective of this research was to investigate the resin-dentin interface of three adhesive systems, Scotchbond Multi-Purpose®, Optibond® and Denthesive Bond II® by scanning electron microscopy. The adhesives and their respective composite resins were applied inside the cervical root canal of human incisors and canines according to manufacturer recommendations. The teeth were embedded in acrylic resin and sliced transversally to the root canal and perpendicularly to the resin-dentin interface. The adhesive systems Scotchbond Multi-Purpose® and Optibond® had a homogenous hybrid layer and similar characteristics, involving resin penetration of peritubular and intertubular dentin matrix. Morphological differences of resin tags were seen; Scotchbond Multi-Purpose® had more and longer tags than Optibond®. Denthesive Bond II® did not have the same consistency of bonding. Tubular orifices were not opened and the smear layer was not removed. This was due to the absence of previous acid conditioning of dentin that damages hybrid layer formation. Analysis of the hybrid layer revealed different patterns, suggesting that the attachment was influenced by many factors and a standardization of dentinal substrate was impossible.

Key Words: scanning electron microscopy, adhesive systems.

INTRODUCTION

A fundamental objective of most restorative procedures is to create a union of two dissimilar surfaces: mineralized tooth structure and restorative material. In 1955, Buonocore (1) introduced the acid-etching technique which provides mechanical bonding between acrylic resin restorations and treated enamel surfaces via micromechanical retention. Successful clinical results of acid conditioning of enamel led to early efforts of dentin bonding to include the use of acid etching on dentin with the purpose to obtain adhesion (2). The high water and organic content of dentin, as well as the density, diameter and orientation of the dentinal tubules, make consistent and reliable adhesion to dentin a challenge. In addition to these physical factors, dentin bonding is further complicated by the formation of a smear layer when dentin is cut or ground, occluding the orifices of the dentinal tubules (3).

In the last decade, good results have been obtained using fourth-generation adhesive systems. One of their characteristics is to remove or modify the smear layer after conditioning the dentin which allows resin penetration into the dentinal tubules, peritubular and intertubular partially decalcified dentin, followed by polymerization creating a hybrid resin-reinforced layer (4). Besides this acid-resistant hybrid layer, the bonding interface is a complex structure which is composed of resin tags, adhesive resin, collagen fibers and dentin (5-7).

Thus, the objective of this study was to analyze *in vitro* the resin-dentin interface of fourth-generation adhesive systems, Scotchbond Multi-Purpose®, Optibond® and Denthesive Bond II®, using scanning electron microscopy.

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MATERIAL AND METHODS

The fourth generation adhesive systems and their respective composite resins used in this study were: I - Scotchbond Multi-Purpose Dental Adhesive System® + Z100® (SBMP; 3M, St. Paul, MN, USA); II - Optibond Multi-Use Filled Adhesive with Fluoride Release® + Herculite XRV® (OPB; Kerr, Romulus, MI, USA); III - Denthesive Bond II System® + Charisma® (DT; Kulzer Inc., Irvine, Germany).

Six extracted human maxillary central incisors and canines without endodontic treatment were kept in 1% thymol solution at room temperature. The teeth were washed for 12 hours with tap water to eliminate thymol residues. The cervical root canal, at the cemento-enamel region, was obtained using a high-speed diamond bur with water cooling. The diameter of the root canal was widened 1 mm to obtain dentin depth without pulp residues, followed by washing with tap water and drying (Figure 1).

The adhesive systems were applied into the cervical root canal of 2 teeth for each group. All procedures were carried out according to manufacturer instructions. Briefly, the following steps were used: SBMP: 37% phosphoric acid etchant for 15 s, washing and air drying + SBMP primer, air drying for 5 s and SBMP adhesive application and photopolymerization for 10 s; OPB: 10% maleic acid etching for 15 s, washing and air drying + OPB primer, 30 s brushing, air drying and photopolymerization for 20 s + OPB Dual Cure A (activator) and B (paste) application and photopolymerization for 30 s; DT: primer A mixed with primer B was applied, air drying for 15 s + Dentin/Enamel bonding resin application and photopolymerization for 20 s. The restorative resins were applied according to the incremental technique and photopolymerized for 60 s using an Ultralux Eletronic Dabi Atlante photopolymerizer (Ribeirão Preto, SP, Brazil).

Scanning Electron Microscopy

The cervical root portion of the teeth was embedded in acrylic resin and sliced transversally to the root canal and perpendicularly to the dentin-resin interface using a Struers Ministron microtome. Six 1-mm sections were obtained for each cervical root canal and these sections were washed with 96% alcohol to remove non-polymerized resin. The surfaces were smoothed and decalcified with 5% hydrochloric acid for 45 s to remove smear layer developed during the slicing and smoothing procedures. Finally, the slices were washed for 2 min with tap water according to the method of Joseph et al. (8). The sections were then desiccated with 90% alcohol, stored overnight at room temperature and mounted on stubs. They were then sputtered with 300Å to 500Å of gold palladium. Photomicrographs were taken at 500 to 1,500X magnification, at 15 kV and 20 kV (JEOL JMS 25 - SII, Sony, Tokyo, Japan) (9). Four radial points at the dentin-adhesive system-composite resin interface circumference were observed by the scanning electron microscope, as shown in Figure 2.

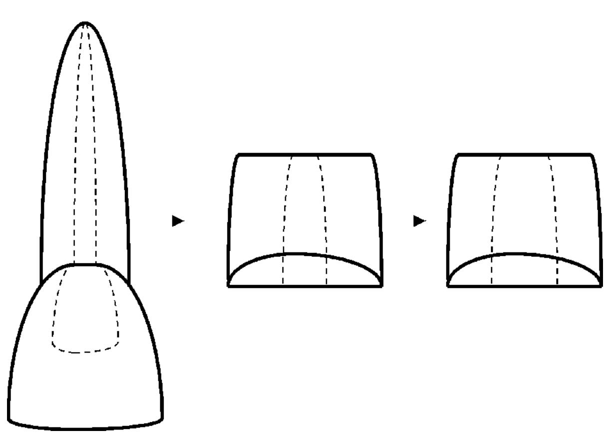

Figure 1. Illustration of root canal tooth preparation. The diameter of the root canal was widened 1 mm with a diamond bur to obtain dentin without pulp residues.

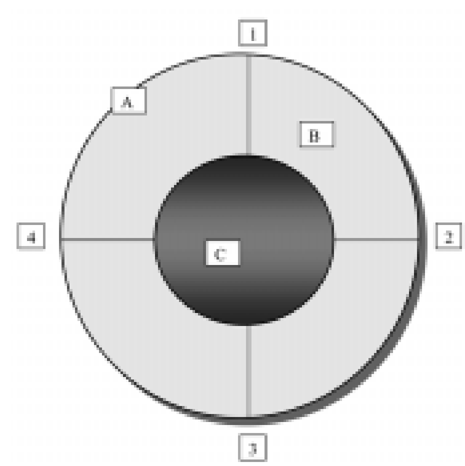

Figure 2. Scheme of the dentin-adhesive system-composite resin interface showing four radial points (1, 2, 3, 4) observed by scanning electron microscopy (A - cement, B - dentin, C - composite resin).

RESULTS

The findings of this study showed true hybridization and resin tag formation inside the dentinal tubules in all three experimental groups. In the SBMP group, there was high primer and adhesive system impregnation inside the peritubular and intertubular dentin, resulting in diffuse resin tags with a consistent and homogenous hybrid layer (Figure 3). Figure 4 shows the inner side of the hybrid layer where the interface had been interrupted. The OPB group presented features similar to those of SBMP, but interface gaps were seen between dentinal tubule walls and tags, with shorter tags and a less continuous hybrid layer (Figure 5). The DT group had different features than the SBMP and OPB groups. The hybrid layer was not homogenous and the length of the resin tags was variable (Figure 6). In some areas in which the hybrid layer thickness was constant, the resin tags were still irregular (Figure 7). Variation of the hybrid layer thickness was observed between the products, among the specimens of the same adhesive and in different areas of the same specimen.

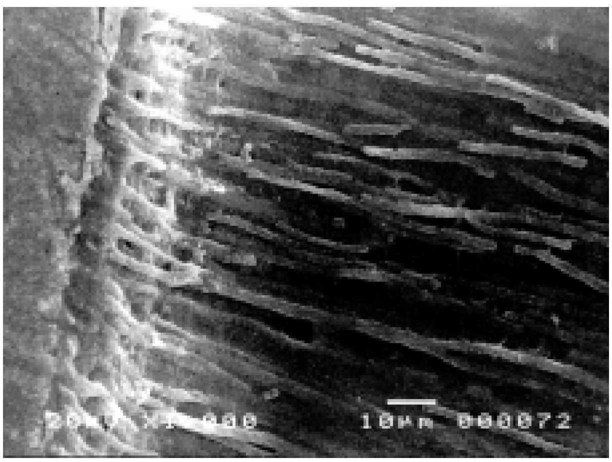

Figure 3. Photomicrography of the Scotchbond Multi-Purpose Dental Adhesive System® hybrid layer with diffuse resin tags with a consistant and homogeneous hybrid layer (1,000X).

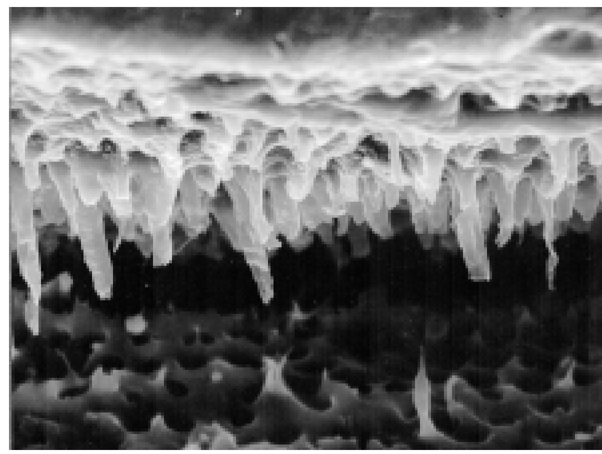

Figure 4. Photomicrography of the Scotchbond Multi-Purpose Dental Adhesive System® showing the inner side of the hybrid layer where the interface was interrupted (1,500X).

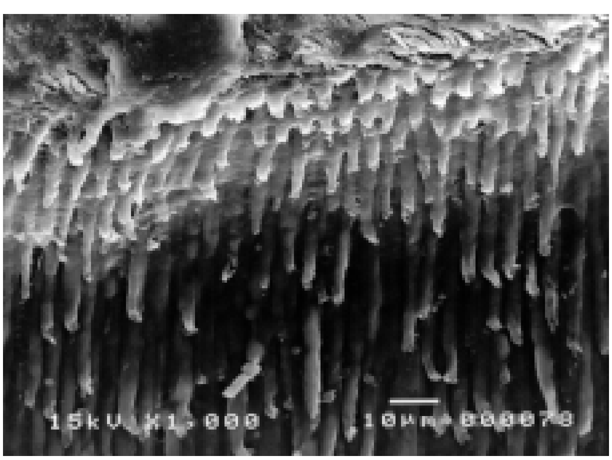

Figure 5. Photomicrography of the Optibond Multi-Use Filled Adhesive with Fluoride Release® hybrid layer. There are interface gaps between dentinal tubule walls and tags, with shorter tags and a less continuous hybrid layer compared to the Scotchbond group (1,000X).

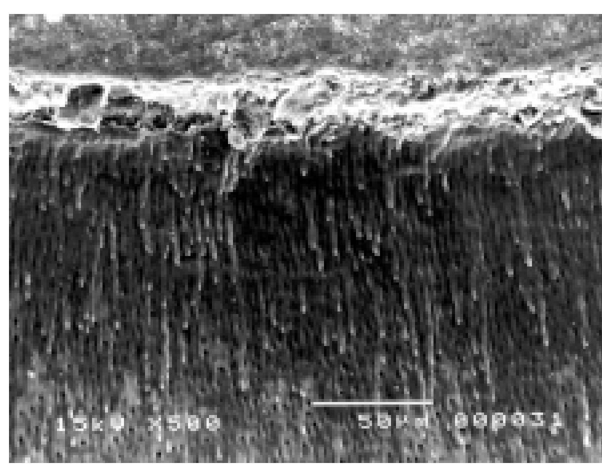

Figure 6. Photomicrography showing the characteristics of the Denthesive Bond II System® hybrid layer. The hybrid layer was not homogeneous and the length of the tags was variable (500X).

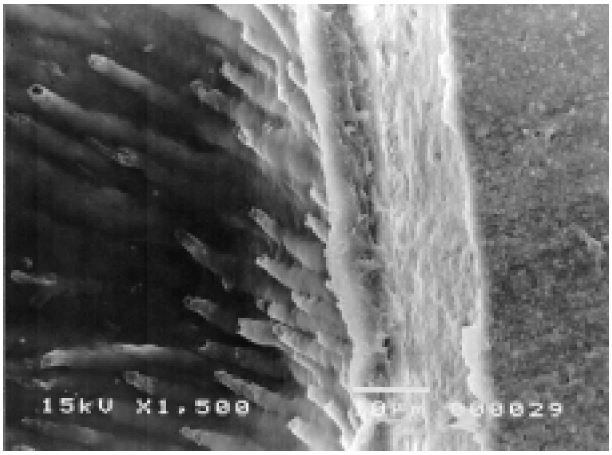

Figure 7. Photomicrography showing the characteristics of the Denthesive Bond II System® hybrid layer. The hybrid layer thickness was constant, but the resin tags were irregular (1,500X).

DISCUSSION

The requirements of an effective dentin adhesive system include the ability to thoroughly infiltrate the collagen network and partially demineralized zone, to commingle and encapsulate the collagen and hydroxyapatite crystallites at the front of the demineralized dentin, and to produce a well-polymerized durable hybrid layer (7,10,11).

Van Meerbeek et al. (12) and Perdigão and Swift (13) reported significant information about how the current generation of adhesive systems bonds to dentin. Although many different types of conditioners, primers and adhesive resins are used, the bonding mechanism of the various etched-dentin adhesive systems are remarkably similar. Acid etching removes the smear layer, opens the dentinal tubules, increases dentinal permeability and decalcifies the intertubular and peritubular dentin. The depth of the decalcification is affected by various factors, including pH, concentration, viscosity and application time of the etchant (14).

The acid treatment apparently creates diffusion paths for the adhesive resin in interfibrillar spaces. Removal of the hydroxyapatite crystals leaves a collagen meshwork that can collapse and shrink because of the loss of inorganic support (14). The process of washing and air drying after acid pre-treatment leads to the loss of calcium and the collapse of demineralized dentin, leaving a protein residue of collagen fibers (13,15,16).

During air drying, water that occupies the interfibrillar spaces previously occupied by hydroxyapatite crystals is lost by evaporation, resulting in a decrease in the volume occupied by the collagen network. Currently, the benefit of the "wet bonding" technique is derived from the ability of the water to retain the collagen framework and intertubular porosity patent for subsequent infiltration of monomers (17).

The infiltration of the dentin surface by dentin primer is thought to facilitate subsequent penetration of the adhesive resin, eventually resulting in perfect seal and encapsulation of the collagen fibrils and remaining hydroxyapatite crystals in the dentin surface layers (7). After the conditioner is rinsed off, a primer containing one or more hydrophilic resin monomers is applied. Primer molecules contain two functional groups: the hydrophilic group has an affinity for dentinal surfaces and the hydrophobic group has an affinity for resin. The primer wets and penetrates the collagen meshwork, raising it almost to its original level, and also increases the surface energy, hence the wettability of the dentinal surface. Unfilled resin is applied to and penetrates the primed dentin, copolymerizing with the primer to form an intermingled layer of collagen resin, termed hybrid layer. Formation of this hybrid layer of dentin and resin, which was first described by Nakabayashi et al. (4), is thought to be the primary bonding mechanism of most current adhesive systems. This resin-dentin interdiffusion zone not only allows adequate mechanical retention of the restoration but also is likely to be provided with a coefficient of elasticity that contributes to the maintenance of the bond (18,19).

In this study, SBMP had a homogenous hybrid layer and more regular resin tags compared to the other adhesive systems, in agreement with Walshaw and McComb (14) and Gordan et al. (20). However,Van Meerbeek et al. (6) reported that the SBMP system produced a hybrid layer with a more variable ultrastructure, with less distinctly outlined collagen fibrils.

In agreement with Van Meerbeek et al. (6), a morphological well-organized hybrid layer of collagen fibrils intermingled with resin in tiny interfibrillar channels was consistently formed by the OPB system. The adhesive system OPB requires a primer polymerization before adhesive resin application. High viscosity of this material is possible by the adhesive resin mixture, OPB Dual Cure A (activator) and B (paste). Thus, high superficial tension is observed because the adhesive wettability capacity is decreased. This creates short tags. SEM reveals that many adhesives form long resin tags within the dentinal tubules of extracted teeth. The tags are impressive in appearance and many convey some information about the wetting characteristics of a material. However, resin tags provide little or no retention unless they are firmly bonded to the tubule walls.

The adhesive systems SBMP and OPB had similar characteristics of dentin bonding, presence of hybrid layer and resin tags. This presumably is due to acid etching prior to adhesive system application. However, with the adhesive system DT, the primer is applied (primer A mixed with primer B) without dentin acid conditioning, responsible for the interface dentin-adhesive system feature.

It has been suggested that poor infiltration of the adhesive resin into the collagen-rich area of the demineralized dentin leaves gaps in the hybrid layer that are vulnerable to degradation (13). If incomplete penetration occurs, water and microleakage can infiltrate these spaces, producing hydrolysis of exposed collagen peptides not protected by hydroxyapatite or resin. This exposed collagen is not only susceptible to degradation from water, but also from bacterial enzymes that cause collagen breakdown. When collagen is protected by hydroxyapatite, peptide enzymes are unable to break down the collagen peptides, possibly contributing to the integrity of the tooth restorative interface.

The interface dentin-adhesive system-composite resin can be clearly seen in SEM photomicrographs, with hybridization and resin tags in all adhesive systems studied. The findings of this qualitative study do not represent a hybridization standard for the adhesive system used, since other factors are also involved including the dentinal substrate and the relationship between dentinal substrate and adhesive system. The hybrid layer analysis revealed different hybridization patterns, suggesting that the attachment seems to be influenced by many factors and a standardization of dentinal substrate is impossible.

ACKNOWLEDGMENTS

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RESUMO

Macari S, Gonçalves M, Nonaka T, dos Santos JM. Avaliação da interface de três sistemas adesivos por meio de microscopia eletrônica de varredura. Braz Dent J 2002;13(1):33-38.

O objetivo deste estudo foi avaliar através de microscopia eletrônica de varredura a interface de três sistemas adesivos, Scotchbond Multi-Purpose®, Optibond® and Denthesive Bond II®. Os sistemas adesivos e suas respectivas resinas compostas foram aplicadas no terço cervical do canal radicular de incisivos e caninos permanentes humanos, de acordo com as instruções do fabricante. As amostras foram incluídas em resina acrílica, cortadas transversalmente ao canal radicular e perpendicularmente à interface resina-dentina e analisadas através de microscopia eletrônica de varredura. Os sistemas adesivos Scotchbond Multi-Purpose® (SBMP) and Optibond® (OPB) apresentaram algumas características similares, camada híbrida homogênea e penetração de resina na dentina intertubular e peritubular. Entretanto, os tags apresentaram diferenças morfológicas, o sistema adesivo SBMP apresentou tags mais longos e em maior quantidade que OPB. O sistema adesivo Denthesive Bond II® não mostrou as mesmas características em sua interface. Os túbulos dentinários não foram abertos e a smear layer não foi removida, devido à ausência do condicionamento ácido prévio da dentina, prejudicando a formação da camada híbrida. A análise da camada híbrida por microscopia eletrônica de varredura revelou diferentes padrões de hibridização, sugerindo que a união dentina-sistema adesivo-resina composta é influenciada por muitos fatores, além disso, a padronização do substrato dentinário é impossível.

Unitermos: microscopia eletrônica de varredura, sistema adesivo dentinário.

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