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Morphological Characterization of the Tooth/Adhesive Interface

Sandra Kiss MOURA
José Fortunato Ferreira SANTOS
Rafael Yagüe BALLESTER

Department of Dental Materials, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil

The purpose of this study was to assess the morphological characteristics of the tooth/adhesive interface using different adhesive systems in MOD restorations under scanning electron microscopy (SEM). The tested hypothesis was that the morphology of the bonding interface would vary in different areas of MOD restorations for the three adhesive systems. MOD cavities were prepared in 12 sound extracted human third molars and restored with Filtek Z250 composite resin and one of the following adhesive systems: Experimental ABF (n=4), Clearfil SE Bond (n=4) self-etching primers and Single Bond etch-and-rinse adhesive system (n=4). After 24-h storage in distilled water at 37ºC, teeth were sectioned and prepared for SEM. The interfacial morphology varied depending on the adhesive system and also on the evaluated area. The null hypothesis was accepted because the morphology of the tooth/adhesive interface reflected the characteristics of both the dental substrate and the adhesive systems.

Key Words: adhesive systems, resin-tooth interface, MOD restoration, scanning electron microscopy.

INTRODUCTION

Bonding to dentin is achieved due to primer and adhesive penetration into demineralized dentin forming the hybrid layer (1). This layer may result from different systems and bonding techniques. Etch-and-rinse adhesive systems remove the smear layer completely and expose the collagen fibrils while for self-etching primers the smear layer is not completely removed but incorporated into the hybrid layer (2).

Self-etching primer adhesive systems do not require a separate acid-etching step. They are less technique sensitive than etch-and-rinse adhesive systems regarding moist control because etching and priming occur simultaneously without rinsing. Although most self-etching systems are not likely to produce a discrepancy between the depths of demineralization and resin infiltration (3), differences may occur, which can leave behind areas more prone to degradation (4).

Because self-etching adhesives do not completely remove the smear layer, questions have been raised regarding the presence and activity of remaining bacteria within the smear layer. Therefore, an antibacterial monomer - MDPB (12-methacryloyloxydodecyl pyridinium bromide)-, which is a quaternary ammonium compound with a methacryloyl group, has been added to the formulation of the primer of an experimental self-etching primer adhesive system (ABF; Kuraray Co., Ltd., Osaka, Japan) (5). By co-polymerization of MDPB with other monomers, the antibacterial agent is immobilized in the polymer matrix and inhibits bacterial growth on material surface by means of non-released immobilized bactericidal agents (6).

Morphological studies are useful to corroborate well-established bonding techniques considering that adhesive systems are introduced to the market at a very rapid rate. Even more, they may help explaining results that mechanical tests alone cannot do based on the quality of the bonding interfaces. Uno and Finger (7) reported that bond strength alone was an inadequate indicator of the efficacy of adhesive restorative systems because good bond strength values were found in areas

Correspondence: Prof. Dr. Rafael Yagüe Ballester, Departamento de Materiais Dentários, Faculdade de Odontologia, USP, Avenida Prof. Lineu Prestes, 2227, 05508-000 São Paulo, SP, Brasil. Tel: +55-11-3091-7840 r.222. Fax: +55-11-3091-7840 r.201. e-mail: ryb@usp.br

with gaps, which are prone to microleakage.

In many clinical situations, enamel and dentin are juxtaposed requiring simultaneous bonding. Variations at bonding interface due to dental substrate and restorative procedures should be investigated. However, no comparative study of the regional morphology at bonding interfaces of restorations is currently available. This study examined tooth/adhesive interface of different adhesive systems in MOD restorations. The tested hypothesis was that bonding interface morphology varies in different areas of MOD restorations.

MATERIAL AND METHODS

This study was approved by the Ethics in Research Committee of the School of Dentistry of the University of São Paulo (protocol 50/02). Twelve sound extracted human third molars were obtained and stored in 0.5% chloramine solution at 4ºC until use.

The roots were embedded in chemically activated acrylic resin (Jet; Clássico Artigos Odontológicos Ltda., São Paulo, SP, Brazil) using plastic rings (13 mm in diameter and 6-mm high) and were rinsed in distilled water. MOD cavities were prepared using water-cooled #4138 diamond burs (KG Sorensen, Barueri, SP, Brazil) and had the following dimensions: occlusal box: 3-mm-wide in the buccolingual direction and 2-mm-deep; mesial and distal boxes: 3-mm-wide in a buccolingual direction, 3 mm in occlusocervical height and 1.5-mm-deep towards the pulp with margins below the cementoenamel junction. Superficial enamel was beveled in nearly 1 mm, so that enamel prism exposure was more oblique to the bonding interface. Cavities were finished with low-speed #4138F diamond burs (KG Sorensen). Teeth were randomly assigned to 3 groups (n=4). The order of restoration placement was chosen at random. The tested materials with their compositions, specifications and manufacturers are shown on Table 1. Single Bond adhesive system was used as a control because its morphology is well established.

The adhesive systems were applied following the manufacturers' instructions and the cavities were restored with Filtek Z250 composite resin (3M ESPE, St. Paul, MN, USA) in 2-mm-thick oblique increments each light-cured from the occlusal surface for 20 s using a visible-light curing unit at 700 mW/cm^2 (Optilux 500; Demetron, Kerr, CA, USA). After 24-h storage in distilled water at 37ºC, the restored teeth were sectioned using a water-cooled diamond disc in a sectioning machine (Labcut 1010; Extec Corp, Enfield, CT, USA) thus providing 3 sections *per* tooth (Fig. 1).

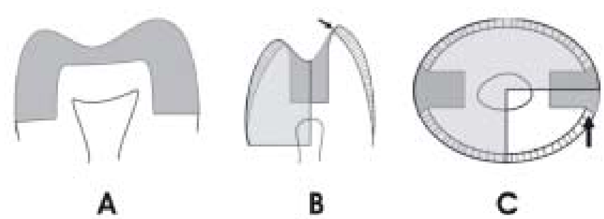

Figure 1. Schematic drawing of tooth sections. A: obtained by bisecting the tooth mesiodistally parallel to its long axis; B: obtained by bisecting one of tooth halves mesiodistally parallel to the longitudinal plane; C: resulting from cutting the obtained fragments (B) transversally to the long axis of the tooth at the middle third. Arrows in B and C show evaluated enamel areas.

Table 1. Tested materials.

Adhesive system	Manufacturer	Composition
Single Bond	3M/ESPE, St. Paul, MN, USA	Bis-GMA, HEMA, ethanol, water, dimethacrylates, camphoroquinone, PAA
Clearfil SE Bond	Kuraray Co., Ltd. Osaka, Japan	*Primer*: MDP, HEMA, water, camphoroquinone, hydrophilic dimethacrylate, N,N-diethanol-p-toluidine; *Bond*: MDP, HEMA, Bis-GMA, silanated colloidal silica, hydrophobic dimethacrylate, camphoroquinone, N,N-diethanol-p-toluidine
Experimental ABF	Kuraray Co., Ltd. Osaka, Japan	*Primer*: MDPB, MDP, HEMA, water, photoinitiator, photoinitiator; *Bond*: MDP, HEMA, Bis-GMA, dimethacrylates, silanated colloidal silica, surface-treated sodium treated sodium, fluoride crystal, photoinitiator

Bis-GMA = bisphenol-glycidylmethacrylate; HEMA = 2-hydroxyethyl methacrylate; PAA = polyalkenoic acid copolymer; MDP = 10-methacryloxydecil dihydrogen phosphate; MDPB = 12-methacryloyloxyodecyl pyridinium bromide.

In preparation for examination under scanning electron microscopy (SEM), the sections were manually wet-sanded using 1200-grit sandpaper (Buhler, Lake Bluff, IL, USA) and polished using: 6-, 3-, 1- and 0.25-µm diamond pastes (Metadi II; Buhler) in wet felt discs. After each polishing, the sections were ultrasonicated in distilled water for 10 min. The specimens were submitted to superficial demineralization with 50% phosphoric acid for 4 s, followed by 10-min ultrasonication in distilled water and immersion in 1% sodium hypochlorite for 10 min. Excess water was removed using filter paper and the sections were stored in a desiccator containing silica gel for 12 h. The surfaces were sputter-coated with gold and examined with a scanning electron microscope (Philips XL30, Philips, Eindhoven, Netherlands).

The technique used in this study is one of the scientific-based methodologies used for specimen preparation for SEM analysis (8,9), in which the samples are stored in hermetically sealed recipients containing silica. The specimens were not fixed, dehydrated in alcohol and dried with HMDS (hexamethyldisilazane) because HMDS is carcinogen and this is a high-cost technique.

To ensure that the morphological features were not artifacts from specimen preparation, replicas of sections were obtained. Impressions from half of the sections were made with an addition silicone impression material (Splash, Discus Dental, São Paulo, SP, Brazil) immediately before storage in the desiccator. After 1 h, the molds were filled with epoxy resin (Epoxide; Buhler) and the replicas were prepared and examined by SEM.

RESULTS

SEM micrographs show the findings on original specimens (Figs. 2-6). Comparison of the original specimens to the resin replicas showed similar morphological characteristics, which suggests that no artifacts were present. Other studies that used the same methodology (8,9) did not find any problem regarding to either specimen preparation or artifact occurrence. Thus, images of resin replicas were not included in this paper.

The interfacial morphology in dentin varied according to the examined area and also to the adhesive system. Hybrid layer and resin tags were observed at the pulp wall (Fig. 2a-2c), axial wall (Fig. 3a-3c) and line angles (Fig. 4a-4c), with particularities for each adhesive. The self-etching systems showed thin hybrid layers (about 0.6- to 2-µm; Figs. 2b and 3c) and some resin tags. Single Bond formed 3- to 4-µm-thick hybrid layers (Fig. 2a) and also resin tags (Figs. 2a, 3a and 4a). The gingival wall showed predominant hybrid layer formation for all adhesive systems (Fig. 5a-5c). Adhesive resin accumulation in the axiogingival line angle (Figs. 4a, 4c) was also observed. There were gaps in dentinal areas of the adhesive/dentin interface (Figs. 2c, 3b, 4b and 4c). The enamel/adhesive interfaces showed good adaptation for all adhesive systems (Fig. 6a-6c). Very similar bonding interfaces were observed for the self-etching adhesive systems (Figs. 6b and 6c).

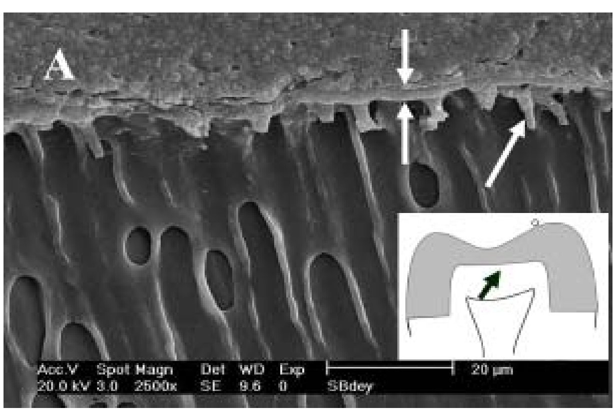

Figure 2a. Resin/dentin interface at the pulpal wall. Single Bond (2500X); Opposing arrows delimitate the hybrid layer; the other arrow indicate resin tags.

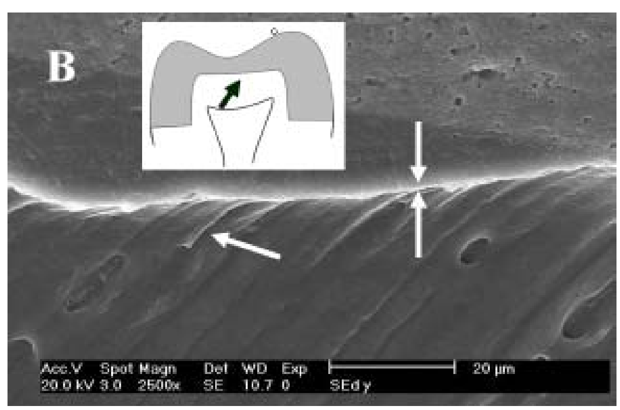

Figure 2b. Resin/dentin interface at the pulpal wall. Clearfil SE Bond (2500X). Opposing arrows delimitate the hybrid layer; the other arrow indicate resin tags.

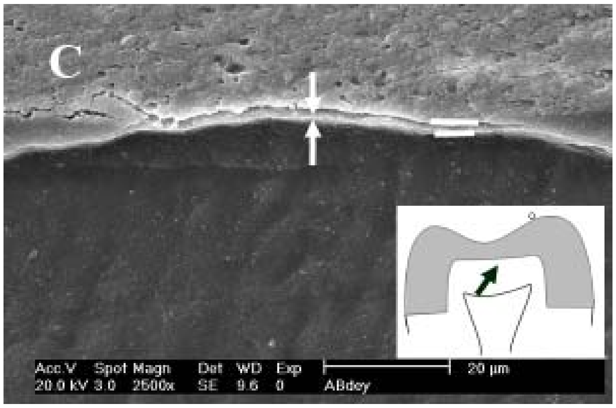

Figure 2c. Resin/dentin interface at the pulpal wall. ABF (2500X). Opposing arrows delimitate the hybrid layer; the bars delimitate the gap observed.

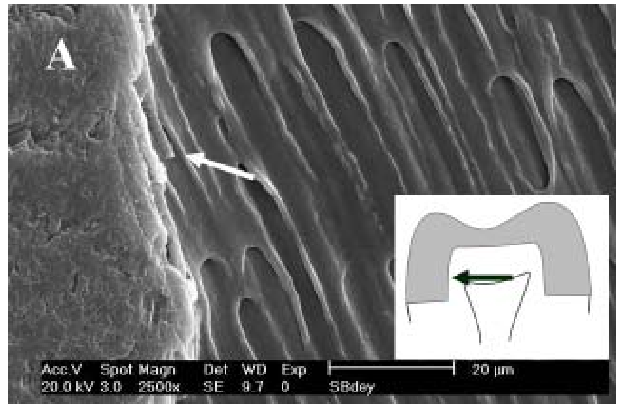

Figure 3a. Resin/dentin interface at the axial wall. Single Bond (2500X). The arrow indicates the formation of resin tags.

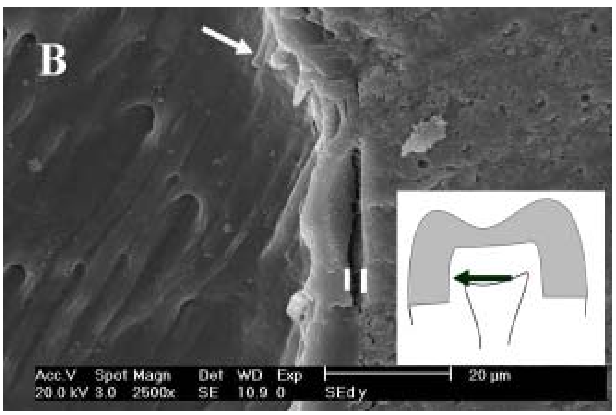

Figure 3b. Resin/dentin interface at the axial wall. Clearfil SE Bond (2500X). The arrow indicates resin tags; the bars delimitate the gap observed in the interface.

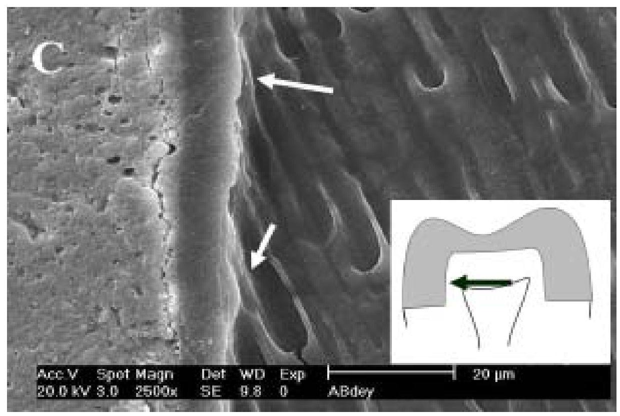

Figure 3c. Resin/dentin interface at the axial wall. ABF (2500X). The arrows indicate the formation of resin tags.

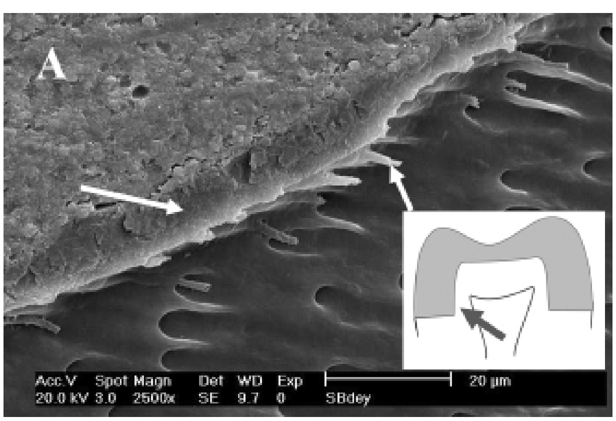

Figure 4a. Resin/dentin interface at the axial-gingival line angle. Single Bond (2500X). Arrows indicate resin tags or excess adhesive material.

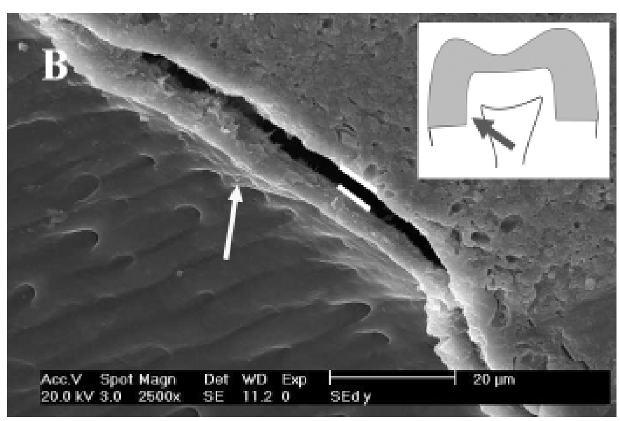

Figure 4b. Resin/dentin interface at the axial-gingival line angle. Clearfil SE Bond (2500X). The arrow indicates resin tags or excess adhesive material; bars delimitate the gap observed.

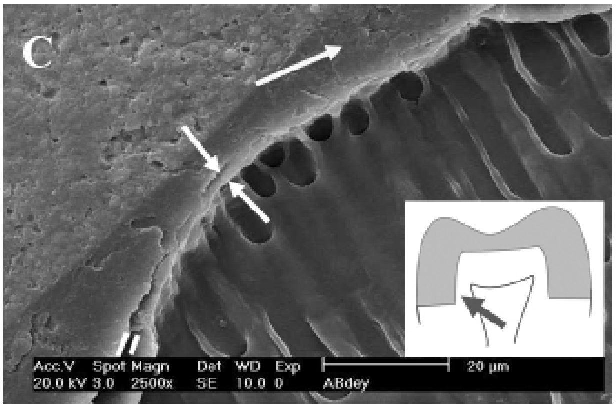

Figure 4c. Resin/dentin interface at the axial-gingival line angle. ABF (2500X). Opposing arrows delimitate the hybrid layer; the other arrow indicates excess material; bars delimitate the gap.

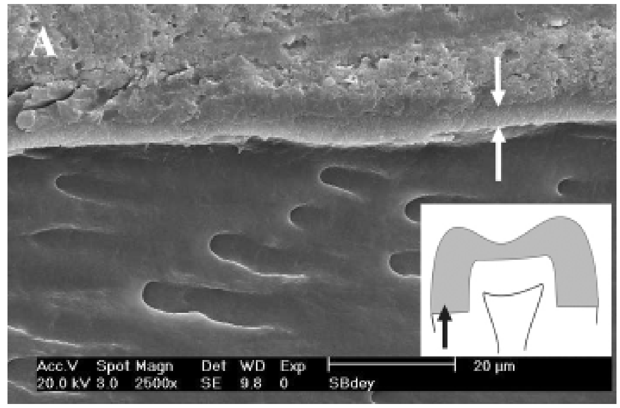

Figure 5a. Dentin/resin interface at the gingival wall. Single Bond (2500X). The opposing arrows delimitate the formed hybrid layer.

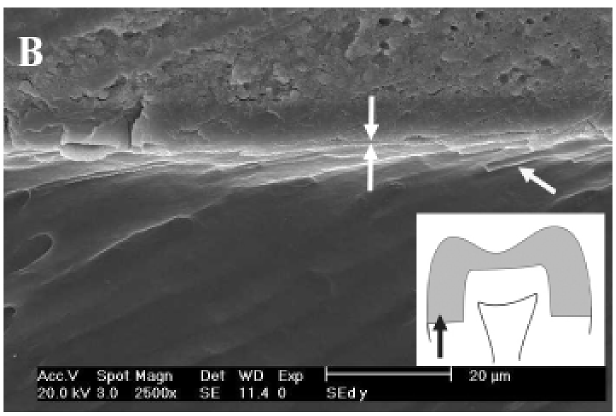

Figure 5b. Dentin/resin interface at the gingival wall. Clearfil SE Bond (2500X). Opposing arrows delimitate the hybrid layer; the other arrow indicates resin tags.

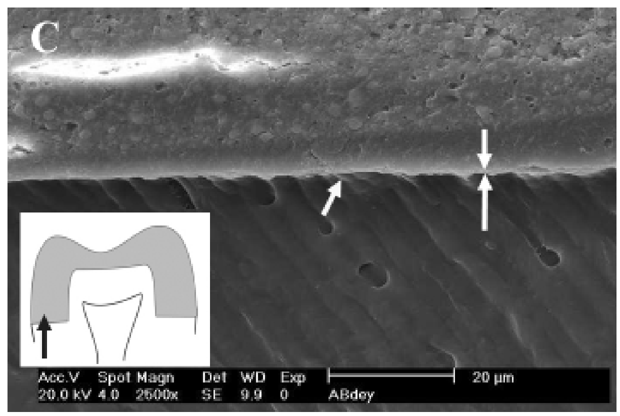

Figure 5c. Dentin/resin interface at the gingival wall. ABF (2500X). Opposing arrows delimitate the hybrid layer; the other arrow indicates resin tags.

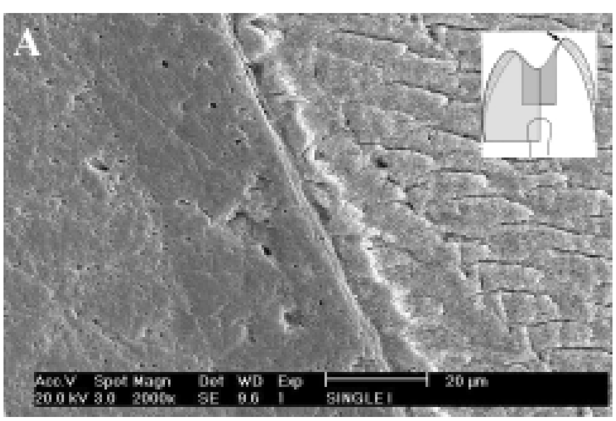

Figure 6a. Adaptation at enamel/resin interface. Single Bond (2000X). The arrow indicates the enamel area that was observed on the section.

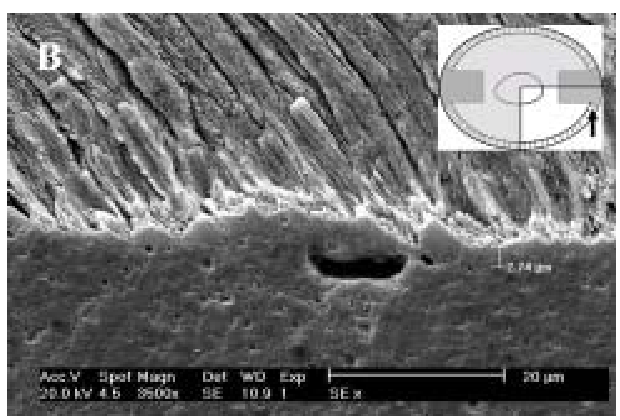

Figure 6b. Adaptation at enamel/resin interface. Clearfil SE Bond (3500X). The arrow indicates the enamel area that was observed on the section.

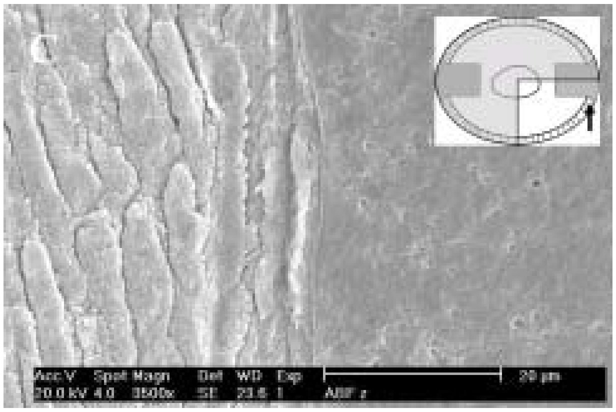

Figure 6c. Adaptation at enamel/resin interface. ABF (3500X). The arrow indicates the enamel area that was observed on the section.

DISCUSSION

The null hypothesis of this study was accepted. The morphological appearance of the bonding interfaces varied among the different areas of the MOD restorations and was influenced by the adhesive system used. Gaps were not found in enamel interfaces and no artifacts were observed compared to the replicas; however gaps/artifacts were observed in dentin. Considering that dentin bonding is likely to be influenced by more factors than enamel (10), it is possible that the quality of dentin bonding was not sufficient to withstand stresses generated during the placement of MOD restorations.

The findings of this study confirmed that enamel is a less critical substrate for bonding than dentin. A possible explanation could be the orientation of enamel prisms after cavity preparation. Prism orientation at the cavosurface margin is generally parallel to the bonding surface. Ikeda et al. (12) showed that this orientation resulted in low tensile bond strength. To minimize these effects, the cavosurface margin was beveled in such a way that exposed enamel prisms were not parallel to the bonding interface. This might have improved bonding, especially for the self-etching systems.

Regarding dentin substrate, resin tags were not observed at the gingival walls of the MOD restorations for all adhesives studied, probably because the parallel direction of dentinal tubules to the plane section might have precluded adhesive system penetration. On the other hand, tags were clearly found in areas where tubule direction was perpendicular or oblique to the surface. This finding is consistent with the results of a previous study (11), which have also reported high bond strength values in these areas, where the intertubular dentin is available in greater proportion to allow the adhesive diffusion.

The morphology of the bonding interface also varied according to the adhesive system used. The self-etching systems produced thinner hybrid layers and fewer resin tags than the etch-and-rinse system. These findings corroborate those of other investigations (5,13,14) and are related to the materials' different bonding mechanisms. Etch-and-rinse adhesive systems remove hydroxyapatite and demineralize dentin to a depth of 3 to 5 µm (15), facilitating the formation of a thick hybrid layer and the removal of peritubular dentin to produce tags with an initial conic shape followed by a cylindrical form. Demineralization depth, hybrid layer thickness and resin-tag formation can also be influenced by the pH of the adhesives etchants. Whereas phosphoric acid gel has a pH of 0.6 (11), MDP present in Clearfil SE Bond and ABF has a pH of almost 2.0 (11, 16), thus leading to less pronounced demineralization, thinner hybrid layers and resin tags without the initial enlargement resulting from demineralization of peritubular dentin. For self-etching systems, as calcium and phosphate ions resulting from demineralization remain in solution, they buffer the primer and limit the depth of demineralization (13, 16).

Clearfil SE Bond and ABF produced similar bonding interfaces. These systems differ only by the presence of MDPB and surface-treated sodium fluoride crystals in the composition of ABF. The thickness of hybrid layers observed in this study using a total-etch system (about 3 to 4 µm) also confirms the values found in the literature (18), as well as the small thickness of the hybrid layer resulted from self-etching adhesive systems (16,17). Perdigão et al. (18) studied the morphology and bond strength of ABF and Clearfil SE Bond. Differences were not found in both aspects, a result that agrees with those found in the present study. A recent *in vitro* study confirmed the ability of a MDPB-containing adhesive system to inhibit the progression of root-surface caries (19) and its good performance in terms of bond strength and curing behavior (20). Further research is needed to investigate the efficacy of MDPB as an antibacterial agent and the surface-treated NaF crystals present in ABF, in clinically relevant situations.

Bonded restorations have represented an important alternative in the demand for esthetic treatments. In order to minimize failure occurrence, it is important to have sound criteria. Moreover, the influence of factors other than those evaluated in this study should also be considered when restoring MOD cavities, such as, light intensity, mode of activation and use of liners with intermediate elastic modulus to the adhesive system and dental structures, in order to achieve a good adaptation to dentin and enamel.

The following conclusions can be drawn: 1) The morphological aspects of the tooth/adhesive interface varied along the cavity walls, thus reflecting the morphological features of the different surfaces exposed after sectioning; 2) The morphological appearance of the tooth/adhesive interface also was compatible with the characteristics of each tested adhesive system.

RESUMO

Este estudo teve o objetivo de observar a morfologia da interface dente-restauração de diferentes sistemas adesivos em cavidades MOD, por microscopia eletrônica de varredura (MEV). A hipótese do estudo foi de que a morfologia da interface adesiva poderia variar nas diferentes regiões da cavidade MOD, para algum dos 3 sistemas adesivos estudados. Preparos tipo MOD foram confeccionados em 12 terceiros molares humanos hígidos e restaurados com resina composta Filtek Z250 e os seguintes sistemas adesivos: ABF (n=4), Clearfil SE Bond (n=4) (sistemas adesivos autocondicionantes) e Single Bond (n=4) (sistema adesivo de condicionamento ácido total). Após 24 h de armazenamento em água destilada a 37ºC, os dentes foram secionados e preparados para MEV. A morfologia da interface de união variou com o sistema adesivo e com a região analisada. A hipótese do estudo foi aceita, pois a morfologia da interface de união refletiu as características do substrato dental e dos sistemas adesivos testados.

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REFERENCES

1. Nakabayashi N, Saimi Y. Bonding to intact dentin. J Dent Res 1996;75:1706-1715.
2. Yoshiyama M, Matsuo T, Ebisu S, Pashley D. Regional bond strengths of self-etching/self-priming adhesive systems. J Dent 1998;26:609-616.
3. Sano H, Shono T, Takatsu T, Hosoda H. Microporous dentin zone beneath resin-impregnated layer. Oper Dent 1994;19:59-64.
4. Carvalho RM, Chersoni S, Frankenenberger R, Pashley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomater 2005;26:1035-1042.
5. Imazato S, Kinomoto Y, Tarumi H, Torii M, Russell RR, McCabe JF. Incorporation of antibacterial monomer MDPB into dentin primer. J Dent Res 1997;76:768-772.
6. Imazato S, Imai T, Russel RRB, Torii M, Ebisu S. Antibacterial activity of cured dental resin incorporating the antibacterial monomer MDPB and an adhesion-promoting monomer. J Biomed Mater Res 1998;39:511-515.
7. Uno S, Finger WJ. Function of the hybrid zone as a stress-absorbing layer in resin-dentin bonding. Quint Inter 1995;10:733-738.
8. Montes MA, de Goes MF, da Cunha MR, Soares AB. A morphological and tensile bond strength evaluation of an unfilled adhesive with low-viscosity composites and a filled adhesive in one and two coats. J Dent 2001;29:435-441.
9. Cardoso PEC, Sadek FT, Placido E, Santos JFF. Microtensile bond strength of current adhesive systems when compared to cohesive strength of sound dentin and a resin-based composite. Mater Res 2004;7:575-581.
10. Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama M, Ebisu S et al. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesive. J Dent Res 1995;74:1679-688.
11. Ogata M, Okuda M, Nakajima M, Pereira PN, Sano H, Tagami J. Influence of the direction of tubules on bond strength to dentin. Oper Dent 2001;26:27-35.
12. Ikeda T, Uno S, Tanaka T, Kawakami S, Komatsu H, Sano H. Relation of enamel prism orientation to microtensile bond strength. Am J Dent 2002;15:109-113.
13. Hannig M, Reinhardt KJ, Bott B. Composite to dentin bond strength, micromorphology of the bonded interface and marginal adaptation of Class II composite resin restorations using self-etching primers. Oper Dent 2001;26:157-165.
14. Phrukkanon S, Burrow MF, Tyas MJ. The effect of dentine location and tubule orientation on the bond strengths between resin and dentine. J Dent 1999;27:265-274.
15. Perdigão J, Lambrechts P, van Merrbeek B, Tome AR, Vanherle G, Lopes AB. Morphological field emission SEM study of the effect of six phosphoric acid etching agents on human dentin. Dent Mater 1996;12:262-271.
16. Oliveira SS, Marshall SJ, Hilton JF, Marshall GW. Etching kinetics of a self-etching primer. Biomaterials 2002;23:4105-4112.
17. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater 2001;17:296-308.
18. Perdigão J, Lopes M, Geraldelli S. Laboratorial characterization of new antibacterial self-etching primer. J Dent Res 2001;80:61 (Abstr. 201).
19. Kuramoto A, Imazato S, Walls AWG, Ebisu S. inhibition of root caries progression by an antibacterial adhesive. J Dent Res 2005;84:89-93.
20. Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial characteristics of an adhesive resin containing antibacterial monomer MDPB. Dent Mater 2002;19:313-319.

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