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Abstract
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Reference

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The ultrastructure of a compomer adhesive interface in enamel and dentin, and its marginal adaptation under dentinal fluid as compared to that of a composite

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Abstract

Objectives: To visualise the ultrastructure of the interface of SCA compomer adhesive and of Optibond composite adhesive in enamel and dentin, and to relate the findings to the marginal adaptation of these two products in mixed class V restorations.

Methods: The ultrastructure was investigated using a scanning electron microscope (SEM) with and without prior argon ion etching, an environmental SEM, a field emission SEM, a confocal laser scanning microscope, and a transmission electron microscope. The marginal adaptation was quantified in mixed class V restorations by using the replica technique and a SEM under simulated dentinal fluid before and after simultaneous mechanical and thermal loading.

Results: The ultrastructure of the compomer adhesive interface differed from those of the composite. However, no significant difference was discerned as regards the percentage of “continuous margin” in the enamel marginal area before loading, and in the dentin area before and after loading (p < 0.05; unpaired t-test). Only after loading, the percentage of “continuous margin” in enamel was significantly (p < 0.05; unpaired t-test) better than that of the compomer.

Significance: The results indicated that the ultrastructure of the adhesive interface allowed no clear conclusions to be drawn as to the quality of marginal adaptation.

Keywords: Dentin; Enamel; Compomer adhesive interface

1. Introduction

Micro-morphological investigation into the adhesive interface is a focal point of research into adhesive systems. These investigations have shown that contrary to opinion hitherto [1,2], dental adhesion is not really of a chemical nature. Rather it is surmised that a micro-mechanical compound is established over the so-called hybrid layer, which is a mixture zone comprising the partly demineralised dentinal surface and the adhesive system which penetrated the micro-porosity of this surface [3–6].

Ultrastructural evidence for the hybrid layer is possible by various means. The morphology of the adhesive interface can be displayed with the aid of a scanning electron microscope (SEM) at relatively high degrees of magnification. In order to differentiate various structures better, the surfaces to be examined can be etched beforehand. Etching is currently carried out chemically using 10% citric acid [7] or 1 N hydrochloric acid [8], followed by deproteinisation with 10% NaOCl. Most frequently however, etching is carried out with argon ions [4,9]. Under this approach the test surface is bombarded with argon ions, whereupon an atom is removed from the surface, typically through collision with an argon ion [10]. As argon etching is effective in a substratum-specific manner, structures of varying composition are removed to varying degrees by means of this procedure, which, on subsequent observation under a SEM, allows a differentiated image of the adhesive interface as compared with an unetched sample [6,11]. It is possible to obtain images of even higher resolution with the aid of a field emission SEM [12] or with a transmission electron microscope [13,14] than with a SEM. In order to avoid all
Table 1

Description of the bonding steps

<table>
<thead>
<tr>
<th>SCA/Compoglass</th>
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<tbody>
<tr>
<td>10 s application of the SCA adhesive on moist enamel and dentin, 20 s penetration, dry with compressed air, light-curing for 10 s</td>
</tr>
<tr>
<td>10 s application of the SCA adhesive on enamel and dentin, 20 s penetration, dry with compressed air, light-curing for 10 s</td>
</tr>
<tr>
<td>Restoration in two layers with Compoglass</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Optibond/Herculite XRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application of Ultra-Etch Gel 35% for 30 s on enamel and 15 s on dentin, that was flushed away using a water spray for 30 s</td>
</tr>
<tr>
<td>30 s application of Optibond Primer on moist enamel and dentin, 30 s penetration, dry with compressed air, light-curing for 60 s</td>
</tr>
<tr>
<td>Application of a mixture of Optibond Dual Cure Paste and Activator on enamel and dentin in a thin coating, light-curing for 60 s</td>
</tr>
<tr>
<td>Restoration in two layers with Herculite XRV</td>
</tr>
</tbody>
</table>

As regards micro-leakage was used as a comparative medium, in combination with the fine hybrid composite Herculite XRV [31,32]. A further intention was to quantify the marginal adaptation of both materials to the enamel and to the dentine before and after long-term loading under the influence of dentinal fluid in mixed class V cavities [27]. In this way it would be possible to establish whether morphological differences have any effect on the quality of marginal adaptation. The hypothesis tested the differences found with respect to the interfacial ultrastructure in enamel and dentine among the compomer and the composite material, and it also tested if these differences will relevantly influence marginal adaptation.

2. Materials and methods

2.1. Marginal adaptation

For the evaluation of marginal adaption, eight caries-free upper premolars, which had been stored immediately after extraction in a thymol 0.1% aqueous solution prior to use in this study, were prepared. First, each tooth was prepared for dentinal fluid simulation. To this end the tips of the roots were sealed with dentine adhesive (Syntac Classic, Vivadent Ets., Schaan, Liechtenstein), followed by two coats of nail varnish. To commence the dentinal fluid simulation—in the form of diluted horse serum—a metal tube, tapered or bevelled at the end, and reaching right into the pulp cavity was bonded to the root of the tooth with the aid of a dentine adhesive (Syntac Classic) and connected to a silicon hose. The pulp chambers of all specimen teeth were evacuated, 24 h prior to cavity preparation, by means of this hose and filled with a solution consisting of one third horse serum and two thirds 0.5 aqueous sodium chloride solution with the aid of a three-way valve. From this point onwards, all specimens were kept continuously connected to a device constructed for the purpose, which exerted a permanent physiological pressure within the pulp of 25 mm Hg until replicas were taken following completion of thermal and mechanical loading [33,34].

Standardised, mixed, wedge-shaped class V cavities with 50% of the margins in enamel and 50% in dentin were prepared in the buccal and palatal surfaces of each of the eight premolars. For the coarse preparation, rotating diamonds were used under water cooling (Amalgam Prep Set Intensiv SA, Lugano, Switzerland). The preparation of cavities was then completed, using magnifying lenses (M5, Wild AG, Heerbrugg, Switzerland) with 12-fold magnification, by means of rotating 25 mm diamonds (Universal Prep Set, Intensiv) also under water cooling.

The prepared teeth were divided at random into two groups of equal size (Table 1). In the compomer group, SCA adhesive (Batch: 648529, Vivadent Ets., Schaan, Liechtenstein) was applied to the preparation and worked in for 10 s using a disposable brush (Table 2). Next it was...
left for 20 s to penetrate, gently dried with compressed air and polymerised for 10 s with halogen light (Optilux 401, Kerr Corp., Romulus, Glendora, CA, USA). This procedure was repeated with a second layer of SCA adhesive. The compomer restoration material (Compoglass, Farbe [shade] A3, Batch: 648537, Vivadent Ets.) was applied to the cavity in two layers, the first layer being laid cervically. Each layer was irradiated for 60 s with halogen light (Optilux 401).

In the composite group, Optibond (Batch 112492/750150, Kerr Corp., Romulus, Glendora, CA, USA) was used as an adhesive system (Table 2), no factory-supplied conditioner was provided by the manufacturer for the adhesive system. For the present study, Ultra-Etch Gel (Ultra-Etch Gel 35%, Lot 24K7, Ultradent Products Inc., Salt Lake City, UT, USA) was used as a conditioner, with an etching period of 30 s on the enamel and 15 s on the dentine. The etch gel was flushed away after 30 s using a water spray. The cavities were dabbed with cotton wool pellets but not totally dried. Primer was applied, briefly dried after 30 s with compressed air, and irradiated for 60 s with halogen light (Optilux 401). Dual Cure Paste and Activator were mixed for 10 s, applied to the cavity in a thin coating and further polymerised for 60 s with a halogen light (Optilux 401). The restoration followed with a fine hybrid composite in two layers (Herculite XR V, Batch: 21142, Kerr Corp.), each layer being polymerised for 60 s with halogen light (Optilux 401).

Finishing work was carried out on both groups immediately after polymerisation, using flexible polishing discs (SofLex, 3M Co., Minnesota, MN, USA) with decreasing grain-sizes under continuous water-spray cooling. Immediately after the fillings were finished, the specimen teeth were stored for a week in the dark, in water at 37°C, and then subjected to 1.2 million loadings of 49 N and 1250 thermocycles of 5–50–5°C in a chewing simulator with a cycle period of 2 min and temperature changeover phases of 5 s [29].

Immediately after finishing, and when loading was completed, impressions of the fillings were taken using silicon impression material (President light body, Coltèn AG, Altstätten, Switzerland). With their aid, epoxy replicas were produced (Stycast, Grace N.V., Westerlo, Belgium), which were examined at 200-fold magnification under a computer-aided SEM (Amray 1810 T, Amray, Dortmund, Germany) using the following criteria as to their marginal quality: “continuous margin” and “marginal gap”. The marginal features, “continuous margin” and “marginal gap”, added up to 100%. The criterion “marginal gap” was further differentiated into “pure marginal gap”, “marginal filling fracture” and “enamel margin fracture”. The percentage differences between the two groups with respect to “continuous margin” were tested as regards their significance with the aid of the unpaired and paired t-test using the corresponding software (Stat View 4.0 Abacus Concepts Inc., Berkeley, California, USA) on a computer (Power Macintosh 8500/180, Apple Computer Inc., Cupertino, California, USA).

### Table 2

<table>
<thead>
<tr>
<th>Product</th>
<th>SCA</th>
<th>Optibond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Vivadent et s.</td>
<td>Kerr Corp.</td>
</tr>
<tr>
<td>Enamel cleaner</td>
<td>HEMA, GPDM, MMEP,</td>
<td>ethanol, H₂O</td>
</tr>
<tr>
<td>Dentine cleaner</td>
<td>Bis-GMA, HEMA, GPDM,</td>
<td>barium–aluminum–</td>
</tr>
<tr>
<td>Primer</td>
<td>Maleic acid HEMA</td>
<td>borosilicate, disodium-</td>
</tr>
<tr>
<td>Adhesive</td>
<td>H₂O</td>
<td>hexafluorosilicate</td>
</tr>
</tbody>
</table>

* Ultra etch 35%, Ultradent Products Inc.

2.2. Ultrastructural investigation of the adhesive interfaces

For the ultrastructural investigation, caries-free, extracted human molars with completed root formation were used, after storage in a 0.1% thymol solution. Platelets of dentine and enamel approximately 1 mm thick, 2 mm deep and 4 mm long were taken from each test tooth with the aid of a slowly rotating diamond disc (Isomet Low Speed Saw 11-1180, AB Bühler Ltd., Chicago, IL, USA) under water cooling. The dentine was cut perpendicular to the direction of the tubules, and the enamel perpendicular to the enamel prisms. The surface of the specimens was polished under water cooling with waterproof SiC paper of increasing grain size up to 4000 grit (Pedmax 2, Struers, Copenhagen, Denmark) and treated with both adhesive systems in similar fashion for the evaluation of marginal adaptation. For the investigation under the confocal microscope 0.01% rhodamine B was applied to the adhesives of both systems [17]. For observation under the scanning and field emission microscope, the specimens were embedded in plastic (Technovit, Heraeus–Kulzer GmbH, Wehrheim, Germany,) so as to avoid artificial splitting between the adhesive and the hard tissue occasioned by procedure-specific drying. After seven days of storage at 37°C, the specimens were cut, vertical to the adhesive joint with a slowly rotating diamond disc (Isomet Low Speed Saw), and the cut surfaces were polished with up to 4000 grit (Pedmax 2, Struers). The ultrastructure of the adhesive interfaces in enamel and dentin was then investigated on the polished cut surfaces by the following methods.

2.3. Scanning electron microscope

After 12 h storage in a desiccator, the above mentioned specimens were fixed to SEM stubs using instant glue, gold sputtered (SCD 030, Balzers Union AG, Balzers, Liechtenstein), and investigated under a SEM (Amray 1810T) at various levels of magnification. Typical images were stored on a computer (Power Macintosh 8100 AV) via a frame
Table 3

Percentages of "continuous margin" (mean (SD)) in mixed class V restorations with 50% of the margins in enamel and 50% in dentin (number of samples per group = 8)

<table>
<thead>
<tr>
<th></th>
<th>Before loading</th>
<th>After loading</th>
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</thead>
<tbody>
<tr>
<td><strong>SCA/Compoglass</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>96.3 (1.0)</td>
<td>83.3 (10.8)</td>
</tr>
<tr>
<td>In enamel</td>
<td>97.6 (1.4)</td>
<td>86.5 (8.5)</td>
</tr>
<tr>
<td>In dentine</td>
<td>99.3 (0.9)</td>
<td>81.5 (21.8)</td>
</tr>
<tr>
<td><strong>Optibond/Herculite XRV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97.6 (3.9)</td>
<td>93.4 (9.2)</td>
</tr>
<tr>
<td>In enamel</td>
<td>100.0 (0.0)</td>
<td>99.5 (0.9)</td>
</tr>
<tr>
<td>In dentine</td>
<td>94.6 (8.8)</td>
<td>85.9 (19.9)</td>
</tr>
</tbody>
</table>

grabber video card (NuVista +, Truevision, Indianapolis, IN, USA).

2.4. Argon ion etching and scanning electron microscope

Before sputtering and location in the SEM chamber, the surfaces of the specimens were etched with the aid of an argon ion etching device (IEV 100, Balzers Union AG, Balzers, Liechtenstein). The following parameters were used: voltage, 15 kV; amperage, 5 mA; etching time, 30 s. Etching was carried out at a 90° angle to the specimen surface under continuous rotation, so as to ensure uniformity. The etched surfaces were photographed under a SEM (Amray 1810 T) at various levels of magnification.

2.5. Field emission scanning electron microscope

The specimens were decalcified for 30 s in 6 N HCl and deproteinised for 10 min in 1% NaOCl [35]. After gold sputtering, the specimens were located in the chamber of the field emission SEM (Hitachi 900, Hitachi, Tokyo, Japan) and photographed at varying levels of magnification under 10 kV EHT.

2.6. Environmental scanning electron microscope

For the environmental SEM (Model E-3, Electro Scan Corporation, Wilmington, MA, USA), the specimens were fixed to SEM stubs using instant glue, and placed in the test chamber moist without special treatment. The test analysis was carried out below 2.5 Torr pressure at various levels of magnification.

2.7. Confocal laser scanning microscope

The specimens were located under an Axioplan-light microscope (Zeiss, Oberkochen, Germany) with an attached argon crypton laser (MRC-600, BIO-RAD, Microscience Division Hemel Hempstead, GB). The specimens were studied in a moist environment with the aid of water immersion objectives at 10- to 63-fold magnification. The digital images were further processed using image processing software (Imaris, Bitplane, Zürich, Switzerland) at a work station (Indigo 2ex, Silicon Graphics, Mountain View, CA, USA) and printed out on a digital printer (Fujix Pictography 3000, Fuji, Tokyo, Japan).

2.8. Transmission electron microscope

Only the Compoglass/SCA system was subjected to evaluation under a transmission electron microscope. To this end, the specimens were placed for 48 h at 4°C in a buffered (pH 7.4) Karnovsky’s fixing solution [36]. After this they were rinsed in a 0.185 M sodium cacodylate buffer (pH 7.4, 350 mOsM). Post-fixation was carried out for 2 h at 4°C in 1.33% osmium tetroxide buffered with 0.067 M S-collidin. The non-decalcified specimens were embedded in Epon 812. After polymerisation, non-decalcified ultra thin sections were made with a microtome (Ultra-cut, Reichert-Jung, Vienna, Austria). These were contrasted with neutral uranylacetate and evaluated under a transmission electron microscope (Phillips 200 TEM, Phillips, Eindhoven, Netherlands) at various degrees of magnification.

3. Results

3.1. Marginal adaptation

Table 3 shows the percentages as regards “continuous margin” of both groups before and after loading. The differences between the two groups did not vary significantly before loading, either in the enamel or in the area of the dentinal margins (p > 0.05). After loading, no significant difference was present in the dentine marginal area between the two groups. In the enamel marginal area on the contrary, Herculite/Optibond fared significantly (p < 0.05) better than Compoglass/SCA. Loading in the case of Compoglass/SCA led to a significant fall-off in marginal quality both in the enamel and in the dentine (p < 0.05). In Herculite/Optibond, significant (p < 0.05) deterioration was only discernable in dentine due to loading. As marginal fractures, excesses and deficiencies in the enamel and fillings amounted to less than 5% in both groups, it was thought unnecessary to give their explicit representation.

3.2. Ultrastructural investigation into adhesive interfaces

In the case of Optibond, a hybrid layer approximately 3–5 μm thick was discernable under the SEM and the environmental SEM, both at the dentine and enamel interfaces (Fig. 1). As the images from both devices were highly similar, it was thought unnecessary to give a representation of the images produced by the environmental SEM. The hybrid layers were more clearly defined by the argon ion etching (Fig. 2). Their presence was unambiguously confirmed by the combination of the field emission SEM and of the confocal laser scanning microscope, as a result of which examination under a transmission electron microscope was considered unnecessary. In the field emission microscope and confocal microscope images, extensive penetration of
the adhesive into the dentinal tubuli was especially apparent. An interesting factor was the penetration by the adhesive deep down into the enamel coating shown by the field emission microscope, which had evidently taken place along the prism border zones (Fig. 3).

In the case of the SCA adhesive, no clear hybrid layer could be attested under a SEM or the confocal laser scanning microscope (Fig. 4), either in the enamel or in the dentine. Also, neither did argon ion etching cause any clear evidence of these structures (Fig. 2). Only field emission scanning electron microscopy (Fig. 3) rendered visible an approximately 500 nm wide structure in the dentine, which was identifiable as a hybrid layer with the aid of transmission electron microscopy (Fig. 5). In the enamel, on the contrary, no hybrid layers could be evidenced under the high-resolution image-production procedures.

4. Discussion

Both the enamel and the dentine are anisotropic with respect to their structure [37]. On account of this anisotropy, the demineralising effect of an acid, and hence the width of the hybrid layer are dependent on the direction of the section [50]. In order to draw a morphologically correct comparison between the adhesive interface morphology and marginal adaptation, fabrication of the specimens for ultrastructural investigation both in the enamel and the dentine was adapted to the predominant direction of the enamel prisms and of the dentinal tubuli in wedge-shaped class V cavities. By way of ultrastructural evaluation methods, a combination of the most important image-producing procedures, described in the literature, was used. This combination was to ensure reliable information about the micro-morphology of the adhesive interface.

The ultrastructure of the compomer adhesive interface differed markedly from those of the composite. In the dentine, the thickness of the hybrid layer amounted merely to around 0.5 μm, which is most probably due to the mild demineralising effect of the self-etching primer [38]. Despite this mild etching, the result was a mere complete removal of the smear plugs in the dentinal tubuli, which could be evidenced by the representation of long adhesive
tags in the dentinal tubuli. This finding does not fit in with any of the adhesion strategy categories defined by Van Meerbeek [38], and should preferably be described as a mixture of categories B and C.

In the case of SCA, no hybrid layer was discernable in the enamel with any of the image-producing procedures. Clearly, the self-etching primer was merely able to remove the smear layer without attacking the structure of the firm enamel surface. The adhesion mechanism is thus best explained by the total wetting of the cleansed, highly reagible enamel surface.

In the case of Optibond, a clear approximate 3 μm thick hybrid layer was apparent in the dentine, which agrees with the observations of other investigators [39]. The adhesion strategy passes here over a pronounced hybrid layer and thus corresponds to category C [38]. Interesting to note, in the case of Optibond this adhesion mechanism was observable not only in the dentine but also in the enamel. Although the formulation of a hybrid layer is today generally associated with dentinal adhesion, the presence of this structure on the enamel is nothing new. The hybrid layer on the enamel was described as far back as the 70s under the name “Mixture zone” [40].

Adhesion to the enamel and dentine should, on the one hand, ensure the retention of the filling in the cavity. While on the other hand, it should guarantee a perfect marginal seal, stable under load, for protection against secondary caries, marginal discoloration and post-operative sensitivity [39]. Retention is also obtainable with partial bonding [41]. Moreover, retention can also—albeit whilst sacrificing sound hard tissue, but nevertheless very reliably—be achieved by means of macro-retentive elements in the form of undercuts, retention pins, etc. Highly efficient adhesives are thus not vital for retention. However, a perfect marginal seal, load-resistant and free from marginal gap is only and solely achievable with the use of highly efficient adhesives. In consequence, evaluation of marginal adaptation is more critical and more relevant clinically than investigations into retention in the form of shear or tensile bond strength tests [42]. In vitro investigations into marginal adaptation in mixed class V cavities under simulated dentinal fluid have the advantage that the marginal seal can be
assessed both in the dentine and in the enamel [27]. In addition, this mode of evaluation correlates well with clinical behavior [43].

The quality of the marginal seal in both the adhesive systems tested was not significantly different before loading. Both materials produced excellent initial sealing of the margins, both in the enamel and in the dentine. SCA showed a tendency towards somewhat better results at the dentinal margin with minimal scatter. In the enamel the situation was the reverse.

In the case of Compoglass, a significant deterioration of approximately 11% “continuous margin” was observed in the enamel, and approximately 18% in the dentine after thermal and mechanical loading. This deterioration was linked to a relatively large increase in scatter at the dentinal margin. In contrast to this, in the case of Optibond, marginal adaptation at the enamel proved to be maximally resistant to loading. There was a similar deterioration in quality in the dentine which was limited to approximately 8% “continuous margin”. When SCA and Optibond were compared, the results at the enamel margin were found to be significantly different after loading. At the dentinal margin on the contrary, no significant difference was attested. Thus, the high elasticity of the Optibond adhesive in conjunction with its dual hardening produced no advantages as regards the dentinal marginal seal pace postulated by other authors [44,45], in contrast to the light-curing, adhesive SCA system, which scarcely forms a layer. This finding matches two-year clinical results which were unable to show any positive influence on the part of the elastic filling concept.

![Field-emission SEM, tooth tissue was etched away (bar represents 3 μm).](image)

(a) Detail of the adhesive interface between enamel (top left, E) and Optibond/Herculite (bottom right, H), showing the hybrid layer in enamel of about 3 μm thickness (original magnification, 5000 ×). (b) Detail of the adhesive interface between Optibond/Herculite (left, H) and dentin (right), showing the hybrid layer in dentin of about 3 μm thickness (original magnification, 5000 ×). (c) Detail of the adhesive interface between enamel (left, E) and SCA/Compoglass (right, S). No hybrid layer is visible (original magnification, 5000 ×). (d) Detail of the adhesive interface between SCA/Compoglass (left, S) and dentin (right). A hybrid layer of about 0.5 μm thickness is visible (original magnification, 5000 ×).
The present study makes it clear that there is no unambiguous relationship between the micro-morphology of the adhesive interface and marginal adaptation. In the area of the enamel, the structural difference between Optibond and SCA could merely be associated with a varying marginal adaptation after loading. In the dentine no significant effects from varying adhesive strategies were detectable on marginal adaptation either before or after loading. Thus, micro-morphological investigations of the adhesive interface are not adequate as a quality criterion for marginal adaptation in the dentine of mixed class V cavities [43].

The present study makes it clear that there is no unambiguous relationship between the micro-morphology of the adhesive interface and marginal adaptation. In the area of the enamel, the structural difference between Optibond and SCA could merely be associated with a varying marginal adaptation after loading. In the dentine no significant effects from varying adhesive strategies were detectable on marginal adaptation either before or after loading. Thus, micro-morphological investigations of the adhesive interface are not adequate as a quality criterion for marginal adaptation in the dentine of mixed class V cavities [43].
sealing. Certainly, the width and the presence of a hybrid layer seem, as far as the present work is concerned, to be of subordinate significance for marginal adaptation, similar to the situation applied to bond strength measurements [7,46].

The successful development of effective adhesive systems must currently be regarded as random. Systematic development of adhesive systems is thus scarcely possible as the success parameters are unknown. It will have to remain reserved for other investigative procedures, such as tunnel microscopy [47,48], rapid cooling [49], dynamic investigations under a confocal microscope amongst others, to define the success parameters of bonding to the dentine and the enamel.

References


