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BORTOLOTTO IBARRA, Tissiana, et al.

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Reference

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Failure analysis of adhesive restorations with SEM and OCT: from marginal gaps to restoration loss

Tissiana Bortolotto · Jose Bahillo · Olivier Richoz · Farhad Hafezi · Ivo Krejci

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Abstract
Objective The objective of this study was to analyse the failure mode of adhesive interfaces by comparing OCT and scanning electron microscope (SEM) analysis of class V restoration margins located on enamel and dentin.

Materials and methods Three groups were tested that differed in the application of a 3-step etch-and-rinse adhesive system (OptiBond FL) prior to cavity filling with restorative composite resin (Clearfil AP-X). After tooth restoration and polishing, the samples were loaded in a fatigue machine, and adhesive interfaces were evaluated with OCT and SEM.

Results Important and complementary information could be obtained with OCT analysis in respect to how marginal defects can propagate inside the cavity, compromising the restoration’s long-term performance. A self-etching effect was observed with OptiBond FL due to the presence of an acidic primer (GPDM) within its composition. Our results could show that areas of bonding and gaps coexisted within the same restoration.

Conclusions When marginal imperfections, or non-continuous margins, were detected by SEM, also imperfections beneath the surface could be observed at the tooth-restoration interface with OCT. Restoration loss occurred above the borderline of 50 % of marginal gaps on enamel and dentin.

Clinical relevance Marginal discrepancies of adhesive restorations can propagate inside the cavity and lead to restoration loss.

Keywords Quantitative margin analysis · Scanning electron microscope · Optical coherence tomography · Bond degradation

Introduction
Both in vitro and in vivo evaluations of restoration margins, as an indicator of the effectiveness of a restorative material or technique, have been the subject of numerous publications [1–7]. In vitro evaluation of marginal adaptation is based on the fact that by identifying defects at the tooth-restoration interface, an early sign of adhesive failure is already affecting the restoration before catastrophic failures like restoration loss can occur. In vivo, marginal adaptation is one of the factors of the United States Public Health Service (USPHS) criteria together with retention, staining, marginal discoloration, surface roughness and sensitivity that is used in most clinical studies to judge on the restoration’s clinical success [7, 8]. In a recent study, a high correlation was observed between clinical and laboratory data of marginal adaptation provided that the same restorative material is considered in both in vitro and in vivo studies [9]. Therefore, the clinical behaviour of restoration margins can be predicted on the basis of in vitro tests on marginal integrity, like shown by Frankenberger and coworkers [10].

However, the relationship between marginal integrity alone and the restorations’ clinical outcome seems to be far
more complicated to demonstrate. One reason may be that in clinical studies, frequent observation periods are of three years, which is too short if we consider that in most cases dentists replace existing class V restorations due to the diagnosis of secondary caries [11] and that failures due to secondary caries are usually observable after 5 years of the restoration’s clinical service [12]. This is the main reason why some in vitro research methodologies have attempted to simulate a 5-year period of clinical function, to compare then the quality of the restoration immediately after placement and after these 5 years of “simulated clinical service” and be able to predict the fatigue resistance and long-term performance of that restoration [13–16].

In the context of marginal integrity, the width and depth of the marginal gap, rather than its solely presence, is claimed as a more significant factor to predict the restoration’s clinical outcome [9]. One limitation of scanning electron microscope (SEM) quantitative analysis is that the presence of marginal gaps is detectable on the restorations’ surface, with no additional knowledge on how much this gap can propagate through the adhesive interface inside the cavity. The detection of a marginal gap is, very likely, the first sign of early failure at the restoration’s adhesive interface and in this sense, it might be of interest to qualitatively analyse restorations with marginal defects to see if the gaps that occur on the surface, propagate or not inside the cavity.

Optical coherence tomography (OCT) is an interferometry imaging technique that maps depth-wise reflections of near-infrared light from tissue to form cross-sectional images of morphological features at the micrometer scale [17]. It is a high-resolution analysis that enables the visualization beyond the surface, without entering into contact with the tissue of interest. Several studies have used this technology in the dental field to judge on the internal adaptation of fissure sealants, class I cavities, defects inside the mass of composite restorations and degree of demineralization of caries-affected dentin [18–22]. However, no study has assessed the effect of marginal gaps on gap depth, justifying why the present investigation can add interesting information to the existing literature.

Therefore, it was the purpose of this study to determine whether marginal adaptation is, or not, a superficial phenomenon without any influence of adaptation on depth of the cavity. The null hypotheses tested were that 1) SEM analysis would not be able to detect differences in marginal gaps amongst groups and that 2) In cavities with non-continuous margins (or marginal gaps), there would never be a gap propagating inside the cavity.

Materials and methods

The adhesive materials used in this study consisted of a 3-step etch-and-rinse adhesive (OFL: OptiBond FL, Kerr, Orange, CA, USA, batch numbers 3271580 and 3437447) and a microhybrid composite resin (Clearfil AP-X, Kuraray, Okayama, Japan, batch number 01067B). The application mode of the adhesive system determined the three testing groups: in gr 1, enamel and dentin were etched with 36 % phosphoric acid (H₃PO₄) prior to the application of the adhesive system, in gr 2, only enamel was etched with H₃PO₄ and in gr 3, no etching with H₃PO₄ neither on enamel nor on dentin was performed before the application of OFL primer and bond.

Twenty-four caries-free human molars stored in 0.1 % thymol solution after extractions were used for the experiment. After scaling and pumicing, the teeth were mounted on custom-made specimen holders with their roots in the centre using a cold-polymerizing resin (Technovit 4071, Heraeus Kulzer GmbH, Wehrheim, Germany) and then randomly assigned to the three above-mentioned experimental groups. Prior to the mounting procedure, the apices were sealed with two coats of nail varnish. To simulate dentinal fluid flow, a cylindrical hole was drilled into the pulp chamber approximately in the middle third of the root and a metal tube with a diameter of 1.4 mm was then adhesively luted using a dentinal adhesive (Syntac Classic, IvoclarVivadent AG, Schaan, Liechtenstein). The pulp tissue was not removed. This tube was connected by a flexible silicone hose to an infusion bottle placed 34 cm vertically above the test tooth. The infusion bottle was filled with horse serum (PAA Laboratories GmbH, Linz, Austria) and phosphate-buffered saline solution (PBS; Oxoid Ltd, Basingstoke, Hampshire, England) diluted in a 1:3 ratio under a hydrostatic pressure of about 25 mm Hg.

Twenty-four hours before starting the cavity preparations, by using a three-way valve, the pulp chambers were evacuated with a vacuum pump and subsequently bubble-free filled with the above solution. As of this moment, the intrapulpal pressure was maintained at 25 mm Hg throughout the testing, i.e. during cavity preparation, restoration placement, finishing and styling.

One U-shaped standardized class V cavity was prepared on the buccal surface of each test tooth with half of the margins located on enamel and half on dentin. Fine diamond burs (Intensiv SA, Grancia, Switzerland) were used under continuous water-cooling. The dimensions of the V-shaped cavities were 3.0–3.5 mm in diameter, 2.5–3.0 mm in height and 1.5 mm in depth. The margin on enamel was bevelled to a crescent-shape with a maximum width of 1.2 mm. The entire cavity was finished using 15 μm finishing diamond burs (Intensiv SA). Then, the cavity preparations were checked for marginal imperfections such as fractures or chipping under an optical microscope (Wild M5, Wild AG, Heerbrugg, Switzerland) at ×12 magnification and corrected if necessary.

After the different etching protocols, OFL was applied in all groups following manufacturers’ instructions. After placement and light-curing of the adhesive system by using a LED
source (L.E.D. Demetron II, Serial No: 792026758, Kerr, Orange, CA, USA) with a relative intensity of 800 mW/cm² (Curing Radiometer, Demetron Research, Danbury, CT, USA) Clearfil AP-X was inserted into the cavity in two layers, the first layer being placed cervically up to one half of the cavity and the second layer occlusally, filling the other half of the cavity. Both layers were light-cured for 40 s each. Immediately after polymerization, the restorations were finished and polished by using flexible aluminium oxide discs with different grain sizes (SofLex PopOn, 3M ESPE AG, Seefeld, Germany). The final polishing was checked using an optical microscope under ×12 magnification and corrected if necessary. The same operator performed the restorations of all three groups.

After storage in the dark in a 0.9 % saline solution at 37 °C for one week, the restored teeth were loaded in a computer-controlled chewing machine [23]. Thermal and mechanical loading was applied simultaneously. Thermal cycling was carried out in flushing water with temperatures changing 600 °C from 5 to 50 °C with a dwelling time of 2 min each. The mechanical stress comprised in total 240,000 load cycles transferred to the centre of the occlusal surface with a frequency of 1.7 Hz and a maximal load of 49 N applied by using a natural lingual cusp taken from an extracted human molar. The rationale for using 240,000 instead of 1.2 million load cycles was to diminish testing time; if 240,000 cycles were enough to induce a decrease of marginal adaptation, then the fatigue test was stopped and samples were removed from the chewing simulator. Simulation of dentinal fluid flow was permanently maintained throughout the loading procedure.

Immediately after loading, the teeth were cleaned with rotating brushes and toothpaste. Then impressions with a polyvinylsiloxane material (President light body, Coltène-Whaledent, Altstätten, Switzerland) were made of each restoration. Subsequently, gold-coated epoxy replicas were prepared for the computer-assisted quantitative margin analysis in a SEM (XL20, Philips, Eindhoven, Netherlands) at ×200 magnification [24]. The marginal quality, expressed in percentages of non-continuous margins (% NCM), was reported for enamel and dentin margins separately. SEM micrographs were procured from representative samples of each group.

Cirrus HD-OCT (Carl Zeiss Meditec AG, Jena, Germany) is mainly used in the ophthalmologic field for in-vivo viewing, axial cross-sectional and 3D imaging and measurement of anterior and posterior ocular structures. Specifications about this OCT are the following: Spectral domain OCT, Optical Source: super luminescent diode (SLD), 840 nm, optical power: less than 725 μW at the cornea, scan speed: 27,000 A-scans per second, A-scan depth: 2.0 mm (in tissue), axial resolution: 5 microns (in tissue), transverse resolution: 15 microns (in tissue), frame rate: more than 20 Hz. It is a computerized instrument that acquires and analyses cross-sectional and three-dimensional tomograms by using spectral domain optical coherence tomography (SD-OCT). The light source is an 840-nm super luminescent light emitting diode (SLD). Light returning from the sample is combined at the detector, which is a spectrometer in SD-OCT. The spectrometer resolves the interference signals throughout the depth of each A-scan immediately by means of a Fourier transform [25]. In order to analyze anterior ocular structures, the software proposes two techniques: Anterior Segment Cube 512×128 and Anterior Segment 5 Line Raster. To scan tooth structures, both scanning modes were tested in a pilot study and due to a superior quality of image; the first scanning mode was selected as tooth scanning method. This scan mode generates a volume of data through 4-mm square grid by acquiring a series of 128 horizontal scan lines each composed of 512 A-scans. It also acquires a pair of high definition scans through the centre of the cube in the vertical and horizontal directions that are composed of 1024 A-scans each. The additional advantage of this scanning mode is that it can create a 3-D image of the data. For data acquisition, each tooth sample was positioned on the right chin rest with a custom-made support, and the restoration was placed parallel to the imaging aperture so that the scanning beam could be oriented at about 90 ° in respect to the restorations’ surface. By using the X–Y controls to move the chin rest, the tooth was displaced until the restoration was visible in the iris viewport (Fig. 1). Then, the distance between the restoration and the imaging aperture was adjusted with the mouse scroll until the tooth-restoration interface was seen in the OCT scan display. By clicking on the capture button, the data was acquired and the image saved. Cervico-occlusal images were obtained from each tooth sample, and a 3D reconstruction of the cavity was also possible. Five OCT images from each sample were selected and quantitative data of high scattering areas along the internal enamel-resin interface could be obtained by using a scoring system with the following scale: 0, no visible infiltration; 2, infiltration not reaching the enamel-dentin junction; 3, infiltration exceeding the enamel-dentin junction; and 4, restoration loss.

![Fig 1 Sample positioning in front to the imaging aperture of the OCT device. The scan pattern (yellow box) is positioned in front of the restoration before image acquisition. Blue line and slice number indicate current fast B-scan (X slice) seen in top scan viewport; magenta line and slice number indicate current slow B-scan (Y slice) seen in middle scan viewport.](image)
Statistical analysis

Statistical analysis was performed with SPSS 14.0 for Windows. Differences amongst the three groups on enamel and dentin margins were tested with nonparametric Kruskal-Wallis and post hoc test at a level of confidence of 95%.

Results

The results of SEM margin analysis after thermo mechanical fatigue stress, expressed as the mean percentages of marginal gaps or non-continuous margins (%NCM) for each group, are detailed in Fig. 2. No significant differences on dentin margins were detected between the groups. On enamel margins, significant differences between groups were observed in percentages of marginal gaps. Significantly higher %NCM were observed at the resin-enamel interface of gr 3 (53.1±19.5, no etching with phosphoric acid on both enamel and dentin substrate) when compared to gr 2 (6.9±8.1 %NCM, enamel etching with phosphoric acid only on enamel) and to gr 1 (3.5±5.1 %NCM, standard application of OptiBond Fl, that is, phosphoric acid applied on both enamel and dentin).

The results (%NCM) of each sample (1 to 8) belonging to groups 1, 2 and 3 are detailed in Fig. 3a–c, respectively. Within the same group, results of marginal gaps on enamel and dentin were not homogeneous. Some samples presented high % of marginal gaps on enamel and low % of marginal gaps on dentin, and vice versa. In respect to restoration loss, one class V restoration (Fig. 3c, sample 3) was lost during the experiment; this restoration presented above 50 % of non continuous margins (NCM) on both enamel and dentin margins (Fig. 3c (sample 3) and Fig. 5).

The results of the OCT analysis are shown in Fig. 4 for enamel margins of the two test groups gr 2 and gr 3. The control group (gr 1) was excluded, as it was not significantly different from gr 2. The group with less % of marginal gaps on enamel (gr 2: 6.9±8.1) also presented significantly more percentages of “no infiltration” along the internal resin-enamel interface (Fig. 4, left bar, blue score), indicating that when less marginal gaps were observed on enamel margins, less infiltration was observed beneath the restoration as well.

Gaps that propagated inside the cavity were significantly more frequent in specimens that presented high percentages of marginal gaps. Said differently, gr 3 presented the lowest percentage of “no infiltration” beneath the margins (Fig. 4, left...
bar, red score); at the marginal level, this group also presented the highest % of marginal gaps (53.1±19.5), indicating that when more marginal gaps were observed on enamel margins, more infiltration was observed beneath the restoration as well.

The most negative factor influencing restoration survival was the presence of high percentages of non-continuous margins (Fig. 2) together with incipient infiltrations beneath the restoration, i.e. infiltrations not reaching the enamel-dentin junction, as it could be observed in gr 3 in respect to gr 2 (Fig. 4, 2nd bar from the left, red score). This was the only group in which restoration loss occurred (Fig. 4, right bar, and Fig. 5).

Important differences in micromorphology could be also observed between OCT scans from samples with low and high % of non-continuous margins. Continuous margins and low scattering areas were characteristic from gr 1 (Fig. 6). Figure 7a, b show how marginal and internal gaps were observed with SEM and 2D and 3D OCT scans, respectively.

**Discussion**

Under function, both chemical and mechanical stresses can result in an alteration of the tooth-restoration interface with time. Proliferation of surface and subsurface flaws may be one major mechanism involved in the mechanical property changes of this interface [26]. Both null hypotheses of the present study could be rejected; SEM analysis could detect differences in the percentage of marginal gaps between groups, and in cavities with non-continuous margins, also gaps propagating inside the cavity could be easily observed with OCT.

Scanning electron microscope margin analysis is a well-known method for the evaluation of adhesive restorations and considered the setup closest to the clinical situation [27]. Nevertheless, SEM diagnosis based on gold-coated replicas is an expensive and time-consuming way of evaluating dental restorations [27]. It also suffers from both sampling and inter observer variability. Interestingly, the medical field is also confronted to this problem; for instance, pathological diagnosis from histological sections has also a certain degree of uncertainty due to sampling variability. The analysis by itself is highly dependent on the pathologist’s experience, and this is why the diagnosis process is expert-based, rather than evidence-based [17]. This has been one major reason why non-invasive optical diagnosis techniques are increasingly used in the medical field.

Optical coherence tomography (OCT), as an imaging method, is based on a classical measurement technique known as low-coherence interferometry that enables non-invasive, high-resolution, in vivo, two- or three-dimensional cross-sectional imaging of microstructural morphology in transparent and non-transparent biological tissue in situ [28]. The device used in the present study is basically conceived for ophthalmologic use. It acquires 27,000 scans per second with a resolution of around 5 microns for in-vivo viewing, axial cross-sectional, and 3-dimensional imaging and measurement of anterior and posterior ocular structures. Taking into consideration that the presence of a marginal or internal gap is a 3D phenomenon, the OCT used in this study enabled the 3D visualization analysis of the entire margin. This is an additional advantage because the entire tooth sample can be observed in a short amount of time without any additional preparation.

The same composite resin and adhesive system was used in the three groups. The same operator performed all restorations as well; therefore, the only variable was the application mode of OFL. This would enable us to see differences in gap formation that depended on how the adhesive system interacted with
enamel and dentin. The finding that marginal gaps on enamel were significantly higher when phosphoric acid etch was avoided (gr 3) is confirmatory of those of other research groups [29] and might be due to a poor etch pattern on enamel. This was the group in which the maximum of marginal openings was observed. OCT analysis of the same margin showed high scattering areas that propagated inside the cavity and were interpreted as internal openings. The depth of the gap was variable and sample-dependent; however, the 3D visualization of the entire restoration permitted us to corroborate that with higher % of NCM; the depth of the gap also increased. Water uptake favoured by open margins may accelerate matrix degradation by abrading more the surface, increasing the surface area and allowing greater ingress of both water and enzymes [26].

However, marginal gaps on dentin were not increased as expected when phosphoric acid was avoided (gr 2 and 3). This might be due to the presence of an acidic monomer, glycerophosphoric acid methacrylate (GPDM), within the composition of the primer of OptiBond FL that favoured dentinal adhesion even without etching with phosphoric acid. This study could show, once again, that the performance of adhesive systems depend on their composition and not on their category (etch-and-rinse or

Fig. 5 Sample from gr 3. SEM micrographs of enamel (a) and dentin (b) margins after thermomechanical loading. See the presence of gaps along the margin length (arrows). OCT scan (c) of the same tooth sample confirming restoration loss before OCT analysis. RC resin composite, E enamel, D dentin

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self-etch) or number of steps. The present findings showed that the so-called gold-standard 3-step etch and rinse adhesive, OptiBond FL, behaves also as a self-etching adhesive system [30].

To determine if high scattering areas that propagated from the composites surface inside the cavity were due to light distortion or to the real presence of gaps, samples with and without marginal gaps belonging to the same group were observed under OCT for comparison. Brighter and wider white lines could be observed along the resin-tooth interface of samples with high % of NCM (Fig. 7); these lines could be hardly observed or were even absent from the samples with gap-free margins (Fig. 6). Therefore, our OCT observations are in line with those of a recent study [22] and white areas at the adhesive interface did not represent reflection artefacts but the real presence of gaps inside the restoration.

Finally, the 3D scans showed that gap and gap-free adhesive interfaces coexisted within the same cavity, confirming the early findings of Shono and co-workers [31] in respect to how inhomogeneous enamel and dentin substrate and therefore, resin-dentin bonds, can be. This means that within the same cavity, areas of good bonding and adhesive gaps can coexist without compromising restorations’ retention. The statement of Van Landuyt et al [32] that clinical failure of restorations occur more often due to inadequate sealing, with subsequent discoloration of the cavity margins, than due to

Fig. 6 Sample from gr 1. SEM micrographs of enamel (a) and dentin (b) margins. Continuous or gap-free margins can be observed along the margin length. OCT scan of the same tooth sample (c), see that high scattering areas are almost non existent at the tooth-restoration interface, which indicates that no gaps are detected inside the restoration as well. RC resin composite, E enamel, D dentin, EDJ enamel dentin junction.
restoration loss, could be confirmed in the present study as interface defects (marginal and internal gaps) were more frequently observed than lost restorations.

Marginal gaps could be interpreted as an early sign of restoration failure. Meanwhile, only one restoration was lost that presented above 50 % of marginal gaps on enamel and dentin (Fig. 5). Possibly, hydrolytic degradation is accelerated under the presence of more than 50 % of marginal openings on both enamel and dentin margins, increasing the probability of failure due to restoration loss. In addition, the OCT data of gr 3 confirmed that marginal openings also propagated beneath the restoration, evidencing that restoration loss strongly depends on the time needed for an infiltrated margin to propagate along the internal interface.

Nevertheless, and despite this evidence, the scientific literature has still not found an answer to the question of which is the internal interface. For class V restorations filled with OptiBond FL and Clearfil APX, the presence of marginal gaps could be considered as an early sign of adhesive failure that on the long term, led to restoration loss if more than 50 % of marginal openings were detected on enamel and dentin margins of class V restorations.

Conflict of interest The authors declare that they have no conflict of interest.

References


Conclusion

Quantitative OCT evaluation of adhesive interfaces provided with important complementary information to SEM quantitative analysis. Marginal gaps propagated from the margins beneath the restoration and degradation was sample-dependent. For class V restorations filled with OptiBond FL and
25. Cirrus HD-OCT User Manual, Zeiss, Germany