Detection of Interfacial Defects in Adhesive Restorations

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Tokyo, Japan/2010

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This thesis is submitted in partial fulfillment of the requirements for the degree of *Doctor of Philosophy in Dental Science*.

Tokyo, Japan

2010

Dedicated to my family and friends who always supported my work.

Acknowledgments

Preface

This thesis is based on the original research works by the author, to which the following articles refer.

Chapter 1. Makishi P, Shimada Y, Sadr A, Wei S, Ichinose S, Tagami J. Nanoleakage expression and microshear bond strength in the resin cement/dentin interface. The Journal of Adhesive Dentistry 2010;12(5):393-401.

Chapter 2. Makishi P, Shimada Y, Sadr A, Tagami J, Sumi Y. Non-destructive 3D imaging of composite restorations using optical coherence tomography: marginal adaptation of self-etch adhesives. Journal of Dentistry (Submitted)

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Introduction

Adhesive restorative materials extensively influenced modern dentistry and provided for patients and dentists a number of choices to create more esthetically pleasant restorations with minimal intervention (1). Although the immediate bonding effectiveness of most current adhesive materials is quite favorable regardless of the adhesive used (2), dental materials technology still faces the challenge of combining the improvements made in adhesion performance, adhesive reliability and simplified application procedures (3). The most cited reasons for clinical failure of adhesive restorations are loss of retention and marginal adaptation (2).

Clinically, occlusal and proximal wear, surface roughness, surface staining, marginal staining, marginal breakdown and post-operative sensitivity may be associated with polymerization shrinkage in direct restorations (4). Indirect materials have been proposed in attempt to minimize the polymerization contraction and improve the curing of the restorative material. Therefore, the overall durability of those restorations fabricated in dental laboratory is expected to be increased (5).

The bond-durability, especially in dentin is strongly influenced by the stability of the components at restorative material-tooth interface, such as hybrid layer and bonding resin layer. From this point of view, evaluation of nanoleakage channel in the hybrid layer is believed to be very effective to speculate on the bond durability of the materials (6).

In chapter 1, it was investigated the nanoleakage expression and micro-shear bond strength in the resin cement-dentin interface. This study attempted to determine the short-term sealing ability of current adhesive luting agents to dentin and if it was correlated to their bond strength.

Although the clinical performance of bonded restorations is predictable by in vitro studies, the longevity of dental restoratives also depends on a number of patient-, material- and procedure-related factors (5, 7). Clinical assessments of margins quality are carried out by dentists everyday, however the replacement of existing restorations and the treatment planning decisions are very limited to clinical inspection and radiographic assessment (8, 9). Furthermore, radiation hazard of the X-ray to both the patient and the clinician remains an issue.

Optical coherence tomography (OCT) has been reported as a promising high resolution biomedical optical method to detect microstructural details of hard and soft oral tissues noninvasively (10). Swept-source optical coherence tomography (SS-OCT) is a variant of OCT in which the main advantage features are high resolution and very fast (4 seconds) acquisition of 2D and 3D data.

In search for a new protocol that could be applied in vitro and in future in vivo studies, in chapter 2, a three-dimensional imaging technique was used to evaluate marginal adaptation of self-etch adhesives. The use of swept-source optical coherence tomography was investigated for fast observation of marginal integrity as a new potential non-destructive method.

Chapter 1

Nanoleakage Expression and Micro-shear Bond Strength in the Resin Cement-Dentin Interface

Introduction

New developments in adhesive cementing systems for composite and ceramic indirect restorations have greatly contributed to the minimal intervention in dentistry and helped reducing the technique sensitivity of multi-step systems (1, 11). Despite significant improvements achieved in sealing, bonding, biocompatibility and aesthetics, the challenging part of the luting procedure remains to be the bonding to dentin due to the unique structural characteristics and dynamics of this hard tissue (2, 12). Much research has been devoted to the nanoleakage as an important factor that may lead to degradation of the bond to dental tissue (13, 14). This phenomenon may be due to insufficient infiltration of resin into the demineralized collagen network or incomplete polymerization of hydrophilic monomers in the submicron interfacial spaces. Unprotected collagen fibrils may constitute preferential pathways to degradation by oral and bacterial enzymes (15, 16).

Resin cements can be classified into three categories based upon the pre-treatment of the tooth structure; etch-and-rinse adhesives, self-etch adhesives and self-adhesive cements. The self-etch system partially removes the smear layer and does not require etching and rinse steps. This system has been clinically reported to cause reduced tooth sensitivity compared to the etch-and-rinse system (17). Although it is less technique sensitive, degradation of resin-dentin bonds may be expected to occur due to the presence of hydrophilic monomers in the self-etch adhesive system (18).

Among the various categories of luting agents available in the market, there is a growing interest towards self-adhesive resin cement due to simple handling, esthetic demands and suitability for indirect all-ceramic works. The self-adhesive properties are claimed to be based upon acidic monomer that partially demineralize and penetrate into the tooth structure, resulting in micromechanical retention or assumedly chemical bonding (19). Self-etching as well as self-adhesive luting cements have been recommended for many clinical procedures as inlays and onlays. In order to evaluate the sealing ability and bond-strength of resin cements, the role of different factors have been investigated, including; seating pressure during luting, (20) application of additional layer of hydrophobic resin (18) or the use of stimulated pulpal pressure (21). However, there is a lack of qualitative and quantitative information on the sealing ability of the resin-dentin interface for self-etch primer resin cements and self-adhesive resin cements.

The aim of this *in vitro* study was to determine the short-term sealing ability of two current adhesive luting agents to dentin and their bond strength. Field Emission Scanning Electron Microscopy (FE-SEM) and Energy-dispersive X-ray Spectroscopy (EDS) analyses were performed in order to evaluate the nanoleakage formation along the resin-dentin interface for these cements. The null hypothesis tested was that there was no significant difference in silver uptake and bond strength between the two cements.

Materials and methods

Two resin cements were used in this study; a self-adhesive resin cement (RelyX Unicem, 3M ESPE, St. Paul, MN, USA) and a self-etch resin cement (Panavia F2.0, Kuraray Medical, Tokyo, Japan). The resin cements were handled in the dual-cure mode

and in accordance with the manufacturers' instructions. Chemical composition of all the materials used in the experiment is reported in **Table 1.1**.

Twenty-six extracted intact human third molars were collected after the individual's informed consent as approved by the Institutional Review Board of Tokyo Medical Dental University. The teeth were stored at 4° C in saline saturated with a small amount of thymol until the experiment. The occlusal third and root of each tooth were removed by means of a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. The occlusal superficial dentin surface, without any enamel remnants, was finished with #600 silicone-carbide paper under running water to create a standardized smear layer.

Table 1.1 - Materials used in this study

Material	Brand (Lot#)	Composition	Application Technique	Manufacturer
Resin composite block	Estenia C&B; 00021A	Filler: ultrafine alumina particles, fine alumino-silicate glass Particles. Matrix: UDMA, UTMA, Bis-GMA	Light-cure 60 s both sides. Heat-cure for 15 min at 110° C.	Kuraray Medical, Tokyo, Japan
Etching agent	K-etchant Gel; 00405A	40 wt% phosphoric acid		
Silane-coupling Agent	Clearfil Porcelain Bond Activator; 00208B Clearfil SE Bond Primer; 00722A	3-MPS, bisphenola-polyethoxy-dimethacrylate; MDP, HEMA, hydrophilic dimethacrylates, dl-camphorquinone, N,N-diethanol-p-toluidine, water.	Composite block: apply K-etchant Gel for 5 s; rinse with water; air dry; apply mixture of Clearfil SE primer with Porcelain bond activator for 20 s and gently air dry.	Kuraray Medical, Tokyo, Japan
Self-adhesive resin cement	RelyX Unicem; 56834	Powder: glass fillers, silica, calcium hydroxide, self-cure initiators, pigments, light-cure initiators. Liquid: Methacrylated phosphoric esters, dimethacrylates, acetate, stabilizers, self-cure initiators.	Tooth: No pre-treatment. Cement: Mix capsule for 15 s (Rotomix, 3M ESPE); apply on surface; lute resin block using light pressure; light cure for 40 s from each side.	3M ESPE, St. Paul, MN, USA
Self-etching primer resin cement	Panavia F2.0; 011185	ED Primer 2.0 A: HEMA, 10-MDP, 5-NMSA, water, accelerator. ED Primer 2.0 B: 5-NMSA, accelerator, water, sodium benzene sulfinate. Paste A: 10-MDP, hydrophobic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic dimetacrylate, silanated silica, photoinitiator, benzoyl peroxide. Paste B: hydrophobic aromatic dimethacrylate, sodium aromatic sulfinate, accelerator, sodium fluoride, silanated barium glass.	Tooth: Mix ED primer (A and B); apply without interruption for 20 s; gently air blow. Cement: Mix cement (A and B); lute resin block using light pressure; light cure for 40 s from each side.	Kuraray Medical, Tokyo, Japan

Sample Preparation for Nanoleakage Test

Composite blocks 1 mm in thickness were fabricated by means of compression of a resin composite for indirect restorations (Estenia C&B, Shade DA2, Kuraray Medical) between two glass slides at 1 mm space, followed by photo-irradiation for 60 s using a light curing unit for laboratory (α-Light II, J Morita Co., Kyoto, Japan) and heat curing at 110° C for 15 min in air (KL 100, Kuraray Medical). The blocks were then abraded with #600 silicone-carbide paper under water-cooling in order to create a more retentive surface. Before cementation, each block was cleaned with a phosphoric-acid etchant (K-etchant Gel, Kuraray Medical) for 5 s, rinsed, dried, and the surface treated using a silane coupling agent for 20 s (mixture of equal amounts of Porcelain Bond Activator and SE Bond primer, Kuraray Medical).

Six teeth were randomly assigned to two groups of three teeth each, based on either the self-adhesive or the self-etch resin cement. The resin cements were applied to the dentin, prior to the placement of the resin block. A force was applied until seating of the block was complete. After 5 min of self-curing of the cement, two additional 20 s intervals of light irradiation were performed from the top of the specimens to ensure optimal polymerization. The bonded specimens were stored in water for 24 h at 37° C and vertically sectioned using a diamond saw (Isomet) under water lubrication, across the resin cement-dentin, into approximately 1-mm-thick slabs. Two central slabs were chosen from each tooth, forming a total of six specimens per group. Bonded slabs were ground and polished using wet #600 silicone-carbide paper and diamond pastes down to a size of 1 μm, then coated with two layers of fast-drying nail varnish applied up to within 1 mm of the bonded interfaces. The specimens were immersed in an ammoniacal silver nitrate solution for 24 h, prepared according to the protocol previously described

by Tay et al. (22) After 24 h in total darkness, the slabs were rinsed thoroughly in distilled water and immersed in a photodeveloping solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains.

FE-SEM and EDS analyses

For the FE-SEM, the silver-stained resin-bonded specimens were gently polished down to a size of 1 μm and sonicated for 5 min to remove the superficial silver adsorption. The specimens were coated with a thin layer of osmium and observed using a FE-SEM (S-4500, Hitachi Ltd., Hitachinaka, Japan) at 5000x. Silver detection was carried out by EDS (EMAX-7000, Horiba Ltd., Kyoto, Japan). Initial energy spectra analyses were performed to determine the element composition of the whole area. Additionally, line scans across the resin cement-dentin interface were performed for elements including silver, calcium and silicon. Interfacial images were obtained from each specimen (n=10). Percentage distribution of metallic silver particles at the resin cement-dentin interface were calculated with a digital image analysis software (NIH Image 1.60, Scion Corp., Frederick, MD, USA) in a selected area on each image (h=2.6μm x w=23.5μm) (23). The data were statistically analyzed by Kruskal-Wallis test with the statistical significance defined as $p \leq 0.05$.

Micro-shear bond strength test

Twenty teeth were randomly assigned to two groups of ten teeth each, based on the resin cements used. Hollow cylinders 0.5 mm in height were cut from a micro-bore tygon tubing (Norton Performance Plastic, OH, USA) with an internal diameter of 0.75 mm and used as molds for the resin composite inlay. The resin composite (Estenia C&B) was placed into the tubing lumens on a flat surface covered with a matrix strip,

gently pressed and irradiated for 60 s (α-Light II, J Morita) prior to heat curing at 110°C for 15 min in air (KL 100, Kuraray Medical). Small resin inlay cylinders were obtained and silanized as described above. The resin cements were placed on the bottom of each cylindrical resin inlay and inserted into the tubing which was held on the dentin surface. The resin cements were cured in the same manner as described above. After 24 h water-storage at 37°C, the tygon tubing was removed carefully with a thin steel cutting blade and each dentin slice was attached to the testing device (EZ-test-500N, Shimadzu, Kyoto, Japan) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, CA, USA). The data were statistically analyzed by *t*-test with the statistical significance defined as p≤0.05. Failure mode was observed using optical microscope at a magnification of 40x and a sample corresponding to the predominant failure pattern was observed using SEM at magnification of 1000x.

Results

Nanoleakage evaluation

Typical images of nanoleakage at the resin cement-dentin interface for each material are illustrated in **Figure 1.1**. High-magnification FE-SEM micrographs after silver challenge revealed the existence of various patterns of nanoleakage at different locations; in case of RelyX Unicem, nanoleakage occurred within the resin cement-dentin interface and clusters of silver grains could be observed. A thin layer (morphologically different zone) was detected at the interface of the self-adhesive cement RelyX Unicem, without any resin infiltration into the dentinal tubules (**Figure 1.1a, 1.1b**).

On the other hand, a substantial spotted pattern of nanoleakage could be visualized at the interface between ED primer and dentin, as well as within the primer

layer (**Figure 1.1c, 1.1d**). The presence of a morphologically different zone beneath the primer layer and formation of resin tags were also identified (**Figure 1.1c, 1.1d**). ED primer layer with a thickness of approximately 1.5 to 2 μm was observed in almost all samples.
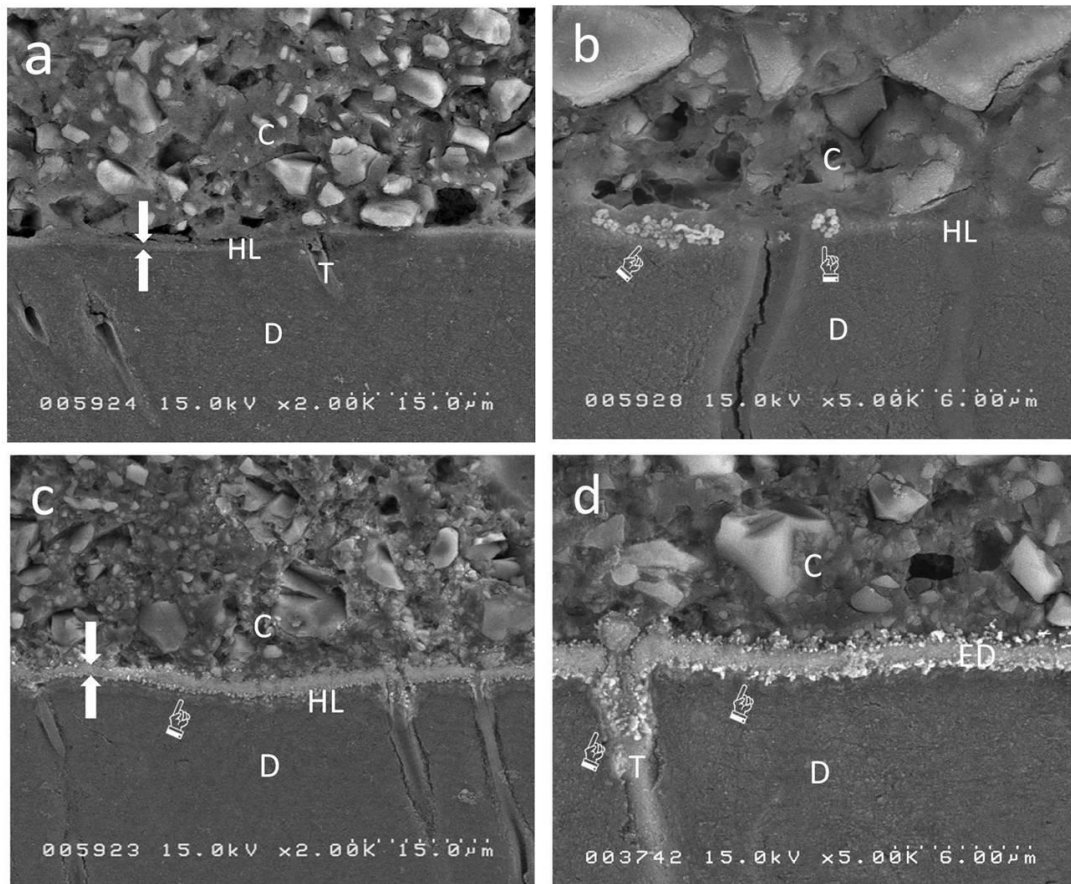
Figure 1.1a - Representative FE-SEM image of nanoleakage at the resin/cement dentin interface in the group RelyX Unicem. A thin layer of morphologically different zone was observed at the resin cement-dentin in (2000x image);
Figure 1.1b - Representative FE-SEM image of nanoleakage at the resin/cement dentin interface in the group of RelyX Unicem showing a cluster pattern of silver grains (5000x);
Figure 1.1c - Representative FE-SEM image of nanoleakage at the resin/cement dentin interface in the group of Panavia F2.0. A thicker 1.5 to 2μm layer of ED primer was observed in the images (2000x);
Figure 1.1d - Representative FE-SEM image of nanoleakage at the resin/cement dentin interface in the group of Panavia F2.0. Deposits of spotted silver particles were found at the ED primer layer and better visualized at 5000x magnification.
C, resin cement; D, dentin; ED, ED primer layer. Silver particles are shown by the finger pointers.

Regardless of the material used, a distinct nanoleakage formation could be recognized at the resin-dentin interface, although it was more prominent for Panavia

F2.0, where silver particles were detected in the primer layer and around the resin tags. There was a significant statistical difference between the two resin cements in terms of nanoleakage expression ($p \leq 0.05$).

Images in which the total percentage distribution of silver tracer within the interface was calculated are shown in **Figure 1**.**2**. The area percentage of silver particle for RelyX Unicem and Panavia F2.0 were 7.4 ± 4.6 and 18.7 ± 8.7, respectively. The corresponding Kruskal-Wallis test mean ranks were 6.8 and 14.2.
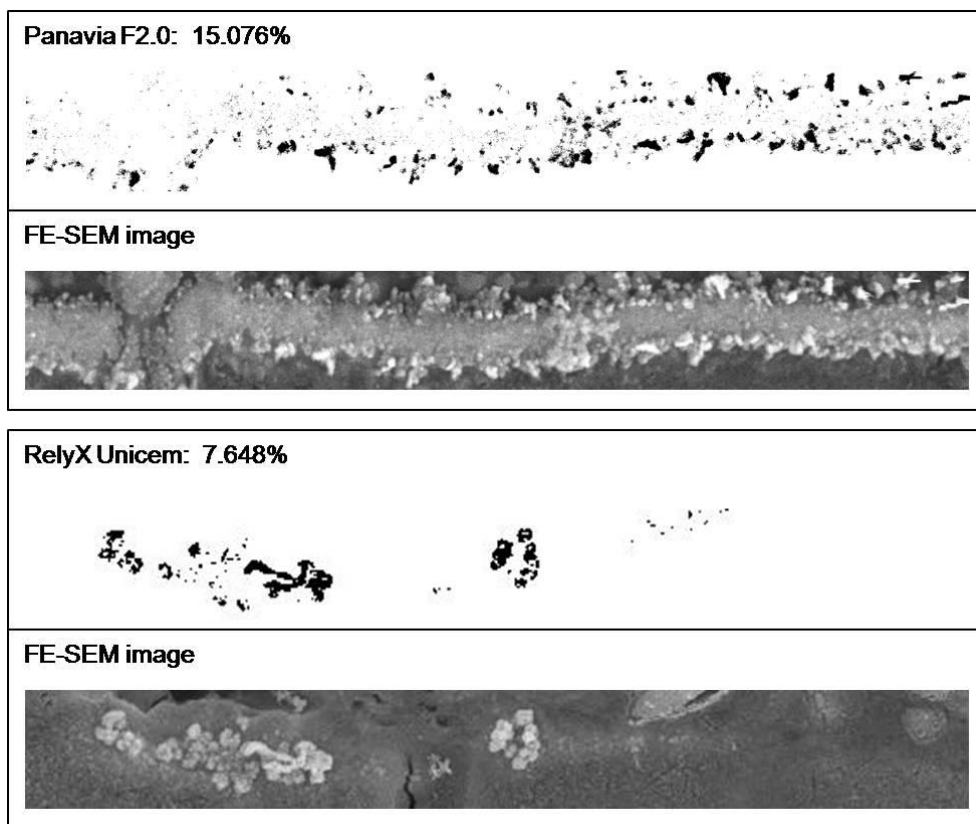
Figure 1.2 - Representative images of silver percentage at the interface, scored by digital image analysis software (NIH Image 1.60, Scion; Frederick, MD, USA).

EDS results for each group are shown in **Figures 1.3** and **1.4** (**Figure 1.3**, RelyX Unicem; **Figure 1.4**, Panavia F2.0). The results reflected a good agreement with those of FE-SEM with regard to the existence of metallic silver. Similar peaks of silver (Ag) were observed on the elemental energy spectra for both materials.
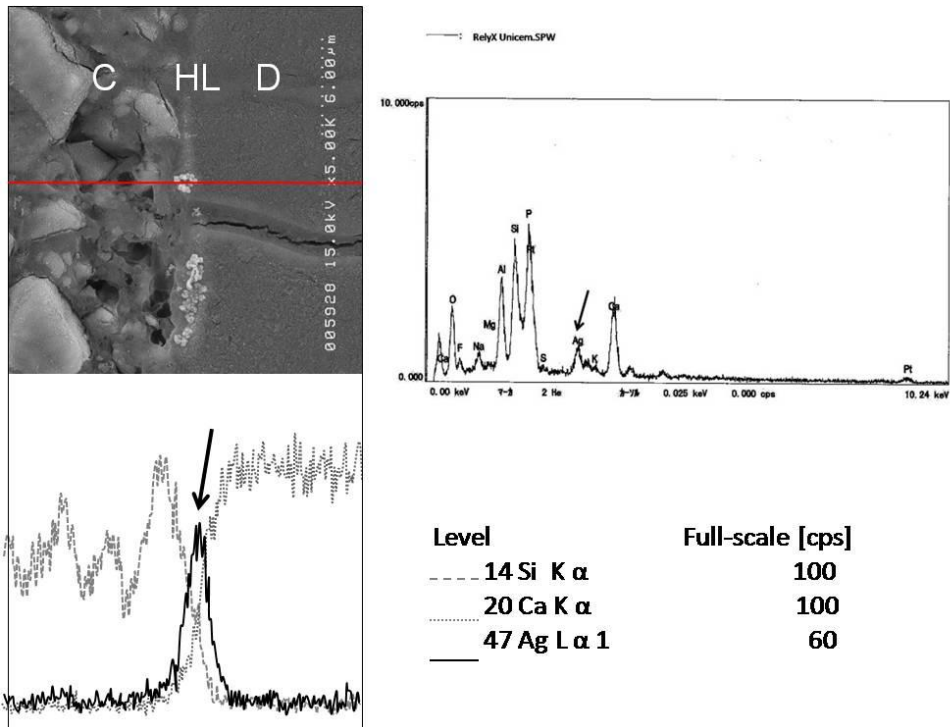
Figure 1.3 - EDS result of the same specimen of RelyX Unicem shown in Fig. 1-1b. A distinct silver peak was observed on the elemental energy spectra (black arrow). Subsequent line scan (red line) could also detect the existence of metallic silver particles. C, resin cement; D, dentin.
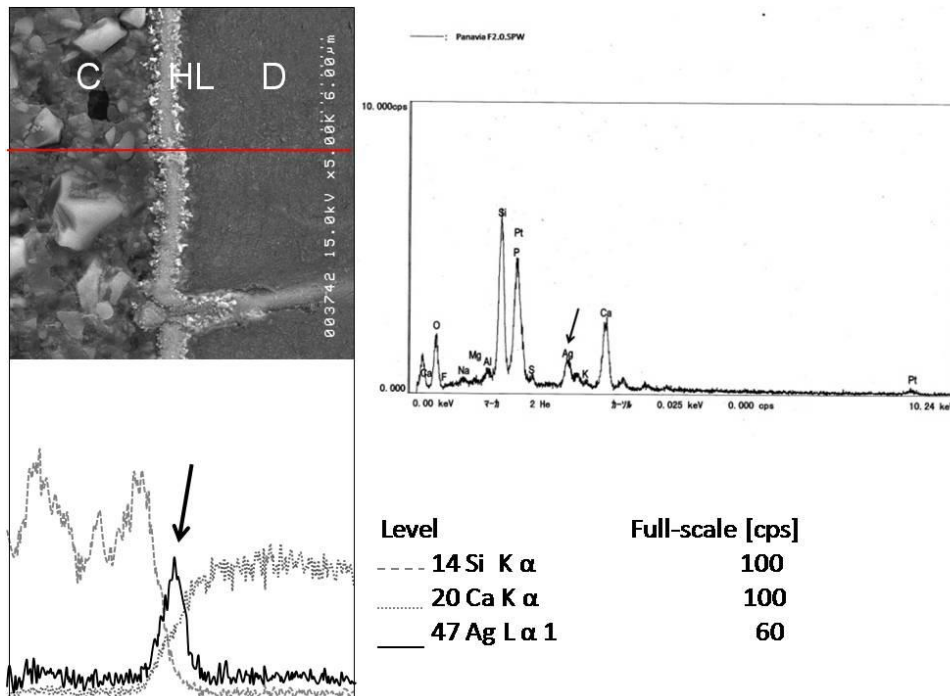
Fig. 1.4 - EDS result of the same specimen of Panavia F2.0 shown in Fig. 1-1d. Similar to RelyX Unicem, a distinct silver peak was observed on the elemental energy spectra (black arrow). The subsequent line scan (red line) also detected the existence of metallic silver particles. C, resin cement; D, dentin.

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Micro-shear bond strength

The means and standard deviations of micro-shear bond strength for RelyX Unicem and Panavia F2.0 were 24.9±4.8 and 26.1±5.3 MPa, respectively. *T*-test showed that there was no statistically significant difference between the two materials (p>0.05). Specimens bonded with RelyX Unicem recorded cohesive failure in resin cement (**Figure 1.5a**), almost for all cases. On the other hand, when Panavia F2.0 was tested, the predominant mode of failure was adhesive failure between tooth substrate and resin cement (**Figure 1.5b**). Typical SEM micrographs of the bonded area on the dentin side after the bond test are shown in **Figure 1.5**.
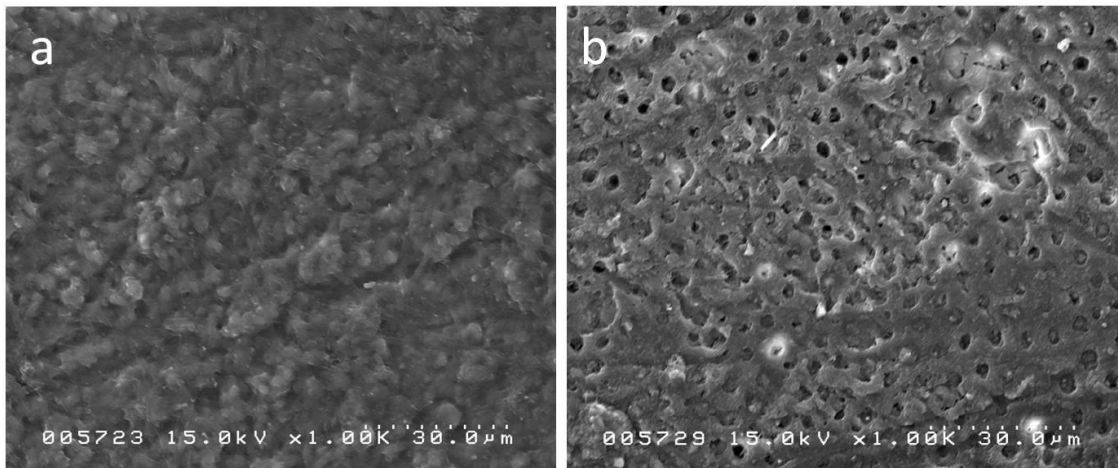
Figure 1.5a - SEM micrograph (1000x) of representative cohesive failure within cement when RelyX Unicem was used.
Figure 1.5b - Dentin tubule orifices are observed as the ED primer of Panavia F2.0 appears to dissolve the smear layer.

Discussion

In this study, the sealing ability and the bonding performance of a self-adhesive resin cement (RelyX Unicem) were tested on human dentin and were compared to a self-etching resin cement (Panavia F2.0). The micro-shear bond strength test was chosen due to the advantages of bonding tests with small and round bonded areas over other common methods, and also with regard to the ease of sample preparation (24-26).

EDS can produce quantitative and qualitative analysis of various elements distribution and is considered to be a sensitive and accurate chemical component detection method (27). Using this method, the probability of misinterpretation due to the electron microscopic edge effects is expected to be lesser (14).

Visual assessment has been traditionally used to evaluate the extent of nanoleakage; however, there are only few standard scoring methods available at the time. In order to score the percentage of silver particles within the interface, this study used a digital image analysis software (NIH Image 1.60, Scion Corp., Frederick, MD, USA). The percentage of silver particles within a selected area was calculated based on the contrast and brightness of each pixel on the digital image.

Using a similar nanoleakage method as in the current study, Yuan et al., (14) evaluated the silver particles penetration within adhesive, adhesive-hybrid layer interface and hybrid layer using (FE)-SEM images and EDS analysis. It was concluded that leakage expression as well as its location was dependent on the dentin bonding system tested. In this study, nanoleakage was detected along the interfacial defects within the resin cements; and the nanoleakage seemed to be more distinct in the self-etching primer resin cement compared to self-etching primer bonding systems that were evaluated in the former study.

RelyX Unicem is a self-adhesive resin cement. According to the manufacturer's data, bonding is achieved through interaction of dentin with the ionized phosphoric acid-methacrylate monomers in the mixture. Ionization may occur either *in situ* from the water of dentinal tubules or from the water produced during the neutralization reaction of the phosphate monomers with basic fillers (19). The bonding mechanism can be considered essentially similar to that of glass-ionomers with an intermediate interfacial

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layer incorporating partially dissolved smear particles (28). Without any pre-treatment, the self-adhesive resin cement showed a better resistance against nanoleakage formation compared to the self-etching resin cement. Although RelyX Unicem is very acidic during the initial minutes after mixing (pH<2 during the first minute), (19) little evidence of dentin demineralization was observed (**Figure 1.1a, 1.1b**); this finding was in agreement with those of the previous studies (29-33). With regard to the high viscosity of the material and limited penetration of the demineralizing components, the application of a constant pressure has been recommended in order to prevent formation of gap at the resin-dentin interface.

In a recent study, (31) RelyX Unicem showed an increased chemical interaction with calcium from hydroxyapatite, which may explain the bonding performance of this material to dentin and better results in the nanoleakage evaluation. In accordance with the bond strength results in this study, Abo-Hamar et al. demonstrated that there was no significant difference between RelyX Unicem and Panavia F2.0 in shear bond-strength to human dentin (34). The SEM images of this study showed that the predominant mode of failure in RelyX Unicem was cohesive failure in the resin cement, while as for Panavia F2.0, the predominant mode was adhesive failure. This finding may partially support the assumption that RelyX Unicem chemically interacts with dentin. However, further investigation is necessary to confirm these speculations.

Some previous studies on RelyX Unicem reported the lowest shear bond strength values compared to other cements when it was used in the self-cured mode. Meanwhile, this cement was the least influenced by thermocycling and demonstrated less sensitivity to variations in handling and aging (35). In this study, RelyX Unicem was used in the dual-cured mode. It has been reported that dual curing cements may

achieve optimal degree of conversion only with additional photo-activation, (36) and that the extent of polymerization may influence the durability of bond to the hard dental tissues (37).

Panavia F2.0 is a self-etching resin cement which contains three amphiphilic monomers (HEMA, MDP and 5-NMSA). Chemical interaction of MDP (a functional monomer in Panavia F2.0) with hydroxyapatite has been shown to be intense and stable (38). This interaction occurs by partial demineralization of the dentin, followed by the monomers adhesion to the remaining hydroxyapatite crystals around the exposed collagen. However, Panavia F2.0 showed substantial amounts of spotted silver depositions, not only at the interface between ED primer-dentin but also at the interface between ED primer-resin cement. It has been suggested that incomplete penetration of the resin monomers into the acid-demineralized collagen layer may be responsible for the formation of nanospaces and silver uptake (16). It has also been reported that the high concentration of hydrophilic and ionic resin monomers in ED primer result in the formation of a highly permeable layer (39). Tay et al. have stated that when water is incompletely removed from the primed dentin, porous anionic hydrogels are formed through copolymerization of HEMA and acidic resin monomers. In addition, the presence of water may result in regions of incomplete polymerization in resin matrix (40). Unpolymerized monomers may have a tendency to react with some staining agents. It is noteworthy that the tracer solution used in this study is an oxidizing agent also commonly used to detect certain organic compounds such as aldehydes, through reduction of the ammoniacal silver nitrate to metallic silver (known as Tollens' reagent) (41). In this regard, a chemical reaction between the uncured monomers and the

ammoniacal silver nitrate could also explain the significant amounts of silver particles for ED primer layer.

A resin coating technique has been introduced by adding a hydrophobic layer of resin over the self-etch primer to overcome the high permeability of such materials. Carvalho et al. reported reduced amount of silver impregnation when on additional layer of low-viscosity bonding resin was applied over the primer, suggesting a reduction in permeability (18).

A study that used simulated pulpal pressure showed a lower micro-tensile bond strength for RelyX Unicem compared to Panavia F2.0. However, in the same study, ED primer showed more permeability compared to smear layer-covered dentin (42). Another study by de Souza Costa et al. reported that no pulpal response was observed after 60 days using RelyX Unicem in human teeth and attributed the findings to the retention of components within the material, maintenance of the smear layer and preservation of normal histological characteristics of the dentin as no etching was required (43).

Clinically, not only the inflammatory response, but also the long-term durability should be concerned as important factors. Traditionally, an ideal bonding system should completely infiltrate and encapsulate the collagen fibrils, protecting them against degradation. On the other hand, the ultimate goal would be to develop a self-adhesive restorative biomaterial that no longer needs an adhesive for bonding to dental tissue. However, there is still a lack of scientific data to prove the long-term durability of self-adhesive resin cements.

The null hypothesis proposed in this study may be partially rejected; the two tested materials showed different leakage patterns, although a similar bonding

performance was observed. The location of nanoleakage was different between the materials. This finding was in agreement with that of another study in that different leakage patterns seemed to be dependent on the bonding system tested (14). Based on the results, it was speculated that RelyX Unicem could provide a better seal.

Conclusions

Within the limitations of this study, the tested self-adhesive resin cement may have a better sealing ability compared to the self-etching resin cement. As for the micro-shear bond strength test, both materials may perform equally.

Chapter 2

Non-destructive 3D imaging of composite restorations using optical coherence tomography: marginal adaptation of self-etch adhesives

Introduction

Advances in dental materials science have enabled functional and aesthetic reconstruction of debilitated tooth structure, along with preservation of dental tissues (11). Recently, simplified adhesive systems and flowable resin composites have become popular for restorative purposes (44, 45), especially because of their easy and fast application procedure.

Although contemporary self-etch adhesives provide excellent bonding to tooth substrate, their major shortcoming is their limited durability *in vivo* (2). While in a short-term, the presence of defects may act as stress raiser (46), in a long-term loss of marginal integrity of resin composite restorations is thought to be a significant factor for clinical failure (6, 47, 48). Insufficient sealing may lead to leakage of oral fluids along the interface between restorative material and tooth substrate and can result in post-operative tooth sensitivity, marginal discoloration and recurrent caries (49).

Current methods to determine marginal adaptation of tooth-composite interface are limited to *in vitro* studies. The most common method for leakage observation of restorative material-tooth is by immersing the bonded samples into a dye solution (organic or silver), followed by multiple slices sections and observation of the depth of dye penetration along the interface using light microscopy, scanning electron microscopy or transmission electron microscopy (8, 16). Creating several slices can provide a more accurate data of leakage formation in the whole cavity; however, conventional leakage evaluation techniques are clinically unfeasible. Moreover, it is

evidenced that leakage is seldom uniformly distributed, and that it is highly dependent on the C-factor as well as the composite volume (50-52).

Clinical assessments of margin quality are carried out by dentists everyday, however the replacement of existing restorations and the treatment planning decisions are very subjective (8). Radiographic assessment is frequently used in the clinic, though a translucent zone on a radiograph can be associated with either the presence of a thick adhesive layer, secondary caries or a gap (9). Furthermore, radiation hazard of the X-ray to both the patient and the clinician remains an issue.

Within the range of noninvasive imaging techniques, optical coherence tomography (OCT) has been reported as a promising high resolution biomedical optical method to detect microstructural details of hard and soft oral tissues (10). Basically, OCT takes advantage of the coherent properties of light. By coupling a low coherence light into a Michelson interferometer, the light is split to the sample and to a reference mirror. When reflections from the reference mirror and backscattered light from the tissue are recombined, an interference signal is detected within the coherence length of the source. Therefore, it produces cross-sectional images of tissue structures as a result of the interaction of partially coherent beam of optical radiation and the tissue component (53).

More recently, extensions of OCT have been developed, such as swept source optical coherence tomography (SS-OCT). This new method combines the capacity of rapidly sweep narrow linewith laser through a broad optical bandwidth with an immediately depth scan calculation by Fourier-transform with reduced noise (54). In dentistry, variations of OCT have found several potential applications in the diagnosis and monitoring of enamel and dentin in health and disease. While several

studies have reported on the characterization of caries under OCT (55), few studies to date have employed this tool to investigate marginal adaptation of composite restorations in three-dimensions.

On the basis of these considerations, in the current study, we investigate SS-OCT as a new tool to evaluate adaptation of composite restorations in class I cavities. For this purpose we used ammoniacal silver staining to improve the contrast in the SS-OCT 3D images.

Materials and Methods

Specimen preparation

Thirty-six freshly extracted bovine maxillary incisors, which were stored frozen prior to the experimental procedure, were used in this study. The teeth were carefully chosen and the enamel was slightly polished with a 1500-grit silicon carbide paper until a cylindrical flat area was obtained in order to eliminate any possible superficial enamel cracks, and create a flat surface for standard cavities. Round-shaped class I cavities (3mm diameter x 1.5mm depth) were prepared with margins located in the buccal enamel of bovine teeth and with cavity floor located in dentin. A high-speed round diamond bur was used to prepare the cavities under water coolant. The bur was replaced after five preparations in order to maintain the cutting efficiency. The usage of teeth in this study was approved by the Institutional Review Board of Tokyo Medical and Dental University.

The cavities were randomly assigned to 3 groups of 12 cavities each, according to the materials used: two-step self-etch adhesive group (SE Bond, Kuraray), all-in-one self-etch adhesive group (G-Bond, GC) and control group (without any adhesive), restored with a flowable resin composite (Clearfil Majesty LV, Kuraray). The

specimens were prepared according to the materials manufacturer's instruction (**Table 2.1**). After the total polymerization, all the specimens were again slightly polished with 1500-grit silicon carbide paper in order to remove the excess of resin and standardize the surface. Root apices were sealed using Clearfil SE Bond and Clearfil Majesty LV and the specimens were coated with 2 layers of nail varnish except for 1mm area around the restoration. The specimens were then stored in water for 24 h at 37°C.

Table 2.1 - Materials used in this study

Material	Brand (Lot#)	Composition	Application Technique	Manufacturer
Two-step, self-etch adhesive	Clearfil SE Bond; (primer) 00921A (adhesive) 01361A	Primer: MDP, HEMA, dimethacrylate hydrophilic, camphorquinone, N,N-diethanol p-toluidine, water. Adhesive: MDP, bis-GMA, HEMA, dimethacrylate hydrophobic, camphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica	Apply the primer on the surface for 20s. Air blow and apply the adhesive. Then, air blow and light cure for 10s.	Kuraray Medical, Osaka, Japan
All-in-one, self-etch adhesive	G-Bond; 0801241	4-MET, methacrylic acid ester, water, acetone, others	Apply one-bottle agent for 10s. Strong air blow for 10s and light cure for 10s.	GC Company, Tokyo, Japan
Flowable resin composite	Clearfil Majesty Shade A2; LV 00004B	Silanated barium glass powder, silanated colloidal silica, TEGDMA, hydrophobic aromatic dimethacrylate	Bulk filling. Light cure for 40s.	Kuraray Medical, Osaka, Japan

Thermocycling procedure

Half of the specimens of each group were randomly selected for thermocycling test (n=6/group). They were fatigued with 5,000 thermocycles between 5°C and 55°C with a dwell time of 30 s in each temperature and a transfer time of 10 s between baths (Cool line CL200 and Cool Mate TE200, Yamato Scientific Co., Tokyo, Japan).

Silver staining

After 24 h water storage at 37°C or 5,000 thermocycles (5°C and 55°C), the specimens were immersed into 50% ammoniacal silver nitrate solution for 24 h. Thereafter they were rinsed thoroughly under running tap water and exposed to

photodeveloping solution for 6 h under fluorescent light, to reduce the penetrating ammoniacal silver nitrate into metallic silver grains.

SS-OCT system

The SS-OCT (Santec OCT-2000®, Santec Co., Komaki, Japan) is a frequency (Fourier) domain technique with a tunable light source. The SS-OCT system used in this study incorporates an external laser probe which power is less than 5 mW within the safety limits defined by American National Standards Institute, and the center wavelength is 1319nm at a 20-kHz sweep rate (56).

The light beam from the laser source is projected onto the sample and scanned across the area of interest using the hand-held probe. Backscattered light carrying information about the microstructures of the sample is collected, returned to the system, digitized in time scale and then analyzed in the Fourier domain to reveal the depth information of the subject (57). Axial resolution of the system is 11 μm in air, which corresponds to 7 μm in tissue assuming a refractive index of about 1.5. A 240 x 240 x 400 pixels 3D image (4mm x 4mm x 2.6mm) can be output within 4 s including data acquisition and process time. The sensitivity of this SS-OCT is 106 dB, while the shot-noise limited sensitivity is 119 dB.

Tomography imaging with SS-OCT

After 24 h of storage or fatigue with 5,000 thermocycles, 3D scans were carried out before and after silver staining using SS-OCT **(Figure 2.1)**. Five cross-sectional images from the 3D scan of each restoration were obtained. The distance between each two slices was approximately 0.6 mm. A total of 360 images were analyzed (n=30). The scanning probe connected to the SS-OCT was set at a fixed distance over the buccal

enamel surface, with the scanning beam oriented 90° with respect to the tooth occlusal plane.

Confocal laser scanning microscope (CLSM)

To assure the presence or absence of gap at the interface between tooth-restorative material, the proximal surface of the stained teeth were cut by a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) and slightly polished with #600 silicon carbide paper and with diamond pastes with particle sizes down to 3 μm under running water. The same interfacial location as the middle slice of SS-OCT cross-sectional images were observed with CLSM (1LM21H/W, Lasertec Co., Yokohama, Japan) at a magnification level of 500x.
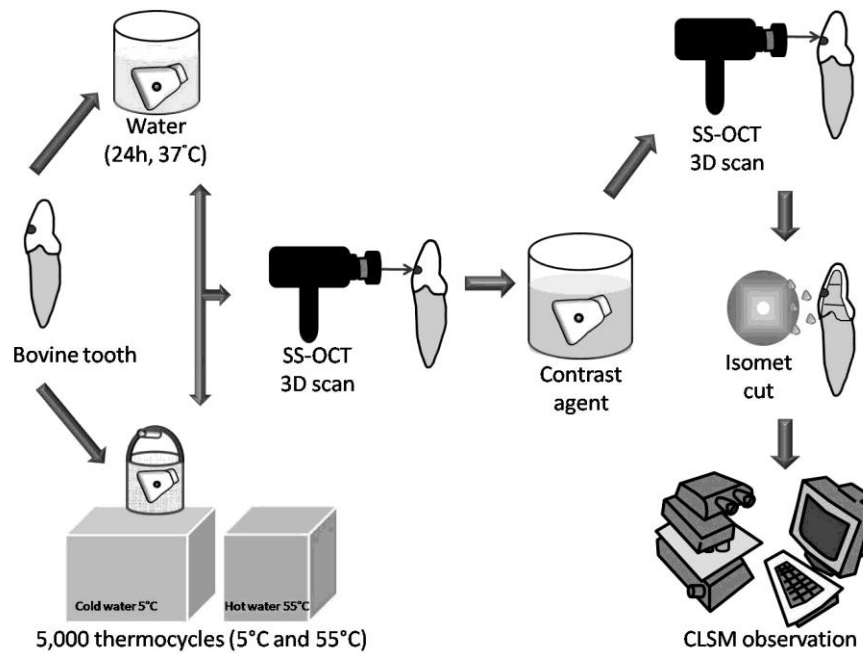
Figure 2.1 - Schematic view of the method used in this study.

Marginal adaptation quantification

Percentage distribution of brighter pixels with significantly higher signal intensity compared to surrounding pixels at the interfacial area was calculated with a digital image analysis software (NIH image 1.60, Scion; Frederick, MD, USA) (58) on

the images obtained with and without a contrast agent **(Figure 2.2; Figure 2.3)**. The data were statistically analyzed with Wilcoxon signed ranks test and one-way ANOVA Post Hoc Dunnett's T3 test at a significance defined as $p \leq 0.05$ using SPSS software.

For the statistical analysis of marginal adaptation between SS-OCT and CLSM in stained samples, the cavity length on the scored data of the 2D image-slices and CLSM images were divided into six equal sections. Indices of sensitivity and specificity for detection of gap presence by SS-OCT were calculated based on the diagnostic results obtained from CLSM.
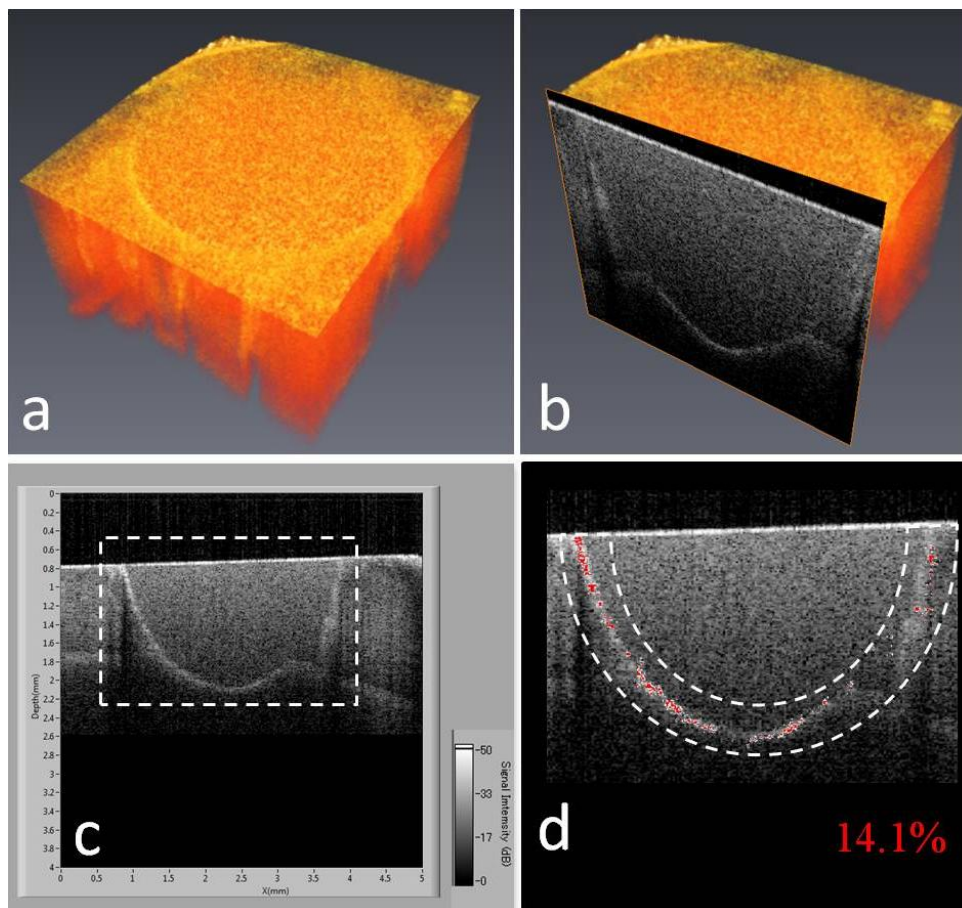
Figure 2.2a - Typical 3D image obtained from SS-OCT and reconstructed by Avizo 6.2 Imaging Software in which the cavity design can be visualized;
Figure 2.2b - 2D image obtained from 3D scan;
Figure 2.2c - 2D sliced-image with selected area to be cropped;
Figure 2.2d - Image from a stained sample and standardized selected area was used to crop the restoration margins of to be analyzed using digital image analysis software.
The percentage distribution of brighter pixels with significantly higher intensity signal at the interfacial zone is visualized by the red spots. The percentage distribution obtained by the digital image analysis software is mentioned on the bottom right corner.
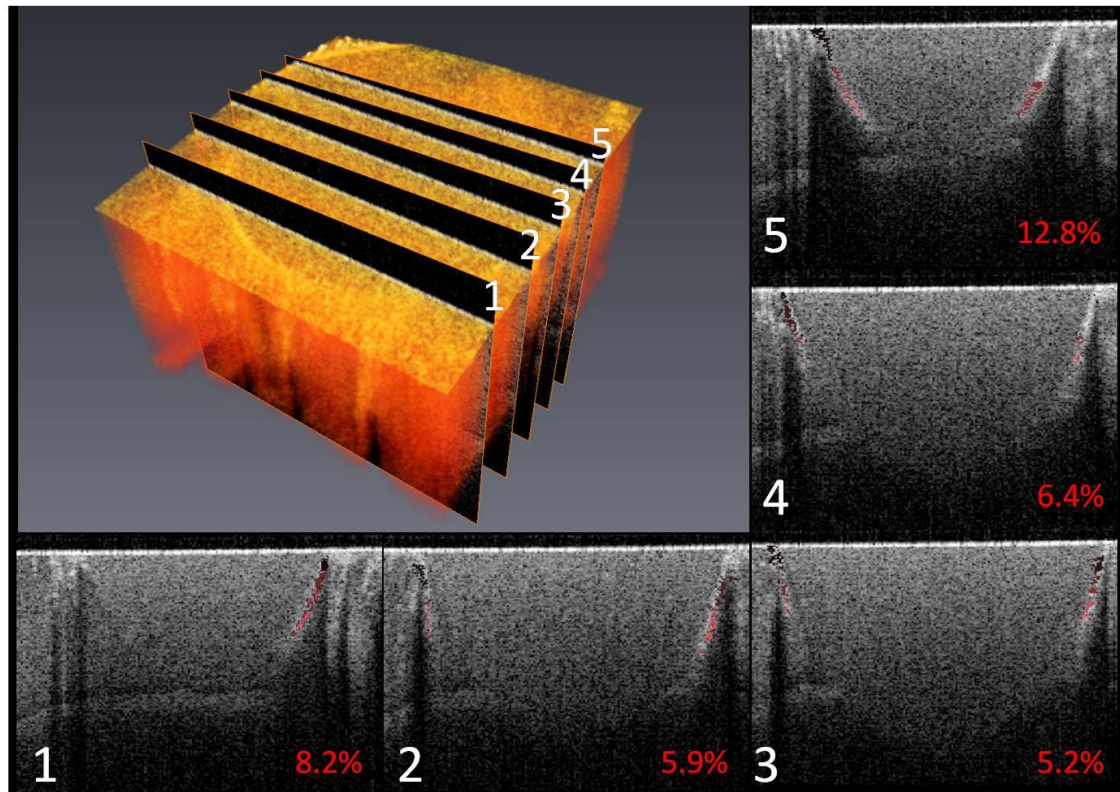
Figure 2.3 - Representative 3D image and corresponding 2D image slices obtained by SS-OCT. Cross-sectional images correspond to the 1, 2, 3, 4 and 5 slices in the 3D image, showing differences in the margins located in the same sample.

Results

Both adhesive materials showed scarce bright areas at the interface before thermocycling. These areas increased with thermocycling. For the control group, more areas with increased brightness were observed along the interface. No significant statistical difference was found between the results of SS-OCT non-stained and those of stained samples when a ranking transformation was applied on the data by Wilcoxon signed ranks test ($p > 0.05$). A significant positive linear correlation was found between stained and non-stained SS-OCT images (correlation coefficient=0.752, $p < 0.05$).

One-way ANOVA Post Hoc Dunnett's T3 test showed that when silver staining was used, there was not a significant statistical difference in percentage of bright areas

between SE Bond and G-Bond after 24 h. No significant statistical difference was found between the two materials in percentage of bright areas distribution after thermocycling as well. However, there was a significant statistical difference within each material before and after thermocycling ($p<0.05$) **(Figure 2.4)**.
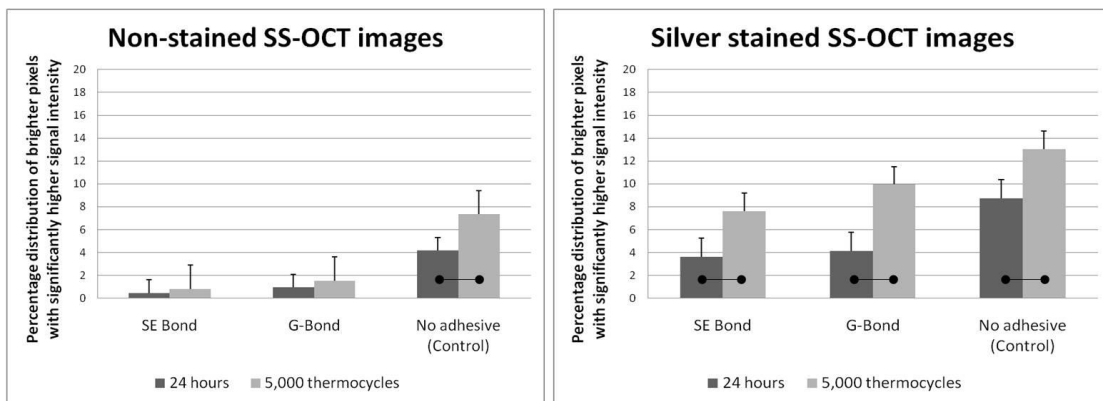
Figure 2.4 - Percentage distribution of brighter pixels with significantly higher intensity signal at the interfacial zone charts. For each group, connecting bars indicate statistical significant difference.

Specimens in the control group after thermocycling were debonded during the cutting procedure for CLSM observation. Gaps around 1μm to 20μm were observed with CLSM. Regions with increased brightness on the images recorded from the stained SS-OCT scanning were confirmed as gap existence by visualization of sectioned samples with CLSM **(Figure 2.5)**.
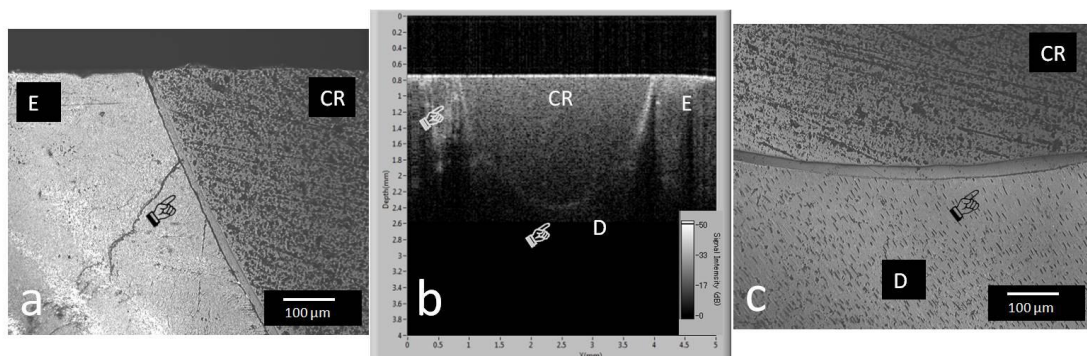
Figure 2.5a; Figure 2.5c - Representative CLSM images from the marginal wall and bottom of the cavity, respectively.
Figure 2.5b - Cross-sectional image from the SS-OCT 3D scan. Finger points show similarities between structures or gap existence. CR, composite resin; E, enamel; D, dentin.

The overall results for sensitivity and specificity, including 24 h storage or 5,000 thermocycling, for the non-stained SS-OCT data compared with CLSM were 0.687 and

1 and for the stained SS-OCT data were 0.804 and 1, respectively. The results for each group can be visualized in **Table 2.2.**

Table 2.2 - Sensitivity and specificity of SS-OCT non-stained and stained samples based on CLSM images.

		SE Bond 24h storage	SE Bond 5,000 thermocyles	G-Bond 24h	G-Bond 5,000 thermocycles
Non-stained	**Sensitivity**	0.75	0.64	0.71	0.66
	Specificity	1	1	1	1
Stained	**Sensitivity**	0.85	0.78	0.85	0.75
	Specificity	1	1	1	1

Discussion

OCT is a high resolution imaging technique that allows micron scale imaging of biological tissue over small distances. It uses low coherence infrared light to perform high-depth resolution for clinical diagnosis and fundamental research studies (53). In dentistry, since 1998 a series of reports appeared with imaging both hard and soft oral tissues (10, 59, 60). Findings in dental restoration imaging by time domain optical coherence tomography (TD-OCT) were previously reported with visualization of gaps as large as 50 μm in size (61).

Although most of the early OCT imaging systems used principles of TD, the SS-OCT used in this study is based on spectral domain. The spectral interference is not measured directly but rather sequentially by time, therefore the image is reconstructed by fast sweeping the laser source (57), enabling high-resolution, rapid 2D and 3D imaging by SS-OCT. The 2D scan is obtained by the combination of depth-resolved backscatter signal intensity profiles along the section of interest on the sample. Adding lateral

scanning capability to the system, allowed 3D acquisition of information of a biological material by combing several 2D scans over the volume of interest (62).

While the optical resolution of SS-OCT remains unchanged independent of the imaging mode, the image resolution of the 2D scans (2001 x 1019) and 3D scans (240 x 240 x 400) are different due to the capacity of the analog/digital (AD) board itself to convert the signal and process the data in the quasi-realtime. Cross-sectional images of the 3D scans can provide good information of the overall restoration with high-speed, although their details are lower if compared to the 2D image-scans.

An important mechanism for distinguishing between different structures in OCT imaging is their composition (60). According to an optical process, when light propagates between two mediums with differences in composition and a great contrast in refractive indices (i.e. air and composite), a portion of light is reflected (63). This phenomenon may explain the increased SS-OCT signal resulting in increased brightness in the presence of gap (air) at the tooth-restorative material interface for the non-stained samples.

On the other hand, in the stained samples silver penetrated into the interfacial gap acts as a metallic contrast agent highly reflecting the light. Ammoniacal silver nitrate staining is a well-established technique to evaluate restorative material-tooth marginal integrity and interfacial analysis (16, 64). The relatively high atomic number of silver allows good contrast to dental structure (65), therefore by using this staining technique a better contrast in the 2D cross-sectional images obtained from the 3D scan was expected to be observed when a gap was present.

Both materials showed scarce bright areas at the interface before thermocycling, indicating a good initial seal. In a recent study, Blunck et al. (66) investigated the

marginal integrity of Class V restorations by thermocycling after different periods of water storage, using G-Bond and SE Bond adhesive systems. In that study, it was found that after 1-year storage and two times of 2,000 thermocycles, differences on the marginal integrity were observed for both materials compared to the baseline. However, it was assumed that there was a good marginal integrity due to the fact that deterioration of the adhesives is generally quite minimal. In the current study, we performed 5,000 thermocycles continuously. Differences in bright areas were observed, indicating increased interfacial gaps compared to the baseline for both materials. The interfacial deterioration was in agreement with the findings of the previous report.

SE Bond is a two-step, self-etching adhesive system. This system is known to have a reliable and predictable bonding to the dentin; however, it has a tendency toward small marginal defects on enamel (11) which might explain the slight brightness in the SS-OCT images, with the majority located in enamel margins. Phosphoric acid etching of enamel prior to the application of the self-etching primer is speculated to improve marginal integrity of this adhesive to enamel (67).

G-Bond is a one-step, self-etching acetone/water based adhesive. This system showed slightly brighter areas at the interfacial area compared to two-step self-etching system; however, no statistically significant difference was observed. It has been reported that the water sorption and subsequent hydrolysis of these adhesives may result in the long term deterioration of the interface (68). In our study, this effect might have been accelerated by 5,000 thermocycles, resulting in more gaps formation along the interface. According to the manufacturer, the flowable resin composite used in this study has high filler loading (81wt%) and relatively low polymerization shrinkage (1.88 lin%). It has been reported that this restorative material exhibited excellent marginal integrity and no

gap formation at the resin-cavity interface for enamel-dentin class I cavities (2mm x 1mm x 2mm) restored with SE Bond adhesive (69). Indeed, this report was partially corroborated by the results of this study, as with a larger cavity (3mm x 3mm x 1.5mm) and only some small gaps could be observed after using the silver stain.

The CLSM direct observation was chosen due to advantages over common methods regarding sample preparation, elimination of the need for vacuum or dehydration and immediate observation, therefore minimizing the risk of technical artifacts (70). In addition, it was possible to verify the sectioning site and trim the specimen, if necessary, so as to reach the exact desired cross-section as imaged by SS-OCT.

It should be pointed out that the results presented in the form of percentage **(Figure 2.4)** in this preliminary study are not directly representing the size (length or height) of the gap, but are rather giving an indication of presence of the gap and the contrast (or visibility) of areas with gap in comparison to the adjacent structure on the images. These areas with increased brightness observed in SS-OCT correlated well with gap presence as confirmed by the CLSM **(Figure 2.5)**.

In this study, the sensitivity and specificity of SS-OCT was measure based in the CLSM results. Sensitivity stands for the proportion of actual gap existence correctly identified as such while specificity measures the proportion of absence of gap correctly identified as such. High sensitivity and perfect specificity were observed for both materials after 24 h water storage. This high specificity was also observed after thermocycling, confirming that when brightness was present, real gap was observed in the CLSM. This result provides valuable information from the clinical point of view.

On the other hand, after the thermocycling test, the number of cases where the actual gap was incorrectly identified as no gap (false negative) was increased, slightly

decreasing the sensitivity of SS-OCT data. It should be noted that the contrast agent would penetrate only as deep as the gap is continued from the superficial margins of the restoration, and therefore it may not reach the isolated small defects in deeper interface. This was confirmed by CLSM observation where occasionally no silver was found at the gaps in the bottom of cavity. It is also speculated that gap formation during the sectioning procedure itself might have also occurred, that appear more prominent after thermocycling.

In *in vitro* studies, the use of micro-CT has been reported for 3D marginal adaptation observation. Although it is a non-destructive method, micro-CT data acquisition can take a long time, during which time the specimen may progressively dehydrate producing false positive gap formation or motion artifacts could be present (71). Clinically, radiographic assessment and visual inspection are the current approaches to evaluate marginal adaptation. However, even considering that superimposition of adjacent structures is avoided, only translucent zones larger than 40 μm are detectable from radiographs. Therefore, misjudgments on replacing restorations can occur (9).

The optical properties of dental composites, such as visible light transmission (translucency) and index of refraction, are more desirable as they better approximate those of the dental tissues with which they form interfaces (59). SS-OCT seems to be a potential technique for examining the structural quality of the teeth restored with composite materials even if no staining is used. Nevertheless, it appears that despite high specificity, staining improved sensitivity in detection of small defects by SS-OCT. SS-OCT can provide fast information of the overall cavity restoration, which can facilitate chair-side diagnosis. As it has a hand-held-probe, different positions and angles of the oral cavity can be observed in real-time and a potential tool for treatment

planning, non invasively to the patient. Furthermore, it seems a promising method applicable to evaluate the life-time of dental materials-marginal restorations integrity *in vitro* and *in vivo*.

Conclusions

Within the limitations of this in vitro study, it is suggested that the combination of SS-OCT and a contrast agent enabled the detection of microgaps along the cavity with a high sensitivity. SS-OCT is a promising tool for fast observation of marginal integrity at the tooth-restoration interface.

General Conclusions

Several factors may influence the interface integrity of adhesive restorations in direct and indirect composite restorations. Morphological changes were observed and discussed in the present studies.

Chapter 1 concluded that the difference in chemical composition of the tested resin cements resulted in different leakage expressions. Moreover, as for the micro-shear bond strength test, both materials performed equally, showing no correlation between leakage expression and bond-strength test.

Chapter 2 concluded that swept-source optical coherence tomography is a potential 3D non-invasive tool for fast observation of marginal integrity with high specificity. A good initial seal was observed, however the interfacial deterioration increased with thermocycling test. The use of a contrast agent improved the sensitivity of that device.

In chapter 1 and 2 a silver staining technique was used to create a better contrast between the restorative material and tooth interface. The results provided a qualitative and quantitative analysis of nanoleakage and marginal integrity.

Although informative and reliable, the methods used in both studies are not comprehensive methods to investigate all the effective attributes of adhesive materials. To further advance the accurate analysis of interfacial defects, the development of a more clinically relevant standardized method is still required.

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Appendix A

Abbreviation	Complete name
3-MPS	3-methacryloyloxypropyl trimethoxysilane;
bis-GMA	bisphenol-A diglycidyl ether dimethacrylate;
HEMA	2-hydroxyethylmethacrylate;
MDP	10-methacryloyloxydecyl dihydrogen phosphate;
TEGDMA	triethylene glycol dimethacrylate.
UDMA	urethane dimethacrylate;
UTMA	urethane tetramethacrylate;