

Self-etching Adhesive Systems: Factors Predicting Clinical Success

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Dedicated to my father
Professor Mohammad T Sadr

In the memory of
Late Professor Stepan Alexanian

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Preface

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

- Chapter 1. Sadr A, Shimada Y, Tagami J. Effects of solvent drying time on micro-shear bond strength and mechanical properties of two self-etching adhesive systems. *Dental Materials* 2007;23:1114-1119.
- Chapter 2. Sadr A, Ghasemi A, Shimada Y, Tagami J. Effects of storage time and temperature on the properties of two self-etching systems. *Journal of Dentistry* 2007;35:218-225.
- Chapter 3. Sadr A, Shimada Y, Lu H, Tagami J. Viscoelastic behavior of dental adhesives: A nanoindentation study. *Dental Materials* (Submitted)

Table of Contents

Introduction	3
Chapter 1	5
Effects of solvent drying time on micro-shear bond strength and mechanical properties of two self-etching adhesive systems	5
Introduction:	5
Materials and methods.....	6
Specimen preparation.....	6
Micro-shear bond test.....	7
Nanoindentation tests	8
Results	11
Micro-shear bond test.....	11
Nanoindentation	11
Discussion	13
Conclusions	16
Chapter 2	17
Effects of storage time and temperature on the properties of two self-etching systems ..	17
Introduction:	17
Methods and materials.....	18
Materials and storage conditions.....	18
Micro-shear bond strength tests	19

Nano-indentation tests.....	21
pH evaluation	21
Statistical analysis	22
Results	22
Micro-shear bond strength	22
Nano-indentation hardness.....	24
pH measurements	24
Discussion	25
Chapter 3	33
The viscoelastic behavior of dental adhesives: A nanoindentation study	33
Introduction	33
Theory	34
Materials and methods.....	36
Sample preparation.....	36
Nanoindentation	37
Data analysis	37
Results	38
Discussion	40
Conclusions	45
Chapter 4	46
Composition and bonding durability of self-etching adhesive systems	46
Introduction	46

Materials and methods.....	46
Results	48
Discussion	50
Conclusion.....	55
Chapter 5	56
General Conclusions.....	56
References	58
Appendix A	68

Introduction

Adhesive dentistry is among the fastest growing fields in the dental science today. Aesthetic restorations with great functionality and durability and at affordable costs are in demand by patients. On the dentist's side, the conservation of natural tissues is highly valued by modern concepts of minimal intervention¹, together with simplified and timewise effective chair-side restorative solutions. However, technique sensitivity is still a major draw back of the adhesive approach.² In addition, with the introduction of newer adhesive materials with different approaches and compositions, selection of the most suitable adhesive is sometimes not easy for the dentist. This hardship may partly be due to the rapid evolution of the field itself and difficulties getting accustomed to new approaches and materials, and partly due to the fact that the bulk retention of a restoration is no longer a concern with most of the available adhesives, and selection of best material may not be easy for the clinicians based on their personal experience. In this regard, it is of vital importance for the researchers to investigate the materials from different aspects; seeking potential weaknesses and developing sophisticated yet clinically relevant techniques for performance evaluation of adhesive materials.

A realistic prediction of clinical success of dental adhesive restorations requires deep understanding of the properties of all the components in the bonded complex from mechanical, chemical and biological point of view on a molecular level and nano-scale, and analysis of the risk factors and contributing factors within the service time of the restoration.

The current adhesive resin materials are classified based on a combination of two major criteria. One of them involves the number of clinical application steps; and the resulting classification has three main categories, one step, two step or three step adhesives. The other criteria, classifies the adhesives according to the strategy and approach of the materials; "etch and rinse" and "self-etch" are the main categories in this view. Self-

etching adhesives are currently presented as one- or two- step adhesives systems. All-in-one systems are the one-step self-etching adhesives that come mixed in a single bottle. Clinically, the most promising approach is the self-etch, with regard to the user-friendliness, technique-sensitivity and minimal conservation concepts. However, this would not necessarily imply that the current self-etching adhesive systems are perfect. In this regard the studies included in this thesis focused on the self-etching systems. Clearfil SE Bond, a two-step self-etching adhesive received as one of the most reliable adhesive systems, has been chosen as the reference bonding system in numerous studies, including those included here.

Chapter 1 investigated the effects of solvent drying time on micro-shear bond strength and some mechanical properties of a one-step and a two-step self-etching adhesive systems. The paper attempted to clarify the technique sensitivity of these systems related to the clinical handling and air-drying step of these materials.

Chapter 2 looked into another clinical factor; the effects of pre-use storage time and temperature on adhesion, mechanical and chemical attributes of the two self-etching systems were investigated.

High bond-strength values have been reported for different classes of adhesive materials by numerous in-vitro studies. Bond-strength results are clinically relevant, but the mechanical factors that affect the clinical performance of adhesive materials may not be limited to the bond-strength. Chapter 3 sought investigation of visco-elastic behavior of the bonding layers from a nano-mechanical point of view and discussed the possible compositional factors.

Chapter 4 summarized a series of studies undertaken by the author, aiming at understanding the long-term bonding performance of the self-etching systems and contributing factors such as fluoride release.

Chapter 1

Effects of solvent drying time on micro-shear bond strength and mechanical properties of two self-etching adhesive systems

Introduction:

The introduction of dentin primers has remarkably improved the efficacy of dentin bonding systems. Dentin primers contain hydrophilic monomers to enhance the wettability and infiltration of hydrophobic resin monomers into the demineralized matrix on the etched dentin surface.³ Self-etching primer systems combine the etching and the priming steps into one; all-in-one systems combine self-etching primer and the bonding agent into one application. Although low technique sensitivity and consistent performance are expected to be achieved hands-down with one-step self-etch adhesives due to their simplified application procedures, some previous studies have indicated controversies on performance of these newly introduced adhesives.⁴

It has been suggested that water, solvents or primer, mixed into adhesive resin result in reduced mechanical properties and accordingly poor bonding performance, thus removing those components from the adhesive seems to be a critical issue.⁵ Nevertheless, giving practical instructions on air-drying of the material while applying in the patient's mouth appears to be difficult due to the restrictive variables such as cavity shapes or configurations which may enhance pooling of the material, tooth position in the mouth, dentin sensitivity to air blast in vital teeth and various air pressures from different air-syringes. Practitioners are expected to use dental materials according to each material's instruction to obtain the best clinical results⁶, however with regard to the above mentioned restrictions, it seems difficult to define criteria for sufficient air-drying and it will be a compromise that the practitioner must make to achieve the best results, particularly for an

all-in-one system.⁷ On the other hand, to-date few studies have investigated the effects of air blowing of the solvent containing agents on material performance.

The aim of this study was to investigate the micro-shear bond strength to dentin, and mechanical properties of one-step and two-step adhesives after different air-drying times of self-etching agents, and the possible relationships between the variables. The null hypotheses tested were: (1) the two adhesives tested perform equally well and show similar characteristics; micro-shear bond strength to human dentin and mechanical properties, and (2) those characteristics are not affected by air-drying time of the self-etching material.

Materials and methods

Specimen preparation

Caries-free human upper premolars were used in this study. The teeth were washed and stored in 0.02% thymol solution immediately after extraction until the experiment time. Superficial dentin slices, each about 2 mm thick were prepared by removing the occlusal enamel and cutting perpendicular to the proximal surfaces using a low speed Isomet saw (Buehler diamond wafering blade 15 HC, Buehler, IL, USA) under running water as coolant. The surface of each dentin slice was then polished under running water by 600grit silicone paper, to create a smear layer on the dentin. Thirty slices were randomly distributed into six groups and treated with one of the adhesive systems (Table 1-1) according to the following procedures.

Table 1-1 Composition, group code and lot no. of the adhesives tested in this study.

Adhesive	Composition	Lot no.	Code
Clearfil SE Bond	Primer: water, MDP, HEMA, CQ, DET, hydrophilic DMA	00206A	SE
	Bond: MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, DET, silanated colloidal silica	00209A	
Clearfil Tri-S Bond	Water, MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, ethyl alcohol, silanated colloidal silica	040219	TS

For SE groups, primer of SE bond (Clearfil SE Bond, Kuraray Medical, Tokyo, Japan) was applied to the dentin surface using a sponge supplied by the manufacturer and rubbed for 20 s. The primed dentin surface was then dried with oil-free compressed air with an air pressure of 4 kgf/cm² from 5 cm above the dentin surface, using a three-way syringe device (Yoshida, Tokyo, Japan) for 2, 5 or 10 s for groups SE2, SE5 or SE10, respectively. After this etching and priming step, the SE bonding agent was applied using sponge pieces provided by the manufacturer and was air spread until a homogeneous layer was observed on the surface using an air pressure of about 2 kgf/cm². For TS groups the self-etching priming and bonding agent (Clearfil Tri-S Bond, Kuraray Medical) was applied to the dentin surface using the sponge and rubbed for 20 s. The treated dentin surface was then dried in a setup similar to that of SE primer as described above to form TS2, TS5 and TS10 groups.

Micro-shear bond test

Prior to irradiation of the bonding resin on each specimen, 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 were placed on the treated dentin surfaces. After 10 s light irradiation with a halogen light cure unit, a hybrid restorative composite (Clearfil APX Shade A3, Kuraray Medical) was carefully inserted into the tubing lumens and irradiated for 40 s according to the manufacturer's instructions. The specimens were then stored in deionized water at 37 °C and the tygon tubing removed after an hour using a feather blade. Twenty-four hours post-bonding, each tooth slice was attached to the testing apparatus (Bencor-Multi-T, Danville Engineering, CA, USA) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, CA, USA) and was tested in a universal testing machine (EZ-test-500N, Shimadzu, Kyoto, Japan). A thin steel wire (0.20 mm diameter) was looped flush between the load cell projection and the resin cylinder making contact with the lower half-circle of the cylinder and touching the tooth surface. The force

was applied at a crosshead speed of 1 mm/min until failure occurred (Fig. 1-1). Care was taken to keep the bonding interface and the center of the load cell in line, parallel to the load cell movement direction and on an imaginary plane parallel to the tooth surface and the wire loop in order to maintain stress orientation as shear at the bonding interface. Ten bond strength values were obtained from the specimens in each group.

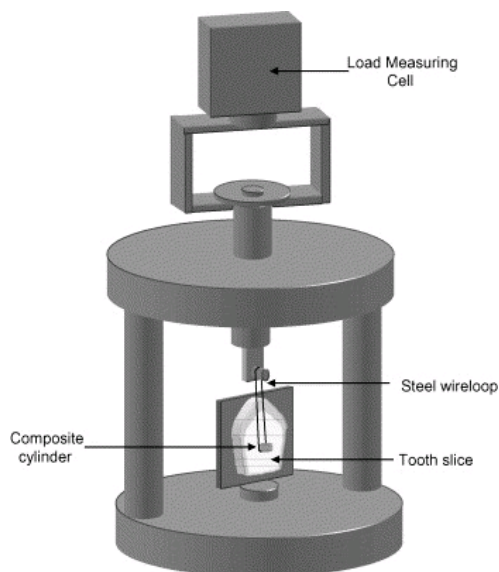


Figure 1-1 Schematic view of micro-shear bond test apparatus

Nanoindentation tests

Six dentin slices (three for each material) were prepared, each slice was surface treated according to one of the groups described above, the bonding was light cured for 10 s after application and a composite layer (about 1 mm thick) was placed over the bonding layer and light cured for 40 s. After storage in deionized water for 24 h at 37°C the specimens were sectioned perpendicular to the bonding surface into two halves. The resulting samples were embedded in epoxy resin with the resin–dentin interfaces facing out, polished under running water on a series of SiC paper with grits ascending from 600 to 1500 and finally polished with diamond pastes of decreasing particle sizes down to 0.25 μm . After polishing, nanoindentation was performed at a constant temperature of 27.5°C with a Berkovich indenter attached to a computer controlled nanoindentation device (ENT-1100, Elionix, Tokyo, Japan).

The positions of indentation points were programmed at the approximate half-width of the bonding layer. The indents were observed with a CCD camera connected to the

device to exclude irregular or unclear shaped indentations. After a series of pilot indentations were performed to select appropriate loading regime, data were obtained from 20 successful indentations on each sample made at a constant loading rate of 10 mgf/s with the load increasing until a maximum value of 250 mgf (around 2.5 mN). The constant rate was maintained by adding a load increment of 0.1 mgf to the current load per 10 ms interval. After this step-loading segment, the maximum load was held for 60 s, followed by the unloading segment in which the load was gradually removed (Fig. 1-2).

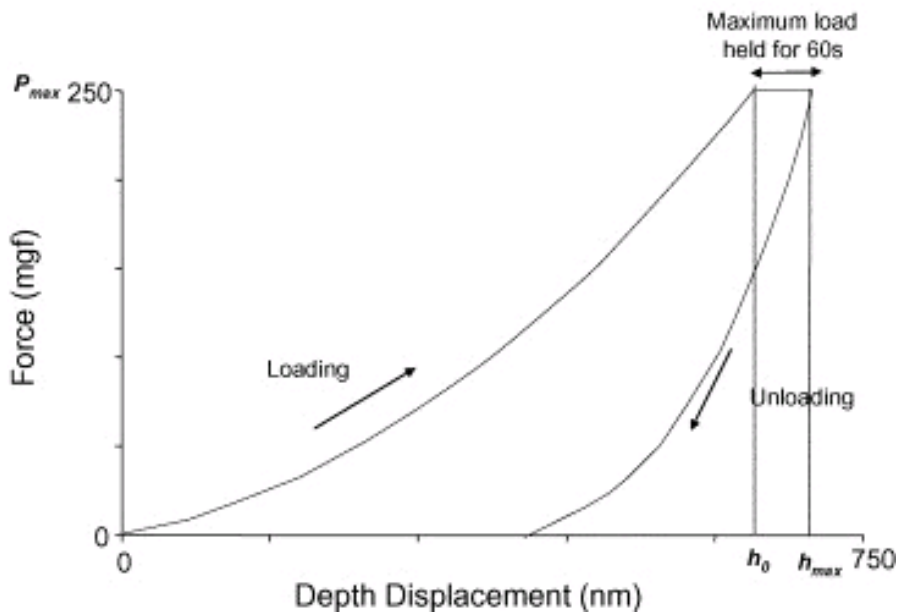


Figure 1-2 Typical load displacement curve with a creep hold segment. The quantities shown are P_{max} : maximum applied load; h_0 : the indenter displacement at the beginning of hold segment (time point 0 s); h_{max} : the indenter displacement before unloading.

Basically, indentation hardness (H) is defined as the stress divided by the projected of the indentation:

$$H = \frac{P}{A_p}$$

where P is the applied load and A_p is the projected area of the indentation which depends on the indenter geometry and the penetration depth that was calculated according to the index provided by the device manufacturer. A hardness value was calculated for each of the indentations based on the position of the indenter just after the maximum load was reached (h_0):

$$H_0 = 0.037986 \times \frac{P}{h_0^2}$$

Indenter instantaneous descent rates were also calculated at different points of time during the hold segment for one typical indentation data set in each group:

$$\frac{dh_i}{dt_i} = \frac{h_i - h_{i0}}{t_i - t_{i0}}$$

where h_i is the position at a given instant (t_i) and h_{i0} is the position at the previous instant (t_{i0}). Strain rate ($\dot{\epsilon}_i$) was calculated from the ratio of the indenter instantaneous descent rate to the instantaneous position:

$$\dot{\epsilon}_i = \frac{\frac{dh_i}{dt_i}}{h_i}$$

Instantaneous hardness (H_i) was also calculated at each corresponding point of time to indicate local stress. Strain rate values were normalized and plotted against hardness to obtain a regression plot on which the deformation behavior was studied and stress

exponent parameter was calculated from the best fit for each material. Hardness was considered as an analogue to stress on the plot.

The data were finally subjected to one-way ANOVAs followed by Tukey's HSD post hoc test at the 95% level of confidence.

Results

Micro-shear bond test

The lowest bond strength means were obtained for TS2 and SE2 groups, which were significantly lower than all other groups. SE10 reached the highest mean value of micro-shear bond strength that was significantly higher than SE2 and all TS groups. The means and standard deviations of hardness values are included in Table 1-2. Post hoc analysis showed that hardness was significantly different between all group pairs, except for SE5 and SE10.

Nanoindentation

The means and standard deviations of hardness values are included in Table 1-2. Post hoc analysis showed that hardness was significantly different between all group pairs, except for SE5 and SE10.

Table 1-2 Bond strength and hardness results for all groups

Group	Bond strength MPa (<i>n</i> = 10)	Hardness MPa (<i>n</i> = 20)
SE2	31.4 (8.8) a	245.1 (5.1)
SE5	42.8 (7.9) bc	270.2 (5.8) f
SE10	46.7 (3.6) b	272.2 (5.0) f
TS2	24.4 (8.5) a	180.1 (9.5)
TS5	39.2 (5.6) cd	212.5 (2.6)
TS10	36.6 (8.1) d	222.8 (3.5)

Values marked with the same alphabetical letters are not significantly different ($p > 0.05$). The numbers in parentheses are standard deviations.

Fig. 1-3 exhibits the time-dependent mean creep displacement for each group during the hold segment. The average values of maximum depth ranged from 587 to 742 nm at the full load in the beginning of the hold segment and from 652 to 837 nm at the end of the hold segment. The highest displacements (depths) at the beginning and during the hold segment were observed for 2 s air-dried samples for each material. Fig. 1-4 is a plot analogous to strain-rate versus stress on which the exponent values are calculated for each material. The best regression fit for the strain-rate versus stress was obtained corresponding to the power law relation ($p < 0.05$ for all fits).

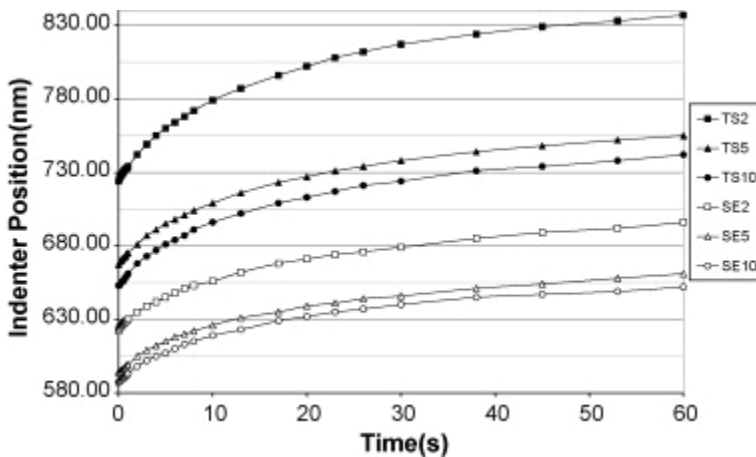
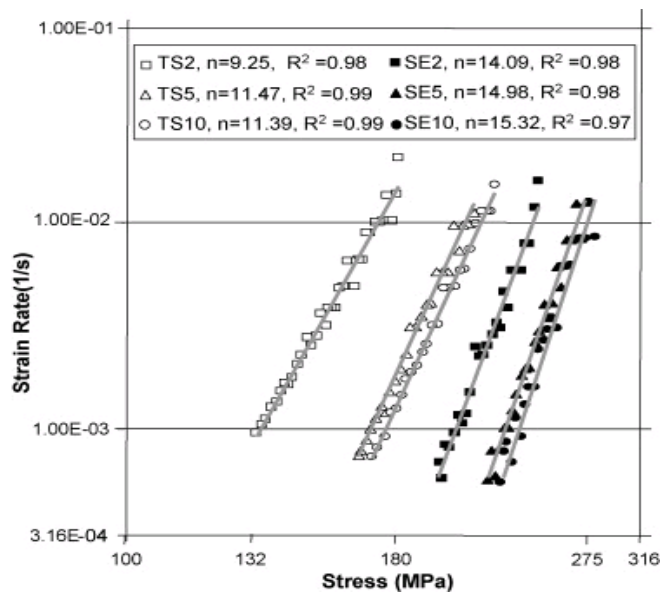


Figure 1-3 Average indenter displacement (penetration depth) under the constant load; from h_0 to h_{max} . Groups with longer air-drying time for each material seem to show more resistance to deformation, however data may not be compared directly due to the variation of the applied stress with depth.

Figure 1-4 Log-log plot of indentation strain rate ($\dot{\epsilon}$) vs. hardness or stress (σ); R^2 -values correspond to the power law regression: $\dot{\epsilon} \propto \sigma^n$, where n is the stress exponent. Regardless of the intercepts, at a given stress a smaller n would mean a higher strain rate.



Discussion

The results of the micro-shear bond strength test indicated that when properly handled, SE bonded more effectively to dentin than TS, and that the air-drying time of the self-etching material can significantly affect the bond strength.

The two adhesives tested were from the same manufacturer, contained the same functional monomers and had similar compositions. Although TS has a higher pH and milder acidity compared to SE (2.7 versus 1.9) and is expected to dissolve lesser mineral content of the dentin, previous studies suggested that higher pH of an adhesive could not consistently be considered as a disadvantage⁸ and thus, it could not be suggested as an explanation for lower bond strength to dentin of TS in the present research.

On the other hand, SE is a two-step self-etching system for which primer is a hydrophilic aqueous solution that should be air-dried to remove the water and solvent content before adhesive is applied. SE adhesive contains a hydrophobic resin with no water or solvent while for TS, as for the one-step self-etching systems, a significant amount of water and solvent is included in the adhesive container and are expected to be removed by air blowing after the adhesive application. Given the harmful effects of water and remaining solvents on the bonding performance as mentioned by several studies⁹⁻¹¹, it could be assumed that one of the reasons for the differences between bonding performance of TS and SE, and also among different groups of each material, was the water and solvent content of the adhesive layer after air-drying. One of the mechanisms by which those ingredients may adversely affect the adhesive performance is by decreasing polymerization efficacy and altering the mechanical properties.¹² In this study, some mechanical properties of the bonding layer in each group were measured to further test the assumptions.

It should be mentioned here that chemical analysis such as measurement of the degree of conversion (DC) is considered to be a reliable technique to determine polymerization efficacy¹³, but it cannot be handled adequately on the bonding layer with a

few micrometers thickness in a bonded complex, such as the bonding layer in the present study. Ultimate tensile strength of cured adhesives has also been used as an indication of mechanical properties of adhesives by some studies and has shown good agreement with the bond strength results.¹⁴ However, the procedure followed to prepare samples for such tests involves neither actual dentin bonding nor air blowing as in real practice.

Microhardness evaluation has been suggested as an alternative to the DC test because of the high correlation found between two methods for a certain resin material.¹⁵

Hardness measurement by nanoindentation has been suggested as advantageous over conventional methods for its high resolution of force and accurate indent positioning. This method has been used to measure the elastic modulus and hardness of the dental adhesives by some previous studies^{14, 16} based on the traditional analyses of penetration data obtained from the unloading curve of the indentations, such as the Oliver and Pharr method¹⁷. However, it has been shown that the complex viscoelastic behavior of polymers and the associated creep, affect the shape of indentation and calculations based on the unloading curve.¹⁸ In the present study, the reported hardness is a relative measure, calculated from displacement data obtained immediately after the maximum load was achieved, rather than the plastic hardness calculated from the unloading curve.¹⁸ In addition, more recent applications of nanoindentation to measuring polymer properties have focused on indentation creep tests and also on dynamic indentation testing. Creep resistance may give an indication of the extent of crosslinking in the network structure of a polymer.^{19, 20} In addition, for a filled resin, creep strain decreases with an increase in the filler content up to an optimum level.²¹

A direct comparison of indenter penetration data between different groups (Fig. 1-3) may be misleading, because local stress applied on the material dynamically changes, depending on the depth of the indenter tip penetration into the material, which was different between groups, in spite of the constant force during the hold segment. In the

present study, a rather simple method described previously²² was employed to calculate strain-rate and stress exponent for nanoindentation creep tests that enabled comparison of indentation creep data obtained for different groups on a plot (Fig. 1-4). It should be noted that the stress exponents were calculated under relatively large local stress amplitudes, higher than 100 MPa, which are likely to be beyond the limits of elastic behavior of the material. In this case, higher stress exponent would mean less damage in the material in response to the stress. In other words, a higher stress exponent in this stress range is associated with higher resistance of the filled resin matrix to the generation of cracks, initiated by submicron flaws of the network structure. Clinically, such data may be of importance in predicting the performance of an adhesive, since the flaws initiate and propagate under mastication forces or composite contraction stress, and could act as critical stress risers promoting interfacial failures that eventually lead to complete failure of the restoration.^{23, 24}

Interestingly, the data scatter and standard deviations of indentations for each test group in the present experiment were small, which can account for the small scale of the test and accurate surface detection of indentations. Moreover, the local microstructure of the resinous matrix and filler distribution could be assumed as homogeneous at the evaluated areas. The results for SE groups indicated that SE2 presented the lowest hardness and the smallest stress exponent of all SE groups. Longer than 2 s air-drying of the primer resulted in significantly higher hardness and larger stress exponents for this material. On the other hand, while there was no statically significant difference between hardness values of SE5 and SE10, a higher stress exponent of SE10 means better resistance to creep strain compared to SE5 and indicates the modest beneficial effects of 10 s long air-drying time of the primer on mechanical properties of the bonding layer. Ten seconds air-drying may have blown away the excess of the unfilled primer liquid from the dentin surface; thus, the bonding layer could be assumed as dominantly composed of bonding agent in this group with a favorable polymerization rate, cross-linking efficiency and optimum distribution of

the filler. The trend for TS appeared to be generally similar to that of SE. The samples of 2 s air-drying showed the lowest hardness and stress exponent and TS10 showed a significantly higher hardness than TS5, suggesting further evaporation of water and solvent in TS10 compared to that of TS5. While, such phenomenon may have been the cause of higher hardness and led to an increase in the filler concentration, beneficial effects of such increase were not observed on the stress exponent. It has been demonstrated by other studies that higher concentration of filler than an optimum level may not further contribute to the properties of the resin^{21, 23, 25, 26}, thus, it is suggested that TS may already have reached an optimal filler concentration by 5 s air-drying.

It has been suggested that the association of hydrophilic monomers with water via hydrogen bonding may preclude its complete removal by desiccation.²⁷ The results of the present study support that hypothesis, suggesting that such bound water may have existed and could not be removed by long air-drying of the TS, helping to explain why TS10 could not achieve mechanical properties and bond strength as high as SE10. While the thickness of the bonding layer has been reported to affect the bonding performance²⁸ and was likely to decrease by longer air-drying of the adhesive in TS group, it was not possible to investigate the effects of bonding layer thickness on the shear bond strength in this study, due to the possible effects of mechanical properties as an interfering variable.

Conclusions

Within the limitations of this study, it was concluded that the tested one-step self-etching adhesive may not perform equally as well as the two-step self-etching material and that proper air-drying of the solvent-containing adhesive material is necessary to achieve best performance; this conclusion is in general agreement with that of a similar recent study [9]. Our null hypotheses were rejected; the two tested adhesives showed different characteristics and the air-drying time of the solvent-containing agent affected their bonding performance and mechanical properties.

Chapter 2

Effects of storage time and temperature on the properties of two self-etching systems

Introduction:

The ultimate goal of a dental bonding system is to achieve a good and durable bond to the dental substrates. In order to be able to achieve this goal in the clinics, it is necessary for the clinicians to be aware of the limitations of these materials.

The shelf-life and stability of dentin bonding systems have been considered in the literature as a potential limitation since the introduction of these systems in the market.²⁹ Chemical deterioration of these mixtures is a reaction that may begin at the moment the material is manufactured and results in reduction of the bonding performance.³⁰

With the creation of later generations of the adhesives with simplified application procedures, i.e. the self-etching primers and the most recent all-in-one adhesives, concerns have risen over the stability of their relatively complicated and so-called “difficult” formulations.^{31, 32}

A number of approaches have been suggested to slow chemical deterioration down and to increase the shelf-life of the bonding systems, including the storage of the material in the refrigerator and the use of chemically stable ingredients for the material.^{30, 33} However, some studies^{31, 34} reported that self-etching primer systems would show deteriorated performance over time, even though the materials were stored according to the manufacturer's suggestions.

The purpose of this study was to evaluate the micro-shear bond strength to enamel and dentin of two adhesive systems; a two-step self-etching primer and a one-step all-in-

one, over a storage period of 60 weeks at three different temperatures. The null hypothesis was that the bonding performance of neither material is affected by the storage time and temperature.

Methods and materials

Materials and storage conditions

Two adhesive systems were evaluated: Clearfil SE Bond (SE) and Clearfil Tri-S Bond (TS). Chemical composition and batch numbers of the materials according to the manufacturer (Kuraray Medical, Tokyo, Japan) are shown in Table 2-1. For each system, three sets of the same batches were used. All packages were kept in the refrigerator until the experiment, which began within 10 days after the materials were received directly from the manufacturer.

Bonding to enamel and dentin was performed immediately after opening each package, following the procedures described below and the results were recorded as the baseline. One set of each material was then stored in the refrigerator at 4 °C (as recommended by the manufacturer), one in an air conditioned laboratory room away from direct sunlight at 23 ± 1 °C and the other in the incubator at 37 °C. For each set, the bonding tests were repeated after 1, 4, 16 and 60 weeks of storage.

Table 2-1 Composition, group code and lot no. of the adhesives tested in this study.

Adhesive	Composition	Lot no.	Code
Clearfil SE Bond	Primer: water, MDP, HEMA, CQ, DET, hydrophilic DMA	00206A	SE
	Bond: MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, DET, silanated colloidal silica	00209A	
Clearfil Tri-S Bond	Water, MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, ethyl alcohol, silanated colloidal silica	040219	TS

Micro-shear bond strength tests

Seventy caries-free human upper premolars were used for the bonding tests in this *in vitro* study. The teeth were washed under running water immediately after extraction and stored in 0.02% thymol solution until the experiment time which was scheduled within 6 months after extraction. At that time, the roots of the teeth were removed and coronal slices, each about 2 mm thick, were prepared by cutting parallel to the longitudinal axis and facial surface using a low speed Isomet saw (Buehler Ltd., IL, USA) under running water as coolant.

The convex enamel surfaces on the outermost buccal or palatal slices were reduced up to 0.5 mm by gently polishing on a 600 grit silicone paper under running water to prepare a flat enamel surface. The outer surface of the underlying slice was also polished to create a standard smear layer on the dentin. Two enamel and two dentin slices were obtained from each tooth and distributed randomly among the corresponding groups. The bonding systems were then applied on the polished surface of each slice following the manufacturer's instructions.

For SE, the self-etching primer agent was applied on the surface using a sponge supplied by the manufacturer and rubbed for 20 s. The primed dentin surface was then dried with oil-free compressed air. After that, the bonding agent was applied using sponge pieces provided by the manufacturer and was air spread until a homogeneous layer was observed on the surface. For TS, the adhesive was applied to the dentin surface for 20 s using a sponge and then was air blown to remove water and solvent from the bonding.

Prior to irradiation of the bonding resin on each specimen, 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 were placed on the treated dentin surfaces. After light irradiation with a halogen light cure unit for 10 s, a hybrid restorative composite (Clearfil APX Shade A3, Kuraray Medical) was carefully inserted into the tubing lumens

and irradiated for 40 s according to the manufacturer's instructions. The specimens were then stored in deionized water at 37 °C and the tygon tubing around composite cylinders were removed after an hour, by gently cutting the tube into two hemicylinders using a feather blade. Special caution was taken to avoid applying any stress to the bonded composite cylinders. Twenty-four hours after bonding, each tooth slice was attached to the testing apparatus (Bencor-Multi-T, Danville Engineering, CA, USA) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, CA, USA) and was tested in a universal testing machine (EZ-test-500N, Shimadzu, Kyoto, Japan). A thin steel wire (0.20 mm D) was looped flush between the load cell projection and the resin cylinder making contact with the lower half-circle of the cylinder and touching the tooth surface. The force was applied at a crosshead speed of 1 mm/min until the failure occurred (Fig. 2-1). Care was taken to keep the composite cylinder inline with the center of the load cell and to keep the wire loop parallel to the load cell movement direction and to the bonded surface in order to maintain stress orientation as shear at the bonding interface. The maximum load at the time of failure was recorded in Kgf which was later converted to bond strength in MPa. A total of 520 bond strength values (10 for each group) were recorded.

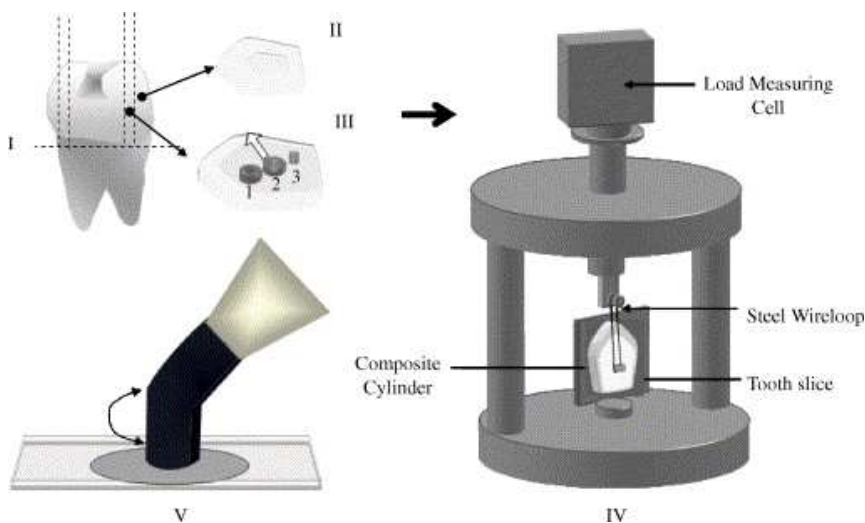


Figure 2-1 Micro-shear bond strength test: (I) the tooth section, (II) enamel slice, (III) dentin slice with (1) tygon tubing, (2) composite inserted into the tubing, (3) after the tubing was removed. (IV) Test apparatus, (V) the bonding resin cured between two glass-slides to form a thin polymer film.

Nano-indentation tests

Hardness values of cured SE bonding resin and TS were determined by the nano-indentation technique. About 0.5 ml (three drops) of SE bonding agent or TS at the baseline or after storage for 60 weeks at 4, 23 or 37 °C was placed on a glass slide. The material was then air blown in order to spread it evenly and in case of TS, to evaporate solvent and water. After that, a micro glass-slide was gently placed over it to create a thin layer with a smooth surface and film thickness of a few microns and to prevent formation of the oxygen inhibition layer. The resulting layer was then light cured for 100 s with light cure probe in close contact with the glass slide and moving all over it (Fig. 2-1). The top micro glass slide was removed after 24 h and the surface hardness of the polymerized bonding resin film was measured in a nano-indentation tester (ENT-1100, Elionix, Tokyo, Japan) under a maximum load of 2 mN reached at a loading rate of 0.1 mN/s. Further details of nano-indentation technique used in the present study are described elsewhere.³⁵ For each group, 12 points were randomly indented on three samples prepared (4 points on each sample).

pH evaluation

At the base line and at the end of 60 weeks storage at different temperatures, pH values of SE primer and TS were determined by a compact digital pH meter (Twin pH, Horiba, Kyoto, Japan). At the beginning of each test schedule, the device was initially calibrated using two standard solutions with pH values of 7.0 or 4.0 (Horiba, Kyoto, Japan), as instructed by the device manufacturer. In order to measure the pH value of each material, a single drop of the material was placed on the meter sensor and the pH was measured and displayed by the device. Due to the resinous nature of the materials tested, after each reading, sensor of the pH meter device was thoroughly rinsed with a solution of ethyl alcohol and deionized water and the device was recalibrated at the room temperature. The test was repeated three times for each group and the average value was calculated and recorded.

Statistical analysis

The bond strength results were statistically analyzed using a two-way analysis of variance (ANOVA) at a significance level of 0.05 with the bond strengths to enamel or dentin as dependent variables and the materials and the storage conditions as factors. One-way ANOVAs with Dunnett's post hoc tests were then used to determine groups with significantly decreased bond strength means compared to the baseline for each material-substrate set. The hardness data were also analyzed by the one-way ANOVA and Dunnett tests. The analyses were performed in the SPSS software (SPSS 13.0 for Windows).

Results

Micro-shear bond strength

Summary charts for bond strength results are presented in Fig 2-2. A time dependent drop off was observed in bond strength of all 37 °C storage groups compared to the groups stored at the other two temperatures.

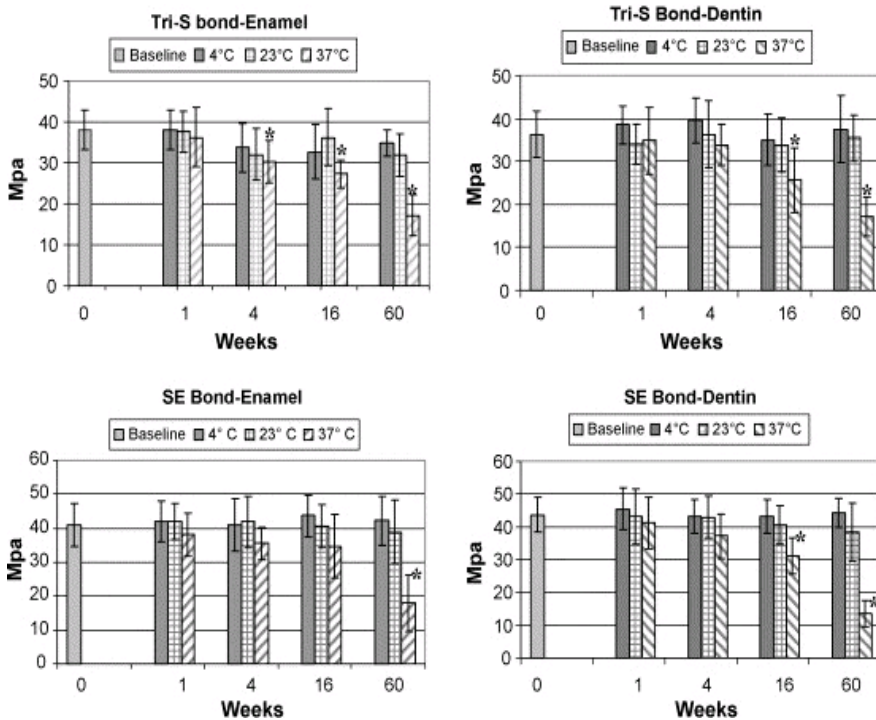


Figure 2-2 Micro-shear bond strength charts. For each chart, groups marked by asterisk showed significantly lower bond strength compared to the baseline. A rapid drop off is apparent in the bond strength for all 37 °C groups, compared to the other two temperatures in each chart.

Table 2-2 Micro-shear bond strength mean values (S.D.) in MPa

Time	Tri S Bond						SE Bond					
	Enamel			Dentin			Enamel			Dentin		
	baseline: 38.0 (4.8)			baseline: 36.3 (5.5)			baseline: 41.0 (6.4)			baseline: 43.7 (5.4)		
	4 °C	23 °C	37 °C	4 °C	23 °C	37 °C	4 °C	23 °C	37 °C	4 °C	23 °C	37 °C
1 week	38.1 (4.8)	37.6 (5.1)	36.2 (7.3)	38.6 (4.4)	34.1 (4.7)	34.9 (7.1)	42.0 (6.0)	42.0 (5.2)	38.1 (6.5)	45.5 (6.3)	43.2 (8.5)	41.2 (8.0)
4 weeks	33.8 (5.9)	32.0 (6.3)	30.4 (5.1) ^a	39.5 (5.1)	36.3 (7.9)	33.9 (4.8)	40.9 (7.9)	41.9 (7.7)	35.5 (4.6)	43.1 (5.1)	43.0 (6.5)	37.2 (6.8)
16 weeks	32.7 (6.7)	36.2 (7.0)	27.3 (3.4) ^a	35.0 (6.0)	33.9 (6.4)	25.7 (7.6) ^b	43.7 (6.2)	40.6 (6.4)	34.7 (9.2)	43.2 (5.2)	40.6 (5.8)	31.1 (5.5) ^d
60 weeks	34.8 (3.3)	31.9 (5.2)	17.2 (4.9) ^a	37.6 (7.7)	35.5 (5.3)	17.3 (4.6) ^b	42.3 (7.3)	38.9 (9.3)	17.9 (8.2) ^c	44.3 (4.4)	38.4 (8.7)	13.5 (4.1) ^d
Overall	35.5 (5.5) ^E	34.9 (6.0)	27.8 (8.7)	37.4 (5.8) ^E	35.0 (6.0)	28.0 (9.5)	42.0 (6.6) ^F	40.9 (7.1)	31.2 (10)	44.0 (5.1) ^F	41.3 (7.4)	30.8 (12)

For each material-substrate set, groups indicated by a letter are significantly lower than the baseline ($n = 10$, $p < 0.05$, Dunnett's t-test). In the overall row, the italic values with different letters are significantly different ($n = 50$, $p < 0.05$, Tukey HSD test).

The two-way ANOVA showed that the bond strength results of both enamel and dentin were significantly influenced by the storage conditions ($F = 18.196$, $p < 0.0001$ for enamel and $F = 26.746$, $p < 0.0001$ for dentin) and by the type of adhesive system ($F = 48.927$, $p < 0.0001$ for enamel and $F = 47.759$, $p < 0.0001$ for dentin). The interactions of these two factors were not significant ($F = 1$, $p = 0.449$ for enamel and $F = 1.41$, $p = 0.162$ for dentin), indicating that the differences that existed between effects of various storage conditions (time and temperature) were not dependent on the type of adhesive systems. One-way ANOVAs used to evaluate the storage conditions effects on each material-substrate set, revealed that statistically significant differences existed between the bond strength results at the baseline and that at other time points for both of the substrates. Post hoc tests showed while no significant differences for either material existed in the groups of storage at 4 or 23 °C, mean bond strengths of SE stored at 37 °C were significantly lower to enamel at 60 weeks and to dentin at 16 and 60 weeks than those at the baseline ($p < 0.05$). For TS stored at 37 °C, bond strength showed significant decrease to enamel from 4 weeks on and to dentin from 16 weeks on, compared to those at the baseline ($p < 0.05$). The detailed results of bond strength tests for all groups are presented in Table 2-2.

Nano-indentation hardness

The results are summarized in Table 2-3. One-way ANOVA revealed no significant differences between the hardness means of SE groups. However, a significant difference was found between TS groups. Dunnett post hoc test disclosed that the adhesive stored at 37 °C showed a significantly lower hardness than that of the baseline.

pH measurements

At the baseline, pH values for SE primer and TS were 1.93 and 2.7, respectively. After 60 weeks of storage, a remarkable drop in pH was observed for both materials stored at 37 °C, as shown in Table 2-4.

Table 2-3 Nano-hardness (S.D.) in MPa of cured resins at the baseline and after 60 weeks of storage at different temperatures

Material	Baseline	4 °C	23 °C	37 °C
SE bonding resin	282.9 (26.1)	277.5 (23.7)	276.5 (15.9)	275.1 (23.6)
Tri-S Bond	214.2(25.6)	213.0 (26.5)	200.4 (14.9)	78.5 (10.8) ^a

N = 10 In each row, groups indicated by letter (a) significantly lower than the baseline (*p* < 0.05, Dunnett's *t*-test).

Table 2-4 pH (S.D.) of self-etching materials at the baseline and after 60 weeks of storage at different temperatures

Material	Baseline	4 °C	23 °C	37 °C
SE primer	1.93 (0.06)	1.93 (0.06)	1.87 (0.06)	1.63 (0.06) ^a
Tri-S Bond	2.7 (0.00)	2.7 (0.00)	2.67 (0.06)	2.57 (0.06) ^a

N = 3 ^aThe pH reduction is remarkable for the materials stored at 37 °C.

Discussion

This study measured the bond strength of adhesives to enamel and to dentin in a relatively large number of groups. The micro-shear bond strength test was chosen because of its advantages over other common bond strength evaluation methods with regard to its ease of sample preparation and suitability for the enamel bonding tests.³⁶ The micro-shear bond strength test in this study was successfully performed for all specimens.

Mode of fracture for the micro-shear bond tests were not reported in this study since there has been some controversy on the reliability of fracture mode data of shear bond test as an indicator of the bonding performance, considering the stress distribution at the bonding interface in this test.³⁷

Similar regions located on the on the axial surfaces of enamel and dentin slices were bonded for all specimens by a single operator at all times to limit interfering effects of those factors on the bond strength, as suggested by other studies.³⁸⁻⁴⁰

The three storage environments in this study were selected among the available laboratory environments with fixed temperatures and according to previous similar studies.^{34, 41} Bond strength values of the materials were evaluated over a period of 60 weeks (14 months). This period is longer than the 1-year storage period selected by similar studies,^{31, 34} which is half of the 2-year shelf-life recommended by the manufacturer.

Previous studies have reported different stability characteristics for different acidic monomers developed by various manufacturers,^{31, 41} thus such selection of materials as in the present study, where they were both produced by the same manufacturer and had similar compositions, made possible to focus better on the possible effects of the differences between the two approaches (two step and all-in-one self-etching) on the performance stability.

Chemical reactions have been attributed responsible for the deterioration of materials over time and are naturally accelerated by heat.^{31, 32, 41} This principally explains the remarkable reduction in bond strength of both materials after several weeks of storage at 37 °C that further continued to 60 weeks, while the materials stored at 4 and 23 °C showed stable bond strength. Moreover, it seems that a temperature beyond the room temperature is the tolerance limit of the materials' performance to chemical reactions. In order to ponder the results, it is necessary to look into the formulations of the materials.

According to the documentations included in the materials packages (Table 2-1), the primer agent of the SE system (SE primer) includes a hydrophilic acidic monomer MDP as the functional monomer, HEMA, DMA and photo initiator CQ, while the bond agent contains filler and bis-GMA in addition to primer ingredients except water. The all-in-one system TS contains all the components named above mixed in a single bottle (Table 2-1). Acidic monomers are the key ingredients of the self-etching primer systems and have various functions in addition to participating in the polymer network, all of which contribute to the final bonding performance to dentin and enamel substrates.^{32, 33}

The etching effects of both systems used in the present study are due to the acidic attribute of the water-MDP solution. MDP is also responsible for priming the dentin by wetting the dental collagen network via infiltration into the dentin. Moreover, MDP has been reported to chemically interact with the HAp (hydroxyapatite) and form low soluble calcium salts.⁴²

The hydrolytic stability of acidic monomers has been questioned by researchers.^{32-34, 41, 43} It was found for acidic methacrylate phosphate MEP (methacryloyloxyethyl dihydrogen phosphate) that both the methacrylate and phosphate ester bonds were hydrolyzed and methacrylic acid (MAA), phosphoric acid or other derivatives were formed.⁴⁴ Although the phosphate ester bond in MDP was found to be significantly more stable than MEP that has a shorter alkylene spacer,⁴⁵ Salz et al. observed a significant

reduction in the concentration of the MDP in an aqueous solution after 16 weeks of storage at 37 °C.⁴¹

It has also been reported for a single-bottle self-etching primer containing water, ethanol and an acidic monomer commonly used in the current self-etching systems (4-MET or 4-methacryloyloxyethyl trimellitic acid) that in addition to the hydrolysis of the methacryloyl ester portion of the acidic monomer, loss of function occurs via esterification of the carboxyl group of the acidic monomer.⁴³ However, esterification of the phosphate group of MDP by ethanol has not been reported by any study to date.

On the other hand some researchers have reported a considerable hydrolytic stability for MDP. In the study by Salz et al., MDP resisted degradation longer than 4-MET, under high temperature and strong acidic conditions (12–16 weeks versus 0–4 weeks).⁴¹ Also, it was concluded in another study that the outstanding hydrolytic stability of the MDP and its interaction with dentin contributed to the long-term durability of the dentin–adhesive interface.⁴²

According to the literature, shelf instability problems of bonding systems are not limited to the acidic monomers; HEMA is also a hydrophilic monomer prone to degradation. HEMA acts as a wetting agent that helps monomers to diffuse into the dentin⁴⁶ and stabilise the collagen fibril network.⁴⁷ It also helps homogeneity of the mixture in the liquid form, particularly in the all-in-one adhesives.⁴⁸ Previous studies have demonstrated a time, temperature and pH dependent decrease in the HEMA content in acidic solutions, due to hydrolysis of the molecules to methacrylic acid (MAA) and ethylene glycol.^{32, 49, 50}

DMA and bis-GMA are dimethacrylates that act as cross-linking monomers and help increase the polymerization rate and improve the mechanical properties of the network. It was reported that under acidic conditions, these dimethacrylates may undergo

similar hydrolysis and degrade under formation of corresponding diols and methacrylic acid.^{41, 45}

In the present study, pH evaluation and nano-indentation hardness measurements at 60 weeks were employed as confirmatory tests to support the supposition that compositional deterioration had occurred in the materials.

It should be noted that hardness of TS cured on the glass surface may be different from that in the clinical situation, because the mineral components from the smear layer may neutralize the acidity of the self-etching systems and influence the polymerization efficacy.⁵¹ However, the method used in this study provided the possibility to measure and relatively compare the hardness of resin polymers³⁵ that were prepared using a standard method for the sensitive nano-indentation technique. Thus, some common problems that occur due to the polishing or desiccation of the bonded dentin samples and may limit the validity of indentations⁵² were avoided.

According to the nano-indentation tests, hardness of TS bottle stored for 60 weeks at 37 °C showed a significant decrease compared to that at the baseline. This reduction in mechanical properties can be explained by the fact that all monomers in an all-in-one system are blended with water. The hydrolytic degradation of methacrylate monomers at higher temperature would affect the hardness of their polymer. In addition to the hydrolytic degradation, it has also been suggested by other studies that in an aqueous environment, an acid–base reaction may take place between the acidic monomer and the amine of photo initiator system,⁴⁵ that would hamper the polymerization process of the resin.

In contrast to TS, such hydrolytic degradation seems improbable for SE bonding resin because it contains no water. This was confirmed where the nano-indentation tests revealed that the storage temperature of SE bonding resin did not affect hardness of its thin polymer film. However, similar to TS, the hydrolytic degradation could have occurred in

SE primer since the acidic monomers and photo-initiator are present in aqueous environment. Although the photo-initiator system in SE bonding resin acts independently from that of primer liquid, a lesser photo-polymerization efficacy would be expected for the final primer–bond mixture if the primer lacks the photo-initiators^{53, 54} or if hydrolytic products such as non-polymerizable acids or diols are present.

The pH drop for both SE primer and TS at 37 °C indicated an increase in the acidity of these materials. It should be mentioned here that an acid–base reaction with amine of photo initiator system in these materials is expected to move the pH higher⁵¹ especially in the early stages of the degradation, however, the remarkable pH drop after the long period of 60 weeks storage at 37 °C indicates a significant increase of the H⁺ ion concentration. This finding is in general agreement with the speculations made above, founded on the hydrolytic interactions that lead to the formation of acids such as phosphoric acid.

In addition, after 60 weeks of storage at 37 °C, a significant phase separation and sedimentation in the SE prime was noted by the operator. This phenomenon may be a result of the formation of polymer particles due to premature polymerization of some monomers, for instance MAA, which is a product of hydrolytic degradation. Although the hydrophilic HEMA is not easily polymerized in the presence of water,⁵⁴ it has been reported that the increase of the temperature significantly raises the polymerization rate of MAA in an aqueous environment.⁵⁵

The premature polymerization of the monomers and deposition of the resulting particles would dilute the concentration of the monomers in the solution. Therefore, the water ratio in the aqueous phase would increase. It has been reported that an increase in water concentration results in further ionization of the acidic monomers that are remaining in the solution, and thereby lowering the pH in the self-etching primer.⁸ This further

justifies the pH change of 0.3 (from 1.93 to 1.63) in the SE primer which means a doubling of the H^+ ion concentration in this solution.

Some researchers have questioned a relationship between the acidity and enamel bonding of the self-etching primers.^{56, 57} In the present study the increased acidity of the SE primer stored at 37 °C for 60 weeks could have resulted in a deeper etching on the enamel, however it seems not to have counterbalanced the compositional deterioration. It is speculated that the deterioration products e.g. the polymer particles in the SE primer not only would be unable to interact chemically with the HAp, improve the polymer network or wet the demineralized dentin, but also would contaminate the bonding surface. Such contamination would prevent an optimal penetration of the bonding resin between enamel crystallites and thus prevent formation of the nanometer sized resin tags that has been suggested⁵⁸ to contribute to high resin–enamel bond strength. Likewise, the contamination would interrupt penetration of the bonding resin into the dentin collagen network and formation of a nonporous hybrid layer.

It has also been suggested for self-etching systems that non-polymerisable hydrolytic products such as phosphoric acid may continue to etch the underlying dentin after formation of the hybrid layer.^{51, 59}

When the confirmatory findings are considered beside the bond strength results, it becomes clear that in a two-step self-etching system, the properties of the bonding resin alone are not adequate to reach an optimal bonding performance if the self-etching primer has undergone chemical changes.

The drawback seemed more challenging for TS which is an all-in-one adhesive system applied in a single-step not followed by a separate bonding agent. It was reported for a single-bottle self-etching adhesive that the degradation of functional monomers after

a long storage period resulted in an increased etching capacity that was not accompanied by resin infiltration or effective polymerization.³¹

However, although separate one-way ANOVAs indicated significant reductions in bond strength earlier for TS than that for SE, the overall two-way ANOVA found no significant interactions between the materials and the storage conditions. This indicated that none of the two systems can be considered as superior over the other one regarding the bond strength stability, during a storage period of 60 weeks at different temperatures.

Moreover, while serious concerns have been expressed over the stability of the single-bottle self-etching adhesives even in optimal storage conditions,^{31, 45} results of the present study confirmed the bond strength performance stability of TS during 60 weeks storage at room temperature or in the refrigerator. These may be attributed to the relative hydrolytic stability of MDP as discussed earlier and to the less aggressiveness and higher pH of TS (2.7), due to the less water content.⁸ The hydrolysis of the ester portion of monomers has been found to be strongly dependent on the acid concentration in an aqueous solution and a pH higher than 2 delayed the hydrolysis of HEMA.⁴ It should also be pointed that in contrast to SE primer, TS did not exhibit a significant phase separation after the 60 weeks storage period at 37 °C.

The changes responsible for the deteriorations are not necessarily identical between the two systems in the present study. Chemo-analytical studies are required to investigate in detail the changes occurred for each material during the storage period. In addition, further research is required to investigate the ultrastructure and durability of the bonds obtained by the aged materials.

Nevertheless, when the bond strength results obtained for each material-substrate at the baseline and those over 60 weeks of storage in the refrigerator (according to the manufacturer's instructions) were pooled, bond strength means to both enamel and dentin

for SE were found to be significantly higher than those for TS (Table 2-2). This finding was in agreement with several other studies that emphasized on performance advantages of the two-step self-etching systems over the all-in-ones.^{48, 60}

In conclusion, the storage time and temperature had significant effects on the performance of both materials; therefore the null hypothesis should be rejected. Effective deterioration was likely to have occurred over time only in the water containing agents and at a temperature beyond the room temperature.

In routine dental practices, adhesives are consumed at a very fast rate.³¹ However, to assure an optimum performance, it is advisable that the clinicians store these materials in the refrigerator, as the manufacturer instructs, particularly in the warmer areas or seasons. Moreover, the dealers need also pay attention to the storage conditions of the materials during long periods of shipping or stocking. After all, development and incorporation of more stable ingredients are desired to formulate dental adhesives in the future.

Chapter 3

The viscoelastic behavior of dental adhesives: A nanoindentation study

Introduction

With the recent developments in adhesive dentistry, evaluating the properties of adhesive materials has received an increasing attention.¹⁴ In order to predict the long term success of an adhesive restoration, it is necessary to critically evaluate the stress and deformation of the components in the bonded complex including the dental substrate, the restorative materials and the interfaces¹⁶, as well as interfacial sealing and biological aspects of the degradation process.⁶¹

Several techniques have been introduced to evaluate the mechanical performance of adhesive resins. The time-dependent viscoelastic response was shown to be an important feature of both natural and many synthetic biomaterials.⁶² While the viscoelastic behavior of restorative composite resins has been investigated and emphasized by several studies⁶²⁻⁶⁵; few studies have measured this behavior of adhesive resins; perhaps because the available conventional methods developed for the bulk materials can not be applied on bonding resins, which should be characterized in the actual in-use thin form.³⁵

Nanoindentation allows the investigation of selected material properties on small amounts of materials, based on the load-displacement data of indentations on a submicron scale. This method was originally developed for measurements of properties such as hardness and Young's modulus for materials that exhibited elastic-plastic behavior.¹⁷ A considerable progress has been recently made on the measurement of viscoelastic properties such as creep compliance and Young's relaxation modulus of thin film polymers using nanoindentation.^{66, 67}

The aim of this study was to characterize the in-plane linear viscoelastic properties of dental adhesive layers using a new technique developed for nanoindentation.

Theory

The equations used in this study for extracting the linear viscoelastic properties from nanoindentation data in the time domain are presented here. The Berkovich indenter attached to the nanoindentation device is considered as a rigid conical indenter. Sneddon⁶⁸ derived the relationship between load and displacement for a rigid conical indenter indenting into an elastic material as,

$$\text{Eq. (1)} \quad h^2 = \frac{\pi(1-\nu) \tan \alpha}{4G} P$$

where P is load, h displacement, α the angle between the cone indenter and the substrate surface, ν the Poisson's ratio, and G the shear modulus. When the material is of linear viscoelastic characteristics, the contact area between the indenter and the material has a time-dependent behavior. That means the boundary between the indenter and the half-space is moving. For this time-varying boundary problem a hereditary integral operator was proposed to determine the time-dependent stresses and deformations⁶⁹. Applying this technique to Eq. (1) leads to the following equation for time-dependent indentation depth in a linear viscoelastic material under a prescribed arbitrary indentation loading history of $P(t)$.

$$\text{Eq. (2)} \quad h^2(t) = \frac{\pi(1-\nu) \tan \alpha}{4} \int_0^t J(t-\xi) \left[\frac{dP(\xi)}{d\xi} \right] d\xi$$

where $J(t)$ is the creep compliance in shear at time t .

On the other hand the creep compliance of a linear viscoelastic material as expressed by the generalized Kelvin model is:

$$\text{Eq. (3)} \quad J(t) = J_0 + \sum_{i=1}^N J_i \left(1 - e^{-\frac{t}{\tau_i}}\right)$$

where J_0 - J_i are compliance numbers, and τ_i retardation time.

Under a ramp loading at a constant loading rate of v_0 , $P(t) = v_0 t$. Substituting Eq. (3) into (2) leads to

$$\text{Eq. (4)} \quad h^2(t) = \frac{1}{4} \pi (1 - \nu) \tan \alpha \left[\left(J_0 + \sum_{i=1}^N J_i \right) P(t) - \sum_{i=1}^N J_i (v_0 \tau_i) \left(1 - e^{-\frac{P(t)}{v_0 \tau_i}} \right) \right]$$

In case Eq. (4) is fitted to the load-displacement curve obtained from nanoindentation, all parameters, J_0 , J_i ($i = 1, \dots, N$) and τ_i can be obtained. The parameters can then be used to determine the creep compliance relation as in Eq. (3). Moreover, the obtained creep compliance $J(t)$ can be used to determine other viscoelastic functions, such as the uniaxial relaxation modulus $E(t)$, which can be determined through the following relation.

$$\text{Eq. (5)} \quad \int_0^t E(\tau) J(t - \tau) d\tau = 2(1 + \nu)t$$

where the Poisson's ratio ν is constant ($\nu=0.3$ in this study).

Materials and methods

Sample preparation

Bonding resins of four adhesive systems were evaluated in the current study: Clearfil SE Bond (CSE), Clearfil Tri-S Bond (CTS) (by Kuraray Medical, Tokyo, Japan), Single Bond (SIB) and Single Bond Plus (SBP) (by 3M ESPE, St. Paul, MN, USA). The composition of each bonding resin is shown in Table 3-1.

A drop of each adhesive resin was placed on a micro-glass slide and then air-blown for 5 s to spread on the glass and remove water or solvents in case where the adhesives contained water or solvents. Another micro-glass slice was placed on the top and pressed against the bottom slide to reach a film thickness. The resin was halogen light cured for 10 s. After the specimens were left in the room temperature ($23\pm 1^{\circ}\text{C}$) for 24 hours, the micro-glass slice on top was removed.

Table 3-1 Compositions of the adhesives tested in this study

Material	Composition	Code
Clearfil SE Bond	Bond: MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, DET, silanated colloidal silica	CSE
Clearfil Tri-S Bond	Water, MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, ethyl alcohol, silanated colloidal silica	CTS
Single Bond	Bis-GMA, DMA, HEMA, Water, Ethanol, PAA	SIB
Single Bond Plus	Bis-GMA, DMA, HEMA, Water, Ethanol, PAA, silane treated silica filler	SBP

Nanoindentation

The resulting thin film of bonding resin polymer was then tested in a nanoindenter system (ENT 1100, Elionix, Tokyo, Japan) with a Berkovich indenter at constant loading rate of 0.1 mN/s up to a maximum load of 1.8 mN. The angle between indenter tip surface and the specimen surface was $\alpha=25^\circ$. The temperature of the testing chamber was held constant at 27.5°C and the specimens were isolated inside the chamber for half an hour before the indentation to reach thermal balance and avoid effects of variable temperature.

During the loading segment 20 load-displacement data points were recorded per second. 10 indentations were programmed and performed on each sample out of which one typical indentation data set, representative of the average value of these indentations, was chosen for the calculations.

Data analysis

The load-displacement curve obtained from the selected indentation was then fitted to determine the best fit parameters of compliance numbers (J_0, J_1, \dots, J_N) and retardation times (τ_1, \dots, τ_N) in Eq. (4) using the ordinary least squares fitting technique (correlation coefficient $R > 0.9999$).

The creep function $J(t)$ determined for each material was then converted to $E(t)$ based on Eq. (5). A numerical approach was carried out to solve the problem. The value observed at $t=18$ s was considered as the Young's modulus of material. The values obtained for Young's modulus using the approach described above were finally compared to the means of values obtained from the conventional output from the default software of the computer attached to the nanoindentation device, using Kolmogorov Smirnov test. Hardness values were obtained from the data at maximum load as previously described.³⁵

Results

The creep compliance formulas as obtained for each material are presented here. For all materials, favorable parameter values for Eq. (4) were found so that a good fit could be established for the experimental load-displacement data ($R > 0.9999$).

$$\text{CSE} \quad J(t) = 0.04(1 - e^{-10t}) + 0.331(1 - e^{-t}) + 0.29(1 - e^{-0.1t})$$

$$\text{CTS} \quad J(t) = 0.14 + 0.795(1 - e^{-0.25t}) + 0.173(1 - e^{-0.025t})$$

$$\text{SIB} \quad J(t) = 0.393(1 - e^{-t}) + 0.485(1 - e^{-0.22t}) + 0.187(1 - e^{-0.047t}) + 0.046(1 - e^{-0.01t})$$

$$\text{SBP} \quad J(t) = 0.032 + 0.377(1 - e^{-t}) + 0.228(1 - e^{-0.1t}) + 0.0264(1 - e^{-0.01t})$$

The load-displacement curve from the experiment and the fitted model are displayed in Fig. 3-1 and the curves corresponding to the $J(t)$ equations are shown in Fig. 3-2.

SIB and CTS showed higher creep compliance compared to CSE and SBP. The Young's modulus values obtained from viscoelastic model and outputs of the default nanoindentation software together with mean hardness values are listed in Table 3-2. SIB and CTS showed lower Young's modulus values compared to CSE and SBP. Kolmogorov Smirnov test on the difference between the observed distributions for the output of the two methods indicated that there was a significant difference between the two. ($p < 0.05$)

Table 3-2 - Hardness and Young's modulus values calculated by the methods discussed in the study.

Group	Modulus based on the visco elastic model (GPa)	Mean modulus by the device (GPa)	Mean hardness at maximum load (MPa)
CSE	4.0	8.51	275
CTS	2.55	5.47	174
SIB	2.42	6.24	162
SBP	4.15	6.89	280

Kolmogorov Smirnov test indicated that there was a significant output difference between the two methods for calculation of Young's modulus. ($p < 0.05$)

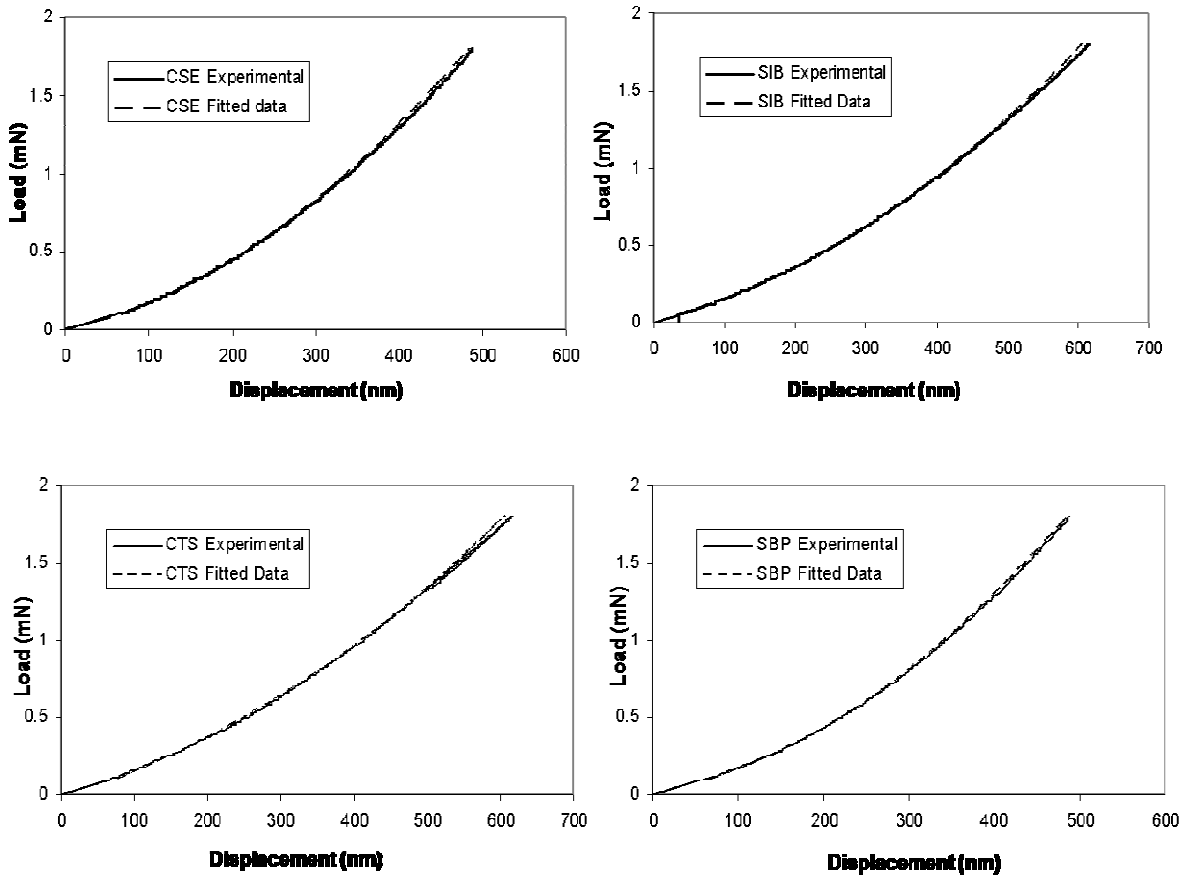


Figure 3-1 Load versus displacement data for the loading segment in a typical indentation for each material. The continuous lines represent raw data from the nanoindentation experiment and the dashed lines show result of curve fitting. Good fitting was achieved for all materials in the selected loading range. ($R > 0.9999$)

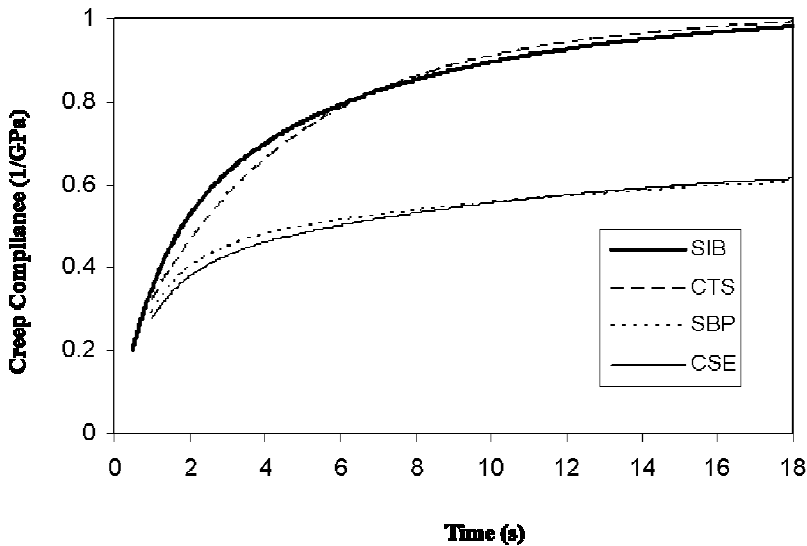


Figure 3-2 Creep compliance curves for each material based on the $J(t)$ relations given in the results. CTS and SIB show higher compliance compared to CSE and SBP. The behaviors of paired materials appear to be very similar.

Discussion

Measurement of mechanical properties by nanoindentation has been suggested as advantageous over the conventional methods for its high resolution of force and accurate indent positioning.^{14, 16, 17} This method has been used to measure the elastic modulus and hardness of the dental adhesives by some researchers^{14, 16, 52}, using traditional analyses of penetration data obtained from the unloading curve of the indentations by the default software of the device, such as the Oliver and Pharr method.¹⁷ These methods are generally based on Sneddon's solution for the relationship between the load and displacement for an axisymmetric indenter indenting into a half-space composed of a linear elastic, isotropic and homogeneous material.⁶⁸

On the other hand, time-dependence is the rule rather than the exception for polymers, even at low temperatures (especially near the glass transition temperature). Well-known viscoelastic models like the generalized Maxwell or Kelvin models, or more sophisticated functions of Kohlrausch-Williams-Watts (KWW) or Wiechert have been shown to successfully describe the behavior of these materials in wide time scales.^{62, 70}

The modulus from the default software of the nanoindenter is determined from unloading portion of the nanoindentation load-displacement curve and the contact area measured at the maximum nanoindentation load. While the method has been very effective and robust for elastic-plastic materials (without time-dependency), measurements on viscoelastic materials using this method have experienced problems.¹¹ The method tends to significantly overestimate the Young's modulus for a viscoelastic material such as polymer. The major reason is that during unloading, the displacement does not follow closely with the decreased load (as in the case for an elastic-plastic materials), due to prior increasing of the applied nanoindentation load and the memory effect of the time-dependent material. As a result, even though the load decreases during initial unloading, the displacement does not decrease at the same pace as the force, and sometimes could even increase during this

initial stage, causing some high unloading slope or even a negative slope, as shown in fig. 3-3, leading to the output of higher modulus than the actual value.^{67, 71}

To take advantage of nanoindentation technique while avoiding the complications associated with the unloading curve, researchers have followed different approaches. A new method was employed in a previous study to evaluate the creep and stress exponent of the adhesives, holding the load constant for a period of time prior to unloading. It was speculated that the results were useful to predict the flaw resistance of the filled adhesive resin matrix.³⁵ Also, by modeling the instrument as a damped harmonic oscillator, it was possible to calculate the values of stiffness and modulus throughout the loading curve.⁶⁶

The generalized Kelvin model used in this study fitted well to the experimental load-displacement data for all materials within the selected loading history of the nanoindentation experiment, indicating the suitability of the linear viscoelastic model for the data in this loading range. The Young's relaxation modulus curves in the current study were calculated by conversion of the creep data to Eq. (5). Even though the method is prone to errors particularly due to the scattering of nanoindentation data at early times, it was suggested that a very good agreement existed between the Young's modulus values obtained using this method and those of the conventional mechanical tests on bulk materials.⁶⁷

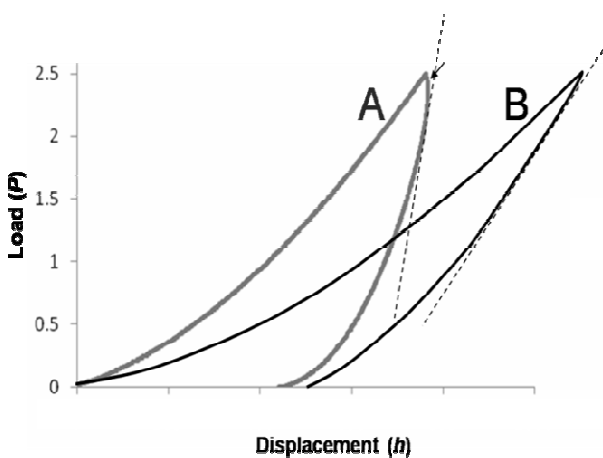


Figure 3-3 Nanoindentation load displacement curves; (A) for SIB a viscoelastic material, and (B) for an elastic-plastic material. Black arrow shows a negative unloading slope at the very beginning of the unloading segment. Dashed lines are the slope of the upper portion of unloading curve or Contact Stiffness ($S=dP/dh$), from which the nanoindentation elastic modulus and plastic hardness are derived (Oliver and Pharr). The time-dependent behavior of (A) has led to an increased contact stiffness slope, which will eventually lead to overestimation of the properties.

In the current study, the modulus values obtained using the viscoelastic model were all lower than those of the default nanoindentation output, and there was a significant difference between the two techniques. The previous nanoindentation studies have reported different values, up to four times higher than the value reported in the current study for CSE and SIB.^{14, 72, 73} Values obtained for the Young's modulus using the conventional nanoindentation techniques, vary significantly depending on the loading rate⁶⁷ and the hold segment before unloading¹⁶; and this should explain the great variance of the previous results for the same material.

The materials in the current experiment were deposited on a glass-slide, in order to minimize the potential interfering factors in this experiment, such as dehydration of dental tissues and rough surface for the nano-scale test after polishing.^{52, 74} It was reported that for a soft film deposited on a hard substrate, the effect of substrate on the nanoindentation measurement could be neglected as long as the final depth of indentation was less than 20% of the film thickness.^{75, 76} In this study, the film thickness of adhesive resins polymerized on a glass slide was around 10 μ m, and the depth of indentations was always less than 700nm; thus, the effect of substrate can be disregarded. Moreover, it has been suggested that the size, composition and distribution of filler particles in the resin exerted variation in the nanoindentation results⁷⁷; results of the pilot investigations and previous studies^{35, 74} suggested that the fillers in adhesive materials used were of size and distribution attributes that had negligible effect on the data scatter among indentation points located within the matrix.

Selection of the four different materials was made in a manner to probe the effects of compositional differences between each two adhesives produced by the same manufacturer. CSE has been known as an established two-step self-etching primer bonding system.^{35, 61, 74} While the primer agent of this material incorporates water and solvent, the bonding agent contains no such ingredients. On the other hand, CTS is an all-in-one

bonding system with a composition similar to that of a hydrophilic combination of primer and bonding agents of Clearfil SE bond. A comparison of the results obtained for the two materials imply that CTS bond has lower modulus of elasticity, lower hardness and higher creep compliance, than those of CSE.

In a polymer matrix, the chemical links established by cross-linking between molecular chains⁷⁸ and monomer conversion⁶³ increase the resistance to plastic flow and creep. It has been demonstrated that residual solvent in adhesive resins can significantly affect the degree of conversion.¹³ It should be noted that, with regard to the sensitivity of the experimental nanoindentation technique and in order to obtain a homogenous film, only the bonding agent of CSE was polymerized instead of a mixture of the two agents. It has been demonstrated that clinically, high mechanical properties of CSE can be achieved when the primer is air blown well and deprived of non-polymerizable components before the bonding agent is applied.³⁵ Moreover, in a clinical setup, the mineral components of dental substrate may neutralize the acidity of the self-etching systems and influence the polymerization efficacy⁵¹, thus the properties of CTS in this experiment is likely to differ from those in clinical situations.

The proximity of the results obtained for CSE and SBP is probably due to their compositional analogy, indicating that from a mechanical point of view they may be similar. Moreover, SIB and SBP are materials with similar compositions, other than that SBP contain a 10% weight fraction of surface treated 5nm diameter filler particles according to the manufacturer. A comparison of the results for these materials confirms that filler addition has significantly contributed to the mechanical properties of SBP, increasing hardness, Young's modulus and creep resistance compared to SIB. The addition of nanofillers resulted in an improvement in creep resistance of structure, which was attributed to the dense network formed by the filler particles with small volume

fraction and surface treatment that effectively restricted the mobility of matrix polymer chains.⁷⁹

It has been suggested that a sufficiently flexible resin layer could resist the polymerization shrinkage stress of the restorative composite, thus a low modulus adhesive resin may be preferred. However, this would be the case probably only when the resin is sufficiently cured and the matrix has no structural defects. A low Young's modulus value might meanwhile be the result of prematurely polymerized and weak structure that has a poor resistance against stress.

Plasticization of methacrylate-based resins is an unfavorable phenomenon often linked to their polarity and porosity, leading to the change of the mechanical properties of the polymers.⁸⁰ Such method as the nanoindentation technique presented in the current study should be useful to describe the long-term changes occurring in the actual bonding layer exposed to moisture, in mechanistic terms suitable for polymers.

The common laboratory bond-strength tests, where the applied force increases to high levels in a short-term until fracture ensues, describe the failure behavior of materials in terms of linear fracture mechanics. Under such loading, the mobility of the crosslinked polymer chains is so limited that the molecular rearrangement in the macroscopic form of the material yield is very restricted. However, the terms may not apply when smaller loads are extended or cyclically repeated over time and relaxation or creep phenomena occur.⁶² Time-dependent effect may continue until the polymer borders the cohesive zone, where a small load is needed to initiate a crack. Studies on the effect of cyclic loading on the adhesives confirm that while a bonding agent may display high bond-strength in the laboratory at the base-line, the cyclic loading would result in a significant reduction of bond-strength^{81, 82}; sometimes with limited or no micro-morphologically detectable defects.⁸³ It has also been suggested that the static creep can be relevant clinically; and thus a useful method for the investigation of the behavior of dental restorative materials.^{62, 63}

Further research is necessary to investigate different aspects of the observed viscoelastic behavior of dental adhesives.

Conclusions

This work discussed a nanoindentation technique for evaluation of mechanical properties of dental adhesives. A good fitting was achieved between the viscoelastic model and the experimental nanoindentation raw data in this study. Adhesive materials exhibit time-dependent creep and relaxation. The attributes depend on the composition of materials and are likely to affect laboratory results and clinical outcome and thus, should not be ignored.

Chapter 4

Composition and bonding durability of self-etching adhesive systems

Introduction

The majority of current adhesive systems may achieve high bond-strength values shortly after polymerization; however, the longevity of bonds is still an area of interest in the adhesive dentistry. As reported by several researchers, a significant decrease of the bond-strength after long-term may reduce the success rate and lead to failure of restorations, thus it is important to investigate the durability of bonding systems.^{84, 85}

The addition of antibacterial and fluoride releasing agents to the chemical composition of adhesives has been recently considered beneficial for durability of the bonds.⁸⁶ Therefore, the purpose of this chapter was to evaluate the micro-shear bond strength to enamel and dentin of three self-etching adhesive systems; a two-step, an anti-bacterial and fluoride-releasing two-step and an all-in-one, over a storage period of one-year after bonding. The null hypothesis was that the bond strength of materials did not change during the storage period.

Materials and methods

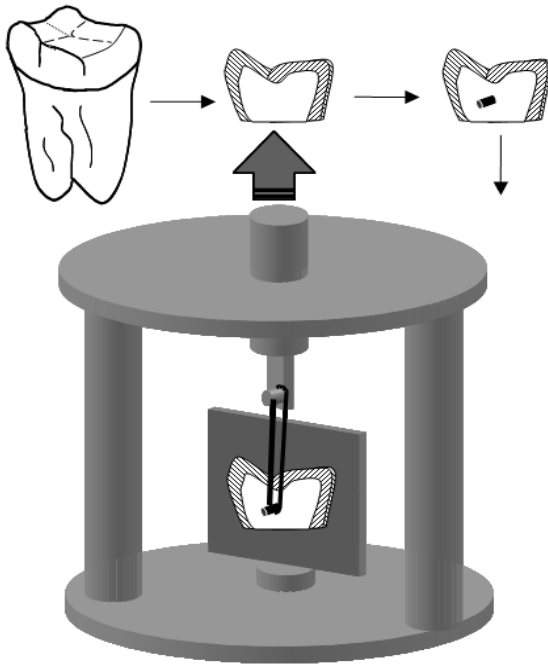
Three self-etching materials were evaluated: two-step adhesives Clearfil SE Bond (SE) and Clearfil protect Bond (PB), and all-in-one adhesive Clearfil Tri S Bond (TS). Composition of each adhesive material according to the manufacturer (Kuraray Medical, Tokyo, Japan) is shown in Table 4-1.

Coronal slices, around 2 mm in thickness, were obtained from human third molar teeth by cutting axially, using a low speed diamond saw (Buehler, Lake Bluff, IL, USA) with water as a lubricant.

Table 4-1 Composition of materials used for the study presented in this chapter.

Material	Composition	Code
Clearfil SE Bond	<i>Primer:</i> water, MDP, HEMA, CQ, DET, hydrophilic DMA <i>Bond:</i> MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, DET, silanated colloidal silica	SE
Clearfil Protect Bond	<i>Primer:</i> water, MDP, MDPB, HEMA, DET, hydrophilic DMA <i>Bond:</i> MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, DET, silanated colloidal silica, Surface treated NaF	PB
Clearfil Tri-S Bond	Water, MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, ethyl alcohol, silanated colloidal silica	TS

In order to prepare flat enamel surfaces, the convex surfaces were reduced by gently polishing on a 600 grit silicone paper under running water. The outer surface of the underlying slice was also polished to create a standard smear layer on the dentin. The enamel and dentin slices were distributed randomly among three groups and treated according to manufacturer's instructions. Prior to irradiation of the bonding, tygon tubes were placed on the enamel and dentin surfaces and photo-irradiated for 10 seconds. The lumens were then carefully filled with a hybrid restorative, Clearfil APX, shade A3



(Kuraray Medical) which was then light cured for 40 seconds. The specimens in each group were randomly divided into 3 subgroups and stored in deionized water at 37°C until the bond-strength tests, scheduled 24 hours (baseline), 6 months and 1 year post-bonding, according to the micro-shear test method described in chapters 1 and 2. The results were then statistically compared within and between material groups using two-way ANOVA and one-way ANOVAs with Tukey and Dunnett post-hoc tests.

SEM micrographs of the resin-dentin interface of SE and PB at the base line and after one year were also obtained; the dentin slices were treated with the corresponding adhesives and a composite build-up around 1mm thick was made on the bonded surface. The samples were cut in a perpendicular direction to the bonding interface after 24 hours. One sample was observed immediately after epoxy resin embedding, dehydration in a glass desiccator, argon ion etching and gold-sputter coating, and another was stored for one year with the bond-strength samples and then observed. The micrographs were obtained at the magnification of x2000, under a scanning electron microscope (SEM, JEOL, Tokyo, Japan), on which the morphological attributes of the interface and changes occurred during the storage period of each adhesive material were studied.

Results

Means and standard deviations of shear bond strength to enamel or dentin for each adhesive at different points of time are summarized in Table 4-2 and graphically displayed in Fig. 4-1. Two-way ANOVA analysis indicated that material type had significant effects on bond strength to both enamel and dentin ($F=3.788$, $p<0.05$ and $F=3.723$, $p<0.05$ respectively). The storage period did not exert a significant effect on the bond-strength to any of the substrates ($F=0.487$, $p=0.616$ and $F=0.135$, $p=0.847$ respectively). A significant interaction between the two factors of material type and storage period suggested that the effect of storage time on the bond strength to dentin depended on the material type ($F=4.356$, $p<0.005$). Meanwhile, the interaction was not significant for enamel ($F=1.697$, $p=0.159$). The comparisons between materials using one-way ANOVA analysis with Tukey post-hoc at the baseline (24h) showed that SE had a significantly higher bond-strength to dentin compared to the other two materials. After one year of storage, the test revealed that PB had significantly higher bond strength to both enamel and dentin only compared to TS. The SEM micrographs obtained for SE and PB interface with dentin at the base-line and after one-year are presented in Fig. 4-3.

Table 2-2 Micro-shear bond strength results in MPa (Mean±SD)

Experiment Time		SE Bond	Protect Bond	Tri S Bond
Dentin	Baseline(24h)	45.2±10.4 ^a	34.5±6.0 ^b	36.0 ±6.1 ^b
	6 Months	43.2±11.2 ^c	38.3±7.0 ^c	35.2±5.9 ^c
	One year	33.9±11.6 ^d	46.6±12.5 ^{e*}	32.5±10.7 ^d
Enamel	Baseline(24h)	39.5 ±5.3 ^f	35.8±6.9 ^f	34.6 ±5.6 ^f
	6 Months	35.9±7.1 ^g	38.8±6.0 ^g	35.2±9.2 ^g
	One year	34.5±8.4 ^{h,i}	40.6±8.9 ^h	29.9±7.7 ⁱ

For each experiment time in rows subgroups indicated by similar letters are not significantly different (p>0.05, Tukey HSD test). For each material-substrate set in columns, subgroup indicated by asterisk is significantly different from other groups (p<0.05, 2-sided Dunnett test).

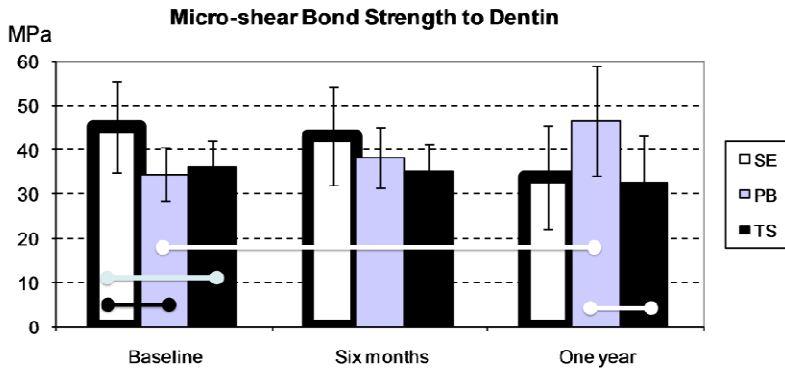
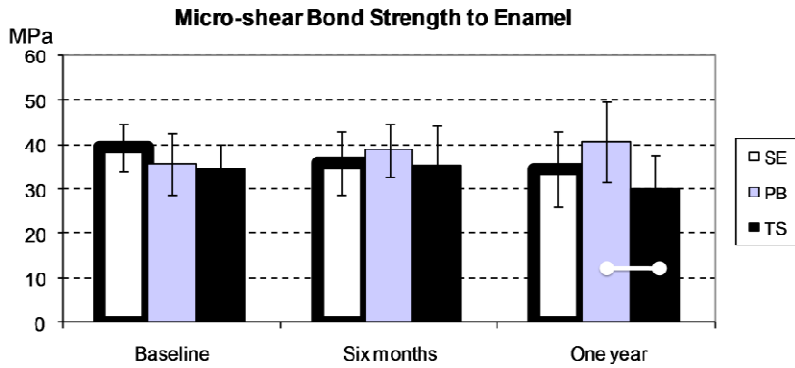


Figure 4-2 Micro-shear bond strength to dentin. Connecting bars indicate significant differences. (One-way ANOVA post-hocs, p<0.05)

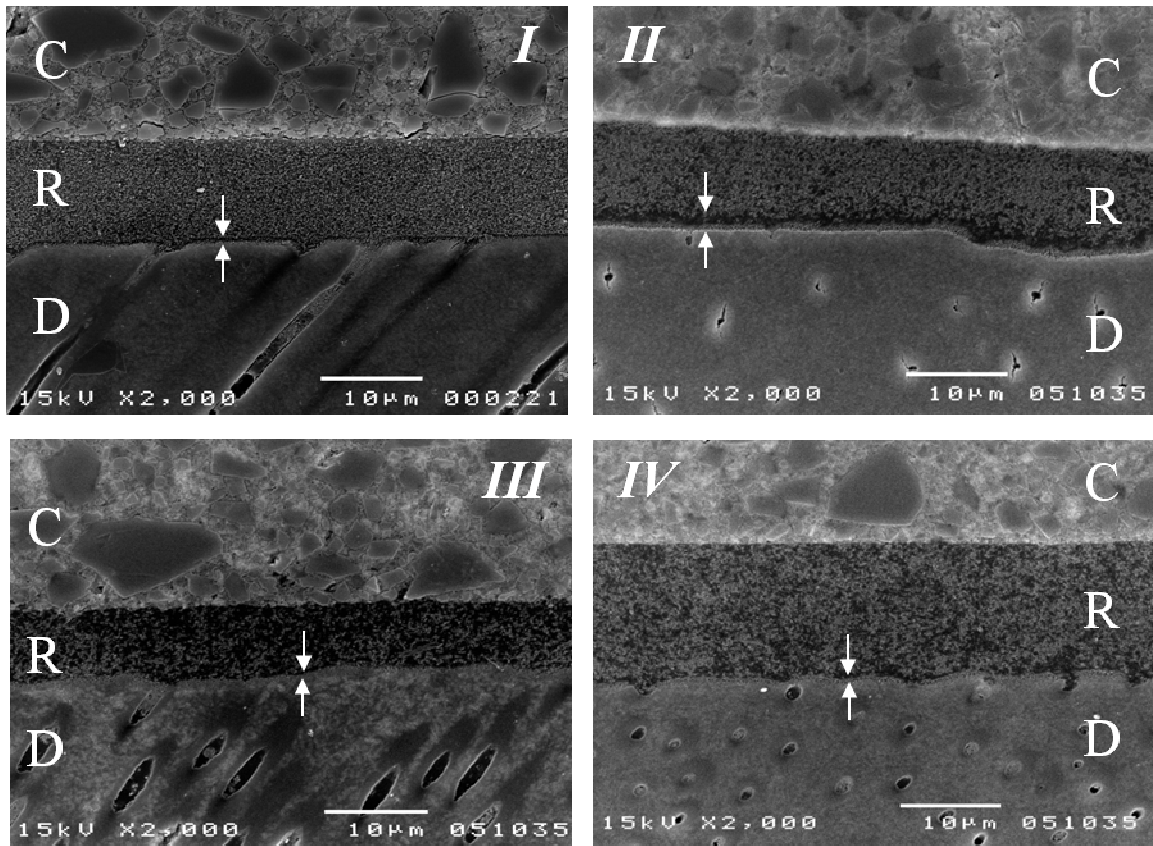


Figure 4-3 SEM micrographs of resin-dentin interface. *I*:SE baseline, *II*:SE one-year, *III*:PB baseline and *IV*:PB one-year. C: Composite, R: Adhesive resin and D: Dentin. Arrows indicate the very interface of adhesive and dentin known as hybrid layer. The thickness and morphological attributes of hybrid layer seems to have changed in SE after one year of storage and the layer seems darker and thicker. PB shows little change over one year.

Discussion

The selection of materials as in the present study, where they were all produced by the same manufacturer and had similar basic compositions, made it possible to focus better on the possible effects of minor differences that existed between the materials. The all-in-one system TS contains all the components of SE system mixed in a single bottle. PB is only different with SE in that the primer incorporates antibacterial monomer MDPB, but lacks photo-initiator; and that sodium fluoride has been added to the bond.

Judging from the results obtained after 24 hours of storage, SE showed higher bond-strength to dentin compared to PB and TS. This was in line with other studies that suggested higher dentin bond strength for SE than that of TS in short-term; and attributed it to the lower mechanical properties of TS due to a lower polymerization efficacy, as a result of incomplete removal of water and solvents.³⁵

Moreover it was suggested that lack of photo-initiator in primer of PB, led to the lower polymerization efficacy of the bonding layer⁵³ and possibly to a lower bond-strength compared to SE at just 24 hours after bonding.

Meanwhile, the bond-strength to enamel at the baseline did not show any differences between materials. This might be due to the effects of bonding substrate, not reflecting the potential differences among adhesives. The bonding mechanism to enamel seems less problematic than that to dentin, the inherent strength of enamel may be the weak link and the brittle nature of the enamel could limit the nominal strength values obtained for the resin-enamel bonds.⁴⁰

Results of the current study showed a slight but not statistically significant decrease in bond strength for SE and TS to enamel during the storage period. While Wang *et al*⁸⁷ had reported a significant drop for bond-strength obtained for SE after a year of storage in sodium chloride, others implied that SE^{88,89} and TS⁸⁹ showed durable bonding to enamel.

During the one-year storage, SE showed stable bond to dentin in the present study. Studies conducted on the effects of long-term direct water exposure on bond strength of SE to dentin reported some drop in the bond strength to dentin; but concluded that SE still performed reliably after one-year direct water exposure.⁹⁰

Susceptibility of resin components to hydrolysis has been identified as a cause for decrease of bond strength. It has been suggested that the outstanding hydrolytic stability of the MDP and its interaction with the enamel and dentin contributed to the long-term

durability of dentin and enamel adhesive interfaces of SE⁴² and TS.⁸⁹ MDP has a special molecular structure that enables chemical reaction with the residual Hydroxyapatite after etching and formation of low-soluble salts.

The materials used in this study are classified as mild self-etching systems with pH values around 2.0 and higher. (2.0, 2.0 and 2.7 for SE, PB and TS respectively), long-term clinical evaluation of SE has suggested that more aggressive etching was not essential for the overall clinical performance of the restorations.⁹¹ In addition to maintaining higher mineral substrate for the chemical interactions, mild acid-etching of dentin bears the advantage of sealing the bonding interface via occlusion of the dentinal tubules and partially blocking the attack by water attack which leads to degradation and failure of the bond.⁷⁹ (Fig. 4-4) Apparently adhesive materials that aggressively etch and deprive the Hydroxyapatite coating of dentin collagen network but lack an effective chemical reaction or adequate hybridization with dentin, undergo a significant loss of bond strength to dentin after storage for a long-time due to the hydrolysis of collagen. Unlike other bonding systems that have a separate acid-etching step, for two-step self-etching materials etching and penetration of the primer monomers occur simultaneously. Some researchers have even further valued TS as an all-in-one material applied in a single step for the simultaneous penetration of all monomers and complete impregnation of the collagen which prevents hydrolysis of collagen.⁹²

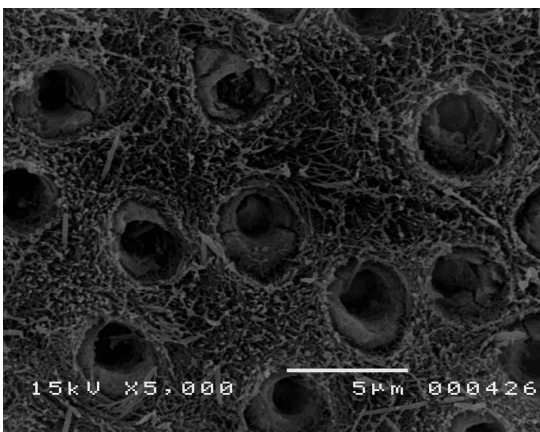


Figure 4-4 Dentin surface after 20 s etching by Clearfil SE Bond primer agent, a mild self-etching system. The dentin (arrows) is partially demineralized and collagen bundles exposed. The tubule orifices are widened however remain occluded by remnants of smear plug. The surface is prepared for application of the bond, which will eventually penetrate to the primed collagen network. MDP will possibly react with the remaining Hydroxyapatite crystals attached to the collagen fibers. (Sadr *et al.*)⁹³

The formation of a homogenous and void-free interfacial zone improves the quality of hybrid layer and contributes to the long-term seal of the dentin bond. Studies on long-term nanoleakage have reported superior leakage resistance for both SE⁹⁴ and TS.⁹⁵ Similar durability results obtained for SE and TS suggested that in spite of the differences that existed between the all-in-one and two-step self etching adhesives used in the current study, their performance may be comparable in the long-run.

In the present study, the only statistically significant change of bond-strength over time was observed for PB to dentin after one year. This is rather an unusual result for a bonding system with good initial bond-strength, where previous studies have mainly reported at best only slight increase of bond strength over long-term storage.⁹⁰

Nakajima et al reported that PB performed superior to SE in that it exhibited no change in bond-strength to dentin over six months of water storage.⁹⁶ The finding was later supported by a TEM investigation on nanoleakage over 6 months of water storage.⁹⁴ SEM findings of the current study strongly supports those findings, where in contrast to PB the interface formed by SE had apparently undergone changes after one year of storage.

It has been suggested that loss of minerals from enamel and dentin during *in-vitro* water storage would lead to deterioration of mechanical properties of teeth.⁹⁷ Such deterioration of the substrate overtime may affect the bond strength; however, fluoride release by adhesive materials might be effective in protection against mineral loss from enamel and dentin surfaces.⁹⁸

It was also reported that fluoride release from PB resulted in increased resistance of sound dentin to acid challenge⁸⁶ and remineralization of the demineralized dentin⁹⁹, compared to other materials that did not release fluoride. It should also be pointed that another study has challenged the remineralizing effect of PB on dentin, but suggested that PB may release sufficient fluoride to inhibit any salivary or dentin matrix-bound esterase

activity that could have been responsible for the decreased bond strength seen in the fluoride-free adhesives. One of the mechanisms suggested for the degradation of bond is the enzyme-catalyzed hydrolysis of the methacrylate-derivatives used in dental adhesives.¹⁰⁰ Nevertheless, the unchanging appearance of the hybrid layer for PB supported either of the assumptions regarding the positive effects of fluoride.

It is noteworthy that significant increase of bond strength to dentin was not observed earlier than a year after bonding; indicating that beneficial effects of additional chemical interactions from fluoride may not be significant except in a long period of time.

The MDPB containing primer has been shown to be promising for inactivating residual bacteria in *in-vitro* and *in-vivo* studies.¹⁰¹ Even though antibacterial effect may seem of little relevance to the current study conducted on caries free teeth *in-vitro*, it is notable that in contrast to the soluble antibacterial agents in some other dentin bonding systems, MDPB incorporates C=C bonds and can be polymerized; thus, it would be immobilized within the polymer structure and would not induce weakness or degradation in the bonding layer through dissolution and substitution by water. PB exhibited significantly lower permeability compared to TS and other adhesives under stimulated pulpal pressure.¹⁰² It was also thought that the fluoride could contribute to the water-repellency of the adhesive layer.

Unlike dentin, for enamel the increase of PB bond strength was not statistically significant after one-year of storage and there was no interaction between factors of material type and storage time. As mentioned for the base-line results, this may be due to the characteristics of enamel, limiting the values of bond-strength. Indeed, confirmation of the effects induced to dental substrates by PB requires further research.

The null hypotheses of the study were rejected. The ranks of materials with regard to the bond strength changed along time, indicating that results of short term bonding test

may not always reflect advantages of the use of different adhesives in long term. The choice of material had significant effect on bond-strength in long-term, particularly for dentin. Cumulating the advantages of two-step self-etching adhesive system incorporating acidic-monomer and filler particles and those of an anti-bacterial monomer and fluoride release, PB was the only material not showing any nominal decrease over time. The use of this material is recommended for adhesive restorations, particularly for high caries risk patients where the recurrent or secondary caries are concerned.

Conclusion

Within the limitations of this in-vitro study, it can be concluded that all tested adhesives showed good performance after one year of storage. The combination of an antibacterial self-etching primer incorporating MDP with a fluoride releasing bonding agent may contribute to durability of bond strength.

Chapter 5

General Conclusions

A number of factors influencing the bond-strength and other attributes of adhesive materials were identified and discussed in the presented studies.

Chapter 1 concluded that air-blowing step of the solvent containing agents of self-etching adhesives affects the outcome of treatment. Moreover, one-step adhesive may not perform equally as well as the two-step self-etching material. It is clinically advisable to perform longer air-drying to make sure the bonding layer is deprived from the non-polymerizable content.

Chapter 2 came to the conclusion that the conditions of self-etching adhesives storage are important factors in performance of the bond, particularly when water is incorporated into the materials with components prone to hydrolysis. It is clinically recommended that the practitioner studies the brochures that come with adhesive materials well and keep them refrigerated, according to the manufacturer recommendation. Any noticeable change in the color, consistency or odor of adhesives could indicate deterioration.

Chapter 3 concluded that the filler addition to adhesive materials was effective in reinforcing the bonding layer, however not all the filler containing adhesives may act favorably under the stresses during the clinical service periods. Compositional factors greatly influence the performance of materials.

Chapter 4 showed that fluoride and anti-bacterial effect of a self-etching adhesive contributed significantly to the adhesion over one-year after bonding. In line with the concept of minimal intervention, the use of such material was recommended for adhesive

restorations that save demineralized non-infected dentin, particularly and for high caries risk patients where the recurrent or secondary caries are concerned.

The studies in chapters 1 to 3 employed nanoindentation techniques for the investigation of material properties. It was shown that the technique had great utility in comparison between materials when a correct approach was selected based on the intrinsic behavior of material. The studies in chapters 1, 2 and 4 included micro-shear bond-strength test. It was also suggested that simple bond-strength tests, although informative and non-substitutable, are not the comprehensive methods to address and compare all the effective attributes of adhesive materials. Development of more sophisticated and clinically relevant laboratory techniques for standardized evaluation of adhesive materials will be a crucial progress in this regard.

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Appendix A

Chemical Abbreviations

Abbreviation	Complete name
4-MET	4-methacryloyloxyethyl trimellitic acid
bis-GMA	bis-phenol A diglycidylmethacrylate
CQ	<i>dl</i> -camphorquinone
DET	<i>N,N</i> -diethanol <i>p</i> -toluidine
DMA	Dimethacrylate
HEMA	2-hydroxyethyl methacrylate
MAA	methacrylic acid
MEP	methacryloyloxyethyl dihydrogen phosphate
MDP	10-methacryloyloxydecyl dihydrogen phosphate
MDPB	12-methacryloyloxydodecylpyridinium bromide
NaF	sodium fluoride
PAA	polyalkenoic acid copolymer