Clinical factors influencing dentin bonding

Miwako Ogata

Promotor: Professor Junji Tagami

Cariology and Operative Dentistry
Department of Restorative Sciences
Graduate School, Tokyo Medical and Dental University

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Preface

This thesis is based on the following original papers, which are referred to the text by the respective chapters.

Chapter 2. Ogata M, Nakajima M, Sano H, Tagami J. Effect of dentin primer application on regional bond strength to cervical wedge-shaped cavity walls. *Operative Dentistry* 24: 81-88, 1999.

Chapter 3. Ogata M, Okuda M, Nakajima M, Pereira PNR, Sano H, Tagami J. Influence of the direction of tubules on bond strength to dentin. *Operative Dentistry* 26: 27-35 , 2001.

Chapter 4. Ogata M, Harada N, Yamaguchi S, Nakajima M, Pereira PNR, Tagami J. Effects of different burs on dentin bond strengths of self-etching primer bonding systems. *Operative Dentistry* 26: 375-382, 2001.

Chapter 5. Ogata M, Harada N, Yamaguchi S, Nakajima M, Tagami J. Effect of self-etching primer vs. phosphoric acid etchant on bonding to bur prepared dentin. *Operative Dentistry* 27: 447-454, 2002

CONTENTS

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Introduction

Adhesive restorations have been widely accepted for both anterior and posterior use in restorative dentistry. Patients' demands for esthetic restorations have caused a recent increase in the use of tooth colored restorative materials. To achieve clinical success with such restorations, good adhesion between restorative materials and tooth substrates is of crucial importance in order to ensure good marginal sealing, reinforcement of the tooth structure, and longer life of the restoration. During the last two decades, a variety of adhesive systems have been continuously developed in order to produce good adhesion to dental substrates. These great advances in the adhesive dentistry have changed the concepts of cavity preparation based on the principals proposed by GV Black (1955) into more conservative and minimally invasive ones, removal of the carious lesion only preserving the intact tooth structure (Fusayama, 1980; Tyas et.al., 2000).

Bonding to enamel was achieved earlier and easier (Buonocore, 1955) because enamel is mostly composed of hydroxyapatite crystals. Although it is possible to obtain predictable and reliable adhesion to enamel, adhesion to dentin, which is the largest part of the tooth, has provided to be more challenging because of heterogeneous nature. Dentin contains approximately 70 % hydroxyapatite, 20 % organic material (mainly type I collagen), and 10 % water by weight (Ten Cate, 1998), and is a highly permeable tissue with numerous dentinal tubules. The mechanism of dentin adhesion, enhanced by hybrid layer formation between the resin and dentin, was proposed by Nakabayashi (1982). The adequate hybrid layer formation is believed to be essential to create a strong and durable bond between resin and dentin.

High bond strengths of recent adhesive systems to dentin have been continuously reported in in vitro studies, all of which were done in the ideal laboratory condition. However, when using adhesives to dentin, the variation of bond strengths is dependent not only on the materials, but also on enormous clinical factors such as dentin depth (Suzuki and Finger, 1988; Tagami et.al., 1990), calcium concentration (Perinka et.al., 1992), age (Tagami et.al., 1993; Burrow et.al., 1994), surface wetness (Prati and Pashley, 1992), relative humidity (Sato et.al, 1991; Nikaido et.al., 1991; Burrow et.al., 1995), saliva and blood contamination (Pashley et.al, 1988; Fritz et.al, 1998; Abdalla and Davidson, 1998; Hebling and Feigal, 2000; Hiraishi et.al., 2003), caries-affected dentin (Nakajima et.al., 1995, 1999a, b, 2000a), sclerotic cervical erosion (Duke and Lindemuth ,1990, 1991; Harnirattisai et.al., 1992; Van Meerbeek et.al., 1992, 1994; Yoshiyama et.al., 1996), or polymerization stress related to cavity configuration (Feilzer et.al., 1987; Yoshikawa et.al., 1999). In order to obtain a successful adhesive restoration, focus on such clinical factors, and control the clinical situations is very important. In this study, several factors that may affect bonding to dentin, which have not been previously investigated, are discussed in the following chapters as outlined below :

In chapter 2, the effect of multiple application methods of self-etching primers on

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regional bond strength to wedge shaped cavity was evaluated and discussed. This primer application technique was designed to compensate the flow-off of the serous primer solution from the cavity walls.

In chapter 3, the influence of the direction of tubules on bond strength to dentin was evaluated and discussed. The dentinal tubules direction on the cavity wall depends on its location, and this may become one of the factors that influence the non-uniformity of bonding to a cavity.

In chapter 4, the effect of the preparation with different types of burs on dentin bond strengths of self-etching primer bonding systems was evaluated and discussed.

In chapter 5, the effect of dentin conditioners on tensile bond strength to dentin prepared in the same manner to chapter four using a self-etching primer system and a phosphoric acid etching system was evaluated and discussed. Through chapters 4 to 5, the effect of bur cutting on dentin bond strength of various adhesive systems were discussed.

And finally in chapter 6, clinical rules to follow for good adhesive performance of self-etching primer bonding systems are reviewed through several studies that evaluated clinical factors on bonding properties of these systems.

Chapter 1

Review of literature

Adhesion to enamel and dentin

After the mechanical tooth preparation with dental rotary instruments, an amorphous layer of organic and inorganic debris, the so-called smear layer is created over the tooth surface (Pashley, 1984). This layer covers the tooth surface, adheres weakly to the underlying enamel and dentin, and cannot be removed by ordinary water spray. Since the introduction of phosphoric acid etching of enamel surface by Buonocore (1955), acid etching has been widely used to treat enamel and produced good adhesion to enamel. Enamel is a highly mineralized tissue, consisting of 96 % mineral and 4 % organic material and water by weight. The inorganic content of enamel is composed of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] that are arranged in a rod-like structure (Ten Cate, 1998). Etching the enamel surface with acid cleans the enamel, removes enamel smear layer, and increases the surface energy and porosity of exposed surfaces through selective dissolution of crystals, which provides a micro-mechanical interlocking between the enamel and resin (Gwinnett and Matsui, 1967).

On the other hand, bonding to dentin has been far more challenging because of the difference in structure of dentin as compared to enamel. Fusayama (1979) advocated the total etching of enamel and dentin with phosphoric acid to remove all smear layer that impedes the direct contact of the bonding material with the dentin (Pashley, 1984; Nakabayashi and Pashley, 1998). Although the bond strength to etched dentin was higher than to non-etched dentin, it was shown to be much lower than to etched enamel (Fusayama et.al., 1979). Dentin contains approximately 70 % inorganic material (mainly hydroxyapatite), 20 % organic material (mainly type I collagen), and 10% water by weight (Ten Cate, 1998). Dentin is a complex hydrated biological composite structure with a highly oriented microstructure dominated by numerous dentinal tubules that extend radially from the pulp throughout the entire thickness of the dentin (Garberoglio and Brännström, 1976; Pashley, 1989; Marshall 1993; Lindén et.al., 1995). Dentin located between the dentinal tubules is called intertubular dentin, which consists of a tightly interwoven network of type I collagen fibrils, in which apatite crystals are deposited. The fibrils are randomly arranged in a plane at roughly right angles to the dentinal tubules, and the apatite crystals (averaging 100 nm in length) are generally oriented with their long axes paralleling the fibril (Ten Cate, 1998). To date, the penetration of resin monomers into demineralized dentin and their polimerization in situ (i.e., hybridization, or hybrid layer formation) have been shown to be essential for good adhesion to dentin (Nakabayashi et.al., 1982). Acid etching of dentin is necessary to remove the smear layer which interferes with the permeation of monomer, and to increase the porosity of intertubular dentin necessary

for monomer infiltration. However, when the etched dentin is excessively air-dried after rinsing off the etchant, the collagen network will collapse and the micro-channels opened by the removal of the apatite crystals will be closed (Pashley et.al., 1993). In order to prevent the collapse of the demineralized collagen network and to create a hybrid layer, the proper application of a primer solution is effective (Sugizaki, 1991; Van Meerbeek et al., 1993). It has been reported that three-step adhesive systems, which consist of acid etching, priming, and bonding procedures, are effective for bonding to dentin (Van Meerbeek et al., 1996).

Current adhesive systems

Contemporary dentin adhesive systems are classified into three-step, two-step, and one-step systems, depending on how the three cardinal steps of etching, priming, and bonding to tooth substrates are accomplished or simplified (Inoue et.al., 2000; Tay and Pashley, 2002). In addition, according to different etching methods, dentin adhesive systems can be classified into four categories as described in Table 1. Although the three-step adhesive systems are reported to be effective for bonding to dentin (Van Meerbeek et al., 1996), the clinical procedures were relatively complicated requiring many steps, leading to technique sensitivity (Sano et.al.,1998). In response to the demand for simplification of adhesive procedures, many techniques have been developed to reduce the application steps and their technique sensitivity.

Currently, two types of two-step adhesive systems have been introduced, and have become popular among clinicians (Haller, 2000). One is the self-priming bonding system, which includes a step of etching enamel and dentin with phosphoric acid, followed by a second step with a priming adhesive solution that combines the primer and the adhesive resin into one liquid (one-bottle adhesive). In order to prevent the shrinking of demineralized collagen fibrils by air-drying, wet-bonding technique should be employed for optimum bonding to dentin (Gwinnett and Kanca, 1992a; Kanca, 1992a, 1992b, 1996). Although the wet bonding technique is an effective and excellent idea, it is technique-sensitive in the clinical situation because it is difficult to produce a uniform wet state on all prepared surfaces, especially in a large, complex shaped cavity restoration. Over-drying or over-wetting of dentin can have undesirable effects for this type of system (Tay et.al., 1996).

The other type of two-step system is the self-etching primer bonding system,

Table 1. Classification of the contemporary dentin adhesive systems

How to etch the cavity	Etching	Priming	Bonding	Step	
Phosphoric acid	Phosphoric acid	Primer	Adhesive	3	Conventional three step system
Phosphoric acid	Phosphoric acid	Priming-adhesive		2	Wet bonding system
Self-etching primer	Self-etching primer		Adhesive	2	Self-etching primer system
Self-etching adhesive	Self-etching adhesive			1	All-in-one

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which was characterized by a self-etching primer followed by an adhesive resin. Self-etching primers combine the etching and priming steps into one procedure. The self-etching primers do not etch as well as a 35% phosphoric acid etchant because of their relatively mild pH (1.5 to 3.0 for self-etching primers, information from the manufacturer ; -0.42 to 0.02 for phosphoric acid etchants, Perdigão et.al., 1996). The self-etching primers are applied to smear layer-covered dentin, followed by brief air-drying and application of the bonding resin. The manufacturers' instructions specify that the primed surface should not be rinsed with water. Therefore, the self-etching primers' acidic component demineralize through the smear layer and diffuse a short distance into the underlying dentin, resulting in the creation of a thin hybrid layer but strong bonds to dentin (Watanabe et.al., 1994b; Chigira et.al., 1994). It has been reported that adhesive systems using self-etching primer produce good adhesion to both cut enamel and dentin (Barkmeier et.al., 1995; Kanemura et.al., 1999; Harada et.al., 2000).

The most recently introduced category of adhesive system is the one-step system. These adhesive systems further combined the three cardinal procedures of etching, priming, and bonding into a single application (all-in-one system) (Tay et.al., 2001). Therefore, the bonding resin solution demineralizes the tooth surface through the smear layer, modify or dissolve the smear layer and diffuse into the underlying enamel and dentin (Inoue et.al., 2000). In general, dentin bond strengths of all-in-one systems are reported to be relatively lower than to the self-etching primer bonding systems (Nakaoki et.al., 2001; Ogata et.al., 2003b).

Clinical factors influencing dentin bonding

In vitro studies showing high bond strengths of current adhesive systems to dentin are continuously reported in the literature (Fujitani et.al., 1992; Gwinnett and Kanca, 1992a; Barkmeier et.al., 1995; Kanemura et.al., 1999; Harada et.al., 2000; Ogata et.al., 2001a, b, 2002). However, most studies were done under ideal condition on sound, flat, polished, freshly cut normal dentin. In constant, sound normal dentin is not the substrate most frequently encountered in clinical situations raising an important question about reliability of these systems on different substrates of clinical scenario. When using adhesives on dentin, the variation of bond strengths is dependent not only the materials, but also on various clinical factors. In order to obtain a successful adhesive restoration, we need to focus on each clinical factor, and attempt to control the clinical situation.

1) Variables of dentin

The most clinically relevant bonding substrates are caries-affected dentin and sclerotic cervical dentin. As a result of an aging process and in a response to mild irritations like cervical abrasion or chemical erosion, there is the continued deposition of intratubular dentin within dentin, resulting in a gradual reduction of the tubule diameter. This continued deposition often leads to complete closure of the tubule that is called

sclerotic dentin (Duke and Lindemuth, 1990, 1991; Ten Cate, 1998). These sclerotic dentin surfaces are reported to be less permeable than the young sound dentin (Tagami et.al., 1992), and difficult to be conditioned by the adhesive systems and promote creation of a thin hybrid layer or relatively lower bond strengths (Duke and Lindemuth, 1990; Harniratisai et.al., 1993; Van Meerbeek, 1992, 1994; Yoshiyama et.al., 1996).

The caries lesion develops by cyclic repetition of the demineralization and remineralization phases in dentin, and the caries-affected dentin is partially demineralized and softer than normal dentin (Fusayama et.al., 1966). The caries-affected dentin has a Knoop hardness that is only half of normal dentin (Ogawa et.al., 1983; Nakajima et.al., 1995, 1999a, 1999b), indicating that it has lost a good deal of its mineral phase, even though the lumina of many of the dentinal tubules are occluded with mineral crystals (Shimizu et.al., 1981). The mineral deposits that occlude the tubules reduce the permeability of the caries-affected dentin (Tagami et.al., 1992). It is reported that the thickness of the hybrid layers created in caries-affected dentin are twice as thick compared in normal dentin (Nakajima et.al., 1995, 1999a, 1999b). However, resin bond strengths to caries-affected dentin and normal dentin depend on the type of adhesive system (Nakajima et.al., 1995, 1999a, 1999b, 2000a).

The regional difference of dentin also affects dentin bonding. Superficial dentin has few dentinal tubules and is composed largely of intertubular dentin (Garberoglio and Brännström, 1976). Deep dentin near the pulp, especially after acid-etching, is mainly composed of funnel-shaped dentinal tubules with much less intertubular dentin. Previous studies have shown that variation in dentin depth and permeability can significantly influence the bond strength of direct restorative systems (Tagami et.al., 1990; Prati et.al., 1991, 1992; Davidson et.al., 1993; Nikaido et.al., 1995). Pereira et.al., (1999) reported that intrinsic wetness related to the dentin region (i.e., superficial vs. deep dentin) could significantly affect regional bond strength.

2) Environmental variables during clinical application of resin

Resin materials are considered to be susceptible to dental moisture contamination, which will unfavorably affect the adhesive resin bonding properties. Most laboratory studies have been performed at room temperature (23°C) and 50 % relative humidity (RH). However, this condition is far from what is experienced in the oral cavity. Yoshida (1983) and Plasmans et.al.(1993), have reported that the environment in oral cavity is far more warm and moist (temperatures of between 27°C to 30°C, and RH of over 75 %) than in the laboratory. Sato et.al (1991), Nikaido et.al.(1991) and Burrow et.al.(1994) reported the negative influence of relative humidity on bond strengths to dentin. They recommended the use of high-speed suction and/or rubber dam when restorations are being placed in a humid condition such as the second molar regions.

Contamination of a cavity by saliva or blood also becomes a clinical problem, especially when a cavity near or at the gingival margin is restored. These contaminants decrease bond strengths and cause increase of microleakage (Pashley et.al., 1988; Fritz

et.al., 1998; Abdalla and Davidson, 1998; Hebling and Feigal, 2000; Hiraishi et.al., 2003). Adhesive systems of recent generations are less sensitive to saliva contamination. With treatment or re-treatment of the contaminated tooth surface by an acidic conditioner has been reported to restore bond strengths, and to decrease microleakage (Fritz et.al., 1998; Hebling and Feigal, 2000; Hiraishi et.al., 2003). On the other hand, contamination by blood is a greater problem than by saliva, because blood decreases bond strengths of adhesives to dentin even after water-rinsing, acidic conditioning, or reconditioning (Shiraishi, 1998). It was reported that the plasma proteins that attach to the dentin surface may not be easily removed by acid etching, because these proteins coagulate with an acidic solution (Nikaido et.al., 1995; Nikaido, 1998). The application of 10 % NaOCl solution or 95 % ethanol (Shiraishi, 1998), or mechanical removal of contaminated dentin with dental bur (Pashley et.al., 1988) are reported to be effective for decontamination of blood. However, regarding the use of these solutions, further discussion should be needed on their influence on the bonding.

3) Variables related to the polymerization shrinkage of resin

It is well known that resin composites shrink during the polymerization. For the adhesive restorations, contraction stress that concentrate at the bonding interface leads the composite-tooth bond disruption (Davidson et.al., 1984a,b; Feilzer et.al., 1987). It was reported that the configuration factor (c-factor), which is the ratio of bonded to unbonded or free surface area, had a remarkable influence on the contraction stress at the bonding interface (Feilzer et.al., 1987). The higher the c-factor, the greater is the potential for bond disruption from polymerization effects. For example, class I restoration with a c-factor of 5 (five bonded surfaces, one unbonded surface) is at much higher risk of bond disruption associated with polymerization shrinkage, particularly along the pulpal floor. Internal stress can be reduced in restorations subject to potentially high disruptive contraction forces, by using incremental filling technique of the composite, or using of the stress-absorbing liner, such as a filled dental adhesive or a flowable composite (Swift et al, 1996; Van Meerbeek et.al, 1993; Unterbrink et.al., 1999; Bayne et. al. 1998; Ogata et.al., 2003a).

Bond strength test

For the evaluation of adhesive products or the influence of experimental variables on bonding property, the conventional tensile or shear bond tests have been used at many laboratories. The bond strength test carried out in the chapters 2 to 5 was the micro-tensile bond test that was developed by Sano et.al. (1994a). The obvious difference between conventional tensile test and this new testing method is the size of the specimen. The diameter of the bonded surface of the conventional tensile and shear test is 3 or 4 mm, is demarcated by a hole punched vinyl tape. As a screening test of new materials or an experimental material, the conventional tests work well, providing useful information about the materials when resin-dentin bond strengths were relatively low (ca. 10-15 MPa).

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However, as bonding techniques and materials improved, the bond strengths became so high that caused cohesive failures in dentin, which means dentin broke within itself, leaving the resin-dentin interface intact (Pashley et.al., 1999). According to Pashley's review article (1995), the frequency of cohesive failures of dentin can be as high as 80% when bond strengths reach 25 MPa. Such failures of the tooth substrate prevent measurement of interfacial bond strengths. A major difficulty of conventional tensile testing is maintaining the alignment during testing, because of the relatively large specimen size (Sudsangiam et.al., 1999). Misalignment of the rod and bonded interface cause incorrect stress concentrations around the interface. The non-uniformity of the stress distribution generated during the conventional tensile test will create areas of high local stress, and fracture will be initiated from a defect at the interface, or in areas of high local stress in the tooth substrate (Sudsangiam et.al., 1999). According to the Griffith defect theory (Griffith 1920), the tensile strength of brittle materials decreases with increasing cross-sectional area (Pashley et.al., 1995). Larger specimens may contain more defects than smaller specimens. The same explanation will be possible at the case of bonded dentin surface areas. Microscopically, the interfaces can contain air bubbles, phase separations, surface roughness and non-uniform film thickness that can lead to non-uniform stress-distributions (Pashley et.al., 1995). Therefore, such cohesive failures in dentin do not mean that the resin-dentin bonds are uniformly stronger than the ultimate strength of dentin, but that the manner in which the bond is stressed is non-uniform, that it is concentrated or focused at one highly localized region where it opens a crack in dentin (Pashley et.al., 1995). The ultimate tensile strength of dentin is reported as over 100 MPa (93.8 - 105.5 MPa, Sano et.al., 1994b). These local stress concentrations often exceed 100 MPa, even though the calculated average bond strength is only 25 MPa (Pashley et.al., 1995).

To avoid cohesive failures of dentin during bond testing, it is necessary to improve stress distributions during testing. The relatively new testing method, such as single plane lap shear test (Watanabe, 1987), micro-tensile bond test (Sano et.al., 1994a), and micro-shear bond test (Shimada et.al., 2002) were produced for the better stress distributions during testing. Sano et.al. (1994a) reported that tensile bond strengths are inversely related to bonded surface area using very small areas. When the tensile bond strength was plotted as a function of bonded surface area, an exponential increase in bond strength was noted with decreasing surface area. As the cross-sectional area of bonded specimens was reduced, the number of cohesive failures of dentin fell to zero at about 2 mm². Below 2 mm², all failures were adhesive.

According to this result reported by Sano et.al. (1994a), original micro-tensile bond testing was designed to permit evaluation of bond strengths between adhesive materials and small regions of 1 mm² of dental tissue. One of the advantages of this technique is that the bonded interface of small specimens has a better stress distribution during loading, so that there are fewer cohesive failures in dentin than are found with

conventional testing method. This is thought to be due to the reduction in the density of crack or defect around the interface. Using the micro-tensile bond test, bond strengths tend to be higher than those of conventional tests. Harada(2000) reported that the bond strength values of Kuraray's self-etching primer systems measured by micro-tensile bond test are over two times higher than the values of conventional testing.

In the original micro-tensile bond test (Sano et.al, 1994a), the occlusal surface of the tooth is ground flat, and the entire surface is treated with an adhesive system. Then a resin composite crown is built up. After 1 day in 37℃ water, the resin-bonded tooth is serially sectioned parallel to the long axis of the tooth, approximately 0.7 mm thick, using a low speed diamond saw. The bonded area is trimmed using a superfine diamond bur to create an hourglass shape with a cross-sectional area of approximately 1 mm². The specimens were then attached to Bencor Multi-T testing device, with a cyanoacrylate adhesive, and subjected to tensile forces at a cross-head speed of 1 mm / min. This original specimen shape was modified to different shapes such as a beam type (Shono et.al, 1999) or an hourglass cylindrical type (Phrukkanon et.al, 1998) by some researchers. Sample preparation for the micro-tensile bond test or the micro-shear test have many complicated steps before loading the specimen. These steps make these testing methods technique sensitive. Due to this reason, the standard deviation of the micro tensile test is higher than the conventional tensile test (Harada et.al,, 2000). Furthermore, the size of the bonded surface area significantly affects the bond strength value of the micro tensile test, because the bond strength value indicates an exponential increase with decreasing bonded surface area (Sano et.al., 1994a). Simple comparison of the raw data without consideration of the testing condition should be avoided.

As mentioned before, conventional testing methods for adhesion require relatively large surface areas for adhesion making it difficult to evaluate differences in regional bond strengths. Since the micro-tensile test permits measurement of bond strengths of relatively small surface areas of 1 mm², this method has been widely used for testing irregular surfaces such as class I, II, and V restorations or different dentin substrates (Nakajima et.al., 1995, 1999a, 1999b; Yoshiyama et.al., 1996, 1998; Pereira et.al., 1997; Ogata et.al., 1999, 2001a, 2001b, 2002; Yoshikawa et.al., 1999). In the studies of the chapters 2 to 5, we evaluated the clinical factors, such as different primer application methods, different dentinal tubules direction, or different smear layer created by different instruments, since those are thought to influence the resin-dentin interface. The micro-tensile bond test's better stress distribution around the interface during loading and relatively small specimen size is the greatest merit for the evaluation of the influence of each factor on the bond strength of resin-dentin interface.

Chapter 2

Effect of dentin primer application on regional bond strength to cervical wedge-shaped cavity walls

Introduction

Recently, current dentin bonding systems with self-etching primers have been produced, yielding major improvements in bonding to tooth substances (Chigira et.al.,1994; Wang and Nakabayashi,1991; Watanabe et.al.,1994; Ikemura et.al.,1996). These adhesive materials attempt to improve the quality of the bond while reducing the bonding procedures. Only one application of etching/primer solution is required to condition enamel and dentin simultaneously, followed by an application of the adhesive resin.

The demand for restoration of root lesions such as wedge-shaped cervical defects and root caries has increased. With the recent use of self-etching primers, these can easily flow off from the cavity, leaving a small amount on the walls of a wedge-shaped defect. This is partly because of the low viscosity of the self-etching primers. In this case, the dentin surface may not be properly treated by the self-etching primer to produce durable and stable bonds. Ferrari et.al.(1996, 1997) described that longer application time of the self-etching primer on dentin creates a more intimate interlocking, and provide an adequate marginal seal. Therefore, it is necessary to devise a suitable method of primer application to a wedge-shaped defect.

Conventional testing methods for adhesion require relatively large surface areas that makes it difficult to evaluate the difference of regional bond strengths. A new bond-testing procedure, called the micro-tensile bond strength test (Sano et.al., 1994a) has been recently developed to permit the measurement of small (ca. 1 mm²) cross-sectional bonded areas. The procedure allows the testing of such as class I, II, and V restorations. Since this method can measure the bond strength of a relatively small surface, it has been widely used to test different dentin substrates. (Nakajima et.al., 1995; Yoshiyama et.al., 1996; Pereira et.al., 1999; Yoshikawa et.al., 1999). In this study, this testing method was used to evaluate the regional bond strength within cervical wedge-shaped cavities.

The purpose of this study was to examine the effect of multiple self-etching primer application of Clearfil Liner Bond 2 and Imperva Fluoro Bond on the regional tensile bond strength to artificial wedge-shaped cavities, as well as to observe the micromorphological appearance of the resin-dentin interface.

Methods and Materials

Eighteen extracted caries-free human upper third molars which were stored frozen, were used for micro tensile testing (Sano et.al., 1994a). Wedge-shaped defects were

Table 1. Adhesive systems used for bonding

System	Ingredients	Code / Lot #	Procedures	Manufacturer
Clearfil Liner Bond 2 (LB)				
LB-primer A	Phenyl-P, 5-NMSA, CQ, ethanol	045	a;b (30s); c;d (20s)	Kuraray Medical, Tokyo, Japan
LB-Primer B	HEMA, water	057		
LB-Bond	MDP, HEMA, Bis-GMA, microfiller	0066		
Imperva Fluoro Bond (FB)				
FB-Primer A	water, acetone, initiator	049604	a;b (10s); c;d (10s)	Shofu Inc., Kyoto, Japan
FB-Primer B	4-AET, HEMA, 4-AETA, initiator	049609		
FB-Bond	4-AET, HEMA, UDMA glass ionomer filler, microfiller	049609		

Procedures : a=mix primer, b=apply primer, c=apply adhesive, and d=light-cure.
Abbreviations; Bis-GMA=bisphenol-glycidil methacrylate; CQ=camphorquinone; HEMA=hydroxyetyl methacrylate; MDP=10-methacryloyloxy methacrylate; NMSA=N-methaeryloxyl-5-aminosalicylic acid; Phenyl-P=2-methacryloyloxyethyl-phenyl hydrogen phosphate; UDMA=urethane dimethacrylate; 4-AET=4-acryloxyethyltrimellitic acid; and 4-AETA=4-acryloxyethyltrimellitate anhydride

prepared in the buccal cervical dentin by means of a medium-grit diamond point (A-18, GC Ltd., Tokyo, Japan) mounted in a high speed turbine under copious air water spray. The dimensions of the cavities were: mesio-distal width,10.0 mm; bucco-gingival height, 5.0 mm ; maximum depth, 3.0 mm. (Fig. 1). The adhesive agents, manufacturers, and batch numbers that were used, and the procedures recommended by the manufacturers are listed in Table 1. The identification of the experimental groups and subgroups, methods of primer application and location of bond strength testing are listed in Table 2. First, the teeth were devided into two groups according to the adhesive systems used for bonding
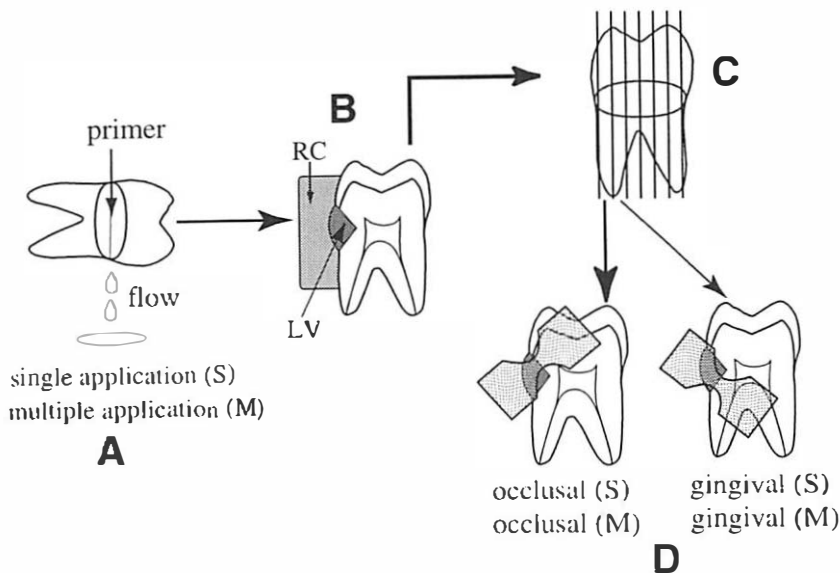
Figure 1. Schematic indication of the methodology used for micro-tensile bond strength testing. A: Single(S) or multiple(M) primer application to the cavity. The teeth were mounted on their distal surface, so as to permit flow-off of the primer from the cavity. B: The resin bonded teeth. RC=resin composite, LV=low-viscosity resin composite. C, D: The sliced specimens that were trimmed alternately to test either occlusal or gingival walls.

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Table 2. Identification of groups and subgroups by material, primer application, location, and their abbreviations

Groups and subgroups	abbreviations
Group (LB) Clearfil Liner Bond 2	
single primer application, occlusal wall	LB-occlusal (S)
single primer application, gingival wall	LB-gingival (S)
multiple primer application, occlusal wall	LB-occlusal (M)
multiple primer application, gingival wall	LB-gingival (M)
Group (FB) Imperva Fluoro Bond	
single primer application, occlusal wall	FB-occlusal (S)
single primer application, gingival wall	FB-gingival (S)
multiple primer application, occlusal wall	FB-occlusal (M)
multiple primer application, gingival wall	FB-gingival (M)

(Clearfil Liner Bond 2 or Imperva Fluoro Bond). The mesio-distal direction of the cavity was kept parallel to the direction of gravity, simulating the supine position of the teeth during dental treatment, and to permit its flow-off from the cavity and to remain slightly on the cavity walls (Fig.1A). The devided teeth were further subdivided into two groups according to the method of primer application (Fig. 1A): group(S), primer was put onto the cavity once with a sponge pellet and the teeth were left untouched during the priming time recommended by the manufacturer (LB: 30 seconds, FB: 10 seconds); group(M), primer was put onto the cavity several times during the priming time recommended by the manufacturer, and its frequency of additional primer application during the priming time of each bonding system was five. The adhesive bonding resins were applied to the cavities and light- cured. The cavities were filled with a low-viscosity resin composite (Protect Liner F, Kuraray Medical, Tokyo, Japan) (Yoshiyama et.al., 1996) and light-cured for 40 seconds. After light-curing, the specimens were stored in 37℃ water for 24 hours. The enamel, dentin and resin composite surfaces were then acid-etched with 37% phosphoric acid gel (K-etchant, Kuraray Medical, Tokyo, Japan), and covered with adhesive resins (Clearfil Photo Bond, Kuraray Medical, Tokyo, Japan) to permit adhesion of additional resin composite (Clearfil AP-X, Kuraray Medical, Tokyo, Japan) to ensure the grips for a micro-tensile bond test (Fig. 1B).

The resin bonded teeth were then serially sectioned into 5-6 slices of approximately 0.7 mm thick parallel to the long axis of the tooth using a low speed diamond saw (Leitz 1600 Microtome, Leica Instruments, GmbH, Heiderbelg, Germany) under water cooling (Fig. 1C). These sections were then trimmed and shaped to form a gentle curve with the narrowest portion at the adhesive interface using a superfine diamond point (c16ff, GC Ltd.,Tokyo,Japan) mounted in a high speed handpiece under copious water spray. Alternate sections were trimmed to test either the occlusal or gingival walls of the each

bonded restoration (Fig.1D). The bonded surface area, that ranged from 0.95 to 1.05 mm^2, was calculated before testing by measuring the width and thickness of each specimen. These specimens were then attached to the testing device (Bencor-Multi-T, Danville Engineering Co., Danville, CA, USA) with a cyanoacrylate adhesive (Zapit, DVA, Anaheim, CA, 91720) which, in turn, was placed in a universal testing machine (Autograph AG-500B, Shimadzu Co., Kyoto, Japan) for tensile testing at a cross-head speed of 1mm / min (Sano et.al., 1994a). After fracture of the bonds, all the specimens were visually inspected to determine the mode of fracture. In addition, representative samples were also observed using a scanning electron microscope (JXA-840, JEOL, Tokyo, Japan) to confirm the accuracy of the visual inspection.

Statistical analysis of the tensile bond strengths were performed using a two-and three-way ANOVA (LB or FB, occlusal or gingival wall, and single or multiple primer application) and Fisher's PLSD test at 95% level of confidence.

For the SEM observation of the resin-dentin interface, four teeth (two for each material) were used and a cervical wedge-shaped defect was produced on the each tooth. Each cavity was treated identically to the bonding procedures mentioned above. The resin bonded samples were then sectioned into two halves, parallel to the longitudinal axis of the tooth, using a low-speed diamond saw. Each specimen was embedded in epoxy resin (Epon 815, NISSIN EM Co., Ltd., Tokyo, Japan), then the cut surfaces were ground with a series of increasingly finer silicon carbide abrasive papers, and highly polished with a diamond pastes (DP-Paste,P, Struers A/S, Denmark) (6 μm, 3 μm, 1 μm). The samples were subjected to 10% phosphoric acid treatment for 3 to 5 seconds (Gwinett and Kanca 1992b; Sano et.al., 1995). The specimens were rinsed with water for 15 seconds and treated with 5% sodium hypochlorite solution for 5 minutes. (Wang and Nakabayashi 1991). After being extensively rinsed with water, the treated specimens were air dried, gold-sputter-coated and observed with the SEM at 10keV. The thickness of the hybrid layers of resin dentin interface of each group was measured on each photograph at 4000x.

Results

The resulting micro-tensile bond strength values (μTBS) and standard deviations are shown in Table 3. Two-way ANOVA analysis revealed that there was a statistically significant interaction between the bonding systems and method of primer application (p = 0.002). Three-way ANOVA analysis revealed that there was no statistically significant interaction between the bonding systems, the cavity walls, and method of primer application (p = 0.8760). With both adhesive systems and methods of primer application, bond strengths to occlusal walls were significantly higher than those to gingival walls (p<0.05). By multiple primer application, bond strength of LB to the each cavity wall rose significantly (p<0.05). However, bond strength of FB indicated no statistical significant difference by altering the method of primer application (p>0.05).

Table 3. Micro-tensile bond strength results (mean ± 1SD (MPa))

	occlusal		gingival
Liner Bond 2			
(S)	29.1 ± 10.8	p<0.05	17.3 ± 6.7
	(n=15)		(n=13)
(M)	37.5 ± 7.9	p<0.05	26.0 ± 8.5
	(n=12)		(n=10)
Fluoro Bond			
(S)	31.6 ± 8.0 [a]	p<0.05	20.8 ± 9.6 [b]
	(n=10)		(n=11)
(M)	29.4 ± 6.9 [a]	p<0.05	17.8 ± 6.4 [b]
	(n=11)		(n=10)

n: number of specimens tested

Groups that are not significantly different are marked with the same superscript letter (p>0.05).

When visually inspected, all specimens showed interfacial adhesive failure. Cohesive failure within dentin or composite were not found. This was confirmed by light microscopic examination (x20). The representative micromorphology of the failure pattern (x750, x1500) was classified as mixed failure within dentin and bonding resin (Fig. 2). The direction of the dentinal tubules for the occlusal groups was almost parallel to the interface, while for the gingival groups, it was almost perpendicular to the interface.

The micromorphology of LB-dentin and FB-dentin interfaces are shown in Figs. 3 and 4, respectively. For the interfaces treated once with LB-primer (groups LB-occlusal(S) and LB-gingival(S)), the thickness of the hybrid layer was about 1 μm, and narrow and short resin tags which did not fill the tubular orifices completely were observed (Figs.3-(a)(b)).
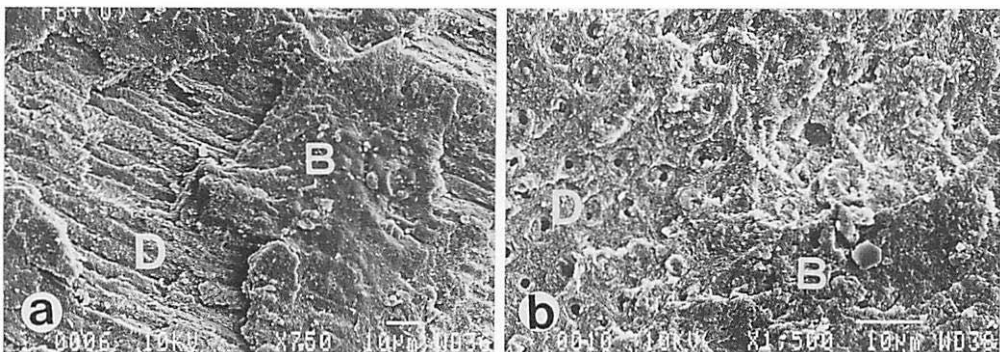

Figure 2. Representative SEM photographs of a fractured specimen. Failure can be seen within bonding resin (B), and dentin (D). a: The direction of the dentinal tubules of the occlusal wall was almost parallel to the interface. b: The direction of the dentinal tubules of the gingival wall was almost perpendicular to the interface. (Original magnification, a:750x, b: 1500x; bar = 10 μm)
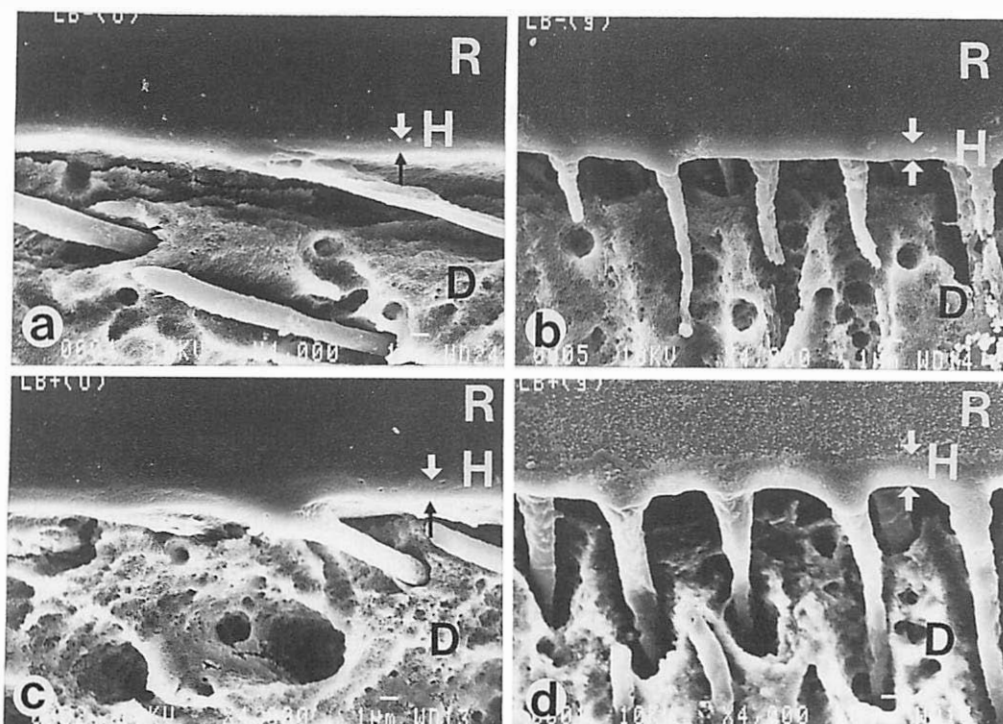
Figure 3. SEM of the Liner Bond 2 / dentin interface after treatment with 10% phosphoric acid and 5% sodium hypochlorite. a, b: Occlusal and gingival walls of the cavity when LB-primer was applied once. Thickness of the hybrid layer (H) is approximately 1 μm (arrows). c, d: Occlusal and gingival walls of the cavity when LB-primer was applied for several times. Thickness of the hybrid layer (H) is approximately 2 μm (arrows). (Original magnification 4000x ; bar = 1 μm)
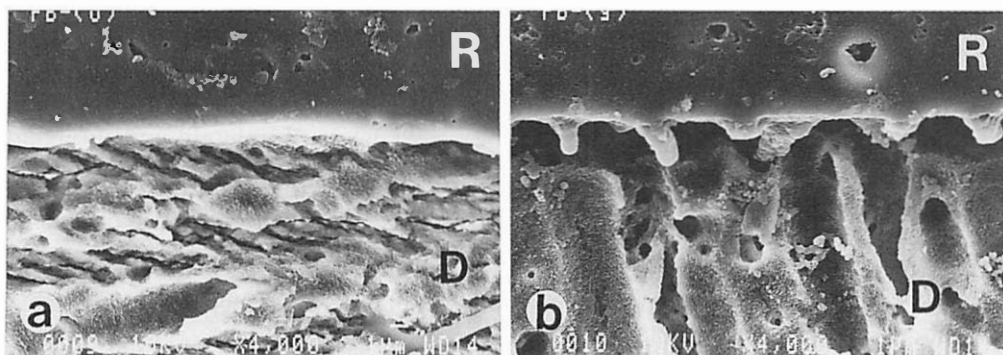
Figure 4. SEM of the Fluoro Bond / dentin interface of the group FB(S) after treatment with 10% phosphoric acid and 5% sodium hypochlorite. a: Occlusal wall of the cavity. b: Gingival wall of the cavity. The hybrid layer is not clearly observed. (Original magnification 4000x ; bar = 1 μm)

For the interfaces treated with LB-primer repeatedly (groups LB-occlusal(M) and LB-gingival(M)), the thickness of the hybrid layer was about 2 μm, and the resin tags were thick and long with a characteristic funnel cone-shape (Figs.3-(c)(d)). For the groups treated with FB, the interfacial micromorphology indicated no difference by altering the method of primer application. The hybrid layer was not clearly observed (Fig. 4).

Discussion

The influence of multiple primer application on the μTBS was evident for the LB groups, since bond strength to each cavity wall increased significantly (Table 3). The priming time recommended by the manufacturer for LB is of 30 seconds in order to achieve maximum bond strength. Recently, Ferrari et.al., (1996, 1997) evaluated the effect of priming time of LB on the micromorphology of the resin infiltrated layer and marginal sealing ability. They concluded that a longer time of primer application on dentin surface created a more intimate interlocking and provided an adequate marginal seal. In this study, when LB-primer was applied only once to the cavity walls, the flow-off of the primer from the cavity caused insufficient amount of primer solution on the dentin surface. Because of this, the dentin surface of these groups might not be treated effectively. However, for the groups which received multiple primer application, LB-primer also flowed off from the cavity but fresh primer was added. Therefore the dentin surfaces of these groups would be treated sufficiently by an adequate amount of primer. In our study, the shortage of primer on the dentin surface was compensated by the multiple application of LB-primer which contributed to the improvement of adhesive bond strength to dentin. The differences between the SEM appearances of the adhesive interface of LB(S) and LB(M) confirmed this concept mentioned above (Fig.3). Comparing the microphotographs of LB(S), the thickness of the resin infiltrated layer was about 1 μm, much thinner than that of LB(M), and showed narrow and short resin tags that did not fill the tubule orifices completely (Fig.3-(b)). This could be because the LB-primer applied only once was unable to dissolve the smear layer sufficiently, obstructing the infiltration of the bonding resin into the demineralized dentin. Meanwhile, for LB(M), the thickness of the hybrid layer was about 2 μm and showed thick resin tags with a characteristic funnel cone-shape. It is highly likely that the LB-primer dissolved the smear layer completely and the bonding resin is able to penetrate into the demineralized dentin sufficiently.

On the other hand, multiple primer application indicated no influence on the μTBS when the cavities were treated with FB-primer (Table 3). The SEM appearances of the FB groups did not show remarkable differences by the alteration of primer application method. For the group(M) of each bonding system, the frequency of primer addition during the priming time of LB and FB was the same. The priming time of FB-primer (10 seconds) is much shorter than LB-primer (30 seconds). Yoshiyama et.al. (1997) reported that LB and FB formed thin hybrid layers of about 1.0 μm thick in cervical dentin, and that the resin tags produced by FB were shorter in length but larger in diameter than those produced by LB. They concluded that FB-primer probably removes the peritubular dentin matrix more efficiently than LB-primer. According to the results of this study, the removal of the peritubular dentin matrix appeared to show no difference between LB and FB. However, thickness of the hybrid layer of FB was thinner than LB. The functional difference of each adhesive-monomer of these primers (LB: Phenyl-P, FB: 4-AET), especially the difference of

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the pH of both monomers (LB: 1.4, FB: 2.5, (information provided by the manufacturers)), may have caused this difference. Ikemura et.al. (1996) reported that the ionized 4-AET in water / HEMA solution would lead to sufficient chemical interaction with dentinal tissue, resulting in good penetration of bonding resin into the superficial dentin at the adhesive interface. High reactivity of 4-AET might have achieved better surface adhesion to dentin. Kanemura et.al. (1996) evaluated the effect of two priming times for the FB-primer on dentin bond strength. They concluded that there was no significant difference of tensile bond strength among the groups treated by FB-primer for 10 seconds or 30 seconds. Therefore, the efficacy of FB-primer may not depend on application time or frequency. The difference of viscosity of the two primer solutions might have partly influenced our result (Contact angle of the each primer to human dentin were: LB; $\theta = 14°$, FB; $\theta = 20°$, water; $\theta = 28°$, (information provided by the manufacturers)). LB-primer is serous and it flows off from the cavity easily, while FB-primer is a more viscous solution. Therefore, more FB-primer should remain on the cavity walls than LB-primer. Yoshiyama et.al. (1996) measured the regional bond strength of LB in natural and artificial wedge-shaped defects of human teeth that were extracted for periodontal reasons. Their results indicated that the tensile bond strengths of bonds made to natural lesions were significantly lower than to artificially created lesions. However, they reported no significant differences between bonds made to gingival walls and occlusal walls of natural or artificial lesions. In our study, we used sound third molars from young patients. With both adhesive systems and methods of primer application, bond strengths to gingival walls were significantly lower than those to occlusal walls. Yoshiyama et.al. (1996) reported that LB formed no resin tags in the tubules in the gingival site of the cavity which were prepared in normal cervical dentin. Our results showed that thickness of the resin infiltrated layer was almost the same as reported by Yoshiyama et.al. (1996), but with tag formation within the tubules. Tagami et.al. (1992) reported that the permeability of old normal dentin was much lower than that of young normal dentin. The orientation of the dentinal tubules within the occlusal wall was generally parallel to the prepared surface, while those of the gingival wall were perpendicular to the interface. Thus, there were more tubules connected to the cut surface at the gingival site than were seen at the occlusal site. Further research should be needed in order to investigate the relation of the direction of dentinal tubules and the dentin bond strength.

In the artificially created wedge-shaped defects, the dentin was normal and the tubules were patent. This probably allowed better resin infiltration. It is unclear why the bond strength of gingival wall decreased in our study. Presumably, the existence of water provided from pulpal side of the dentinal tubule, due to the higher permeability of the young tooth, might have affected negatively the bond strength to the gingival wall. Several studies have demonstrated that natural cervical abraded dentin was difficult to acid-etch for bonding due to hypermineralization of the surface of the dentin (Duke and Lindemuth, 1990, 1991; Harnirattisai et.al., 1992; Van Meerbeek et.al., 1992; Pashley et.al., 1996). In

this study, multiple application of the self-etching primer was intended to supply adequate amount of primer into a wedge-shaped defect in opposition to the flow-off. The priming time recommended by the manufacturer for FB-primer was very short, and maximum frequency which could apply the primer during the priming time of FB was five. Therefore we decided the frequency during the priming time to be five, and applied the same frequency to LB-primer. With this study, it became clear that bond strength to the artificial wedge-shaped defect improved significantly by multiple application of LB-primer. Thus, it is of crucial importance to evaluate the effect of multiple primer application on bonding to natural wedge-shaped defects, as well as to investigate the suitable frequency to achieve the maximum bond strength.

Conclusions

By multiple primer application of Liner Bond 2, bond strength to the artificial wedge-shaped defect improved significantly. However, bond strength of Fluoro Bond indicated no statistical difference by altering the method of primer application. With both adhesive systems and primer application methods, tensile bond strength to the gingival wall was significantly lower than to the occlusal wall. To these defects, multiple primer application may be an effective method to treat the dentin surface properly and to produce strong bonds using the self-etching bonding system.

Chapter 3

Influence of the direction of tubules on bond strength to dentin

Introduction

Bonding of adhesive resin systems to dentin has been widely studied over recent years. When using adhesives on dentin, the variation of bond strengths is dependent not only the materials, but also on such factors as dentin depth (Suzuki and Finger, 1988; Tagami et.al., 1990), calcium concentration (Perinka et.al., 1992), age (Tagami et.al., 1993; Burrow et.al., 1994), surface wetness (Prati and Pashley, 1992), relative humidity (Nikaido et.al., 1991; Burrow et.al., 1995), caries-affected dentin (Nakajima et.al., 1995, 1999), sclerotic cervical erosion (Duke and Lindemuth, 1990, 1991; Harnirattisai et.al., 1992; Van Meerbeek et.al., 1992; Yoshiyama et.al., 1996), or polymerization stress related to cavity configuration (Feilzer et.al., 1987; Yoshikawa et.al., 1999). However, very few reports are available with regard to the influence of the direction of dentinal tubules on bond strength to dentin.

Dentin is a complex hydrated biological composite structure (Lindén et.al., 1995) with a highly oriented microstructure dominated by tubules that converge toward the predentin from the dentino-enamel junction in the crown, and from the cementum in the root. This structural arrangement leads to variation in tubule size, direction and number, as well as quantity of intertubular matrices. The dentinal tubule course, density, and diameter are well known (Garberoglio and Brännström, 1976; Pashley, 1989; Mjör and Nordahl, 1996), and their direction on the cavity wall depends on its location (Cagidiaco et.al., 1997). In class I cavities prepared in molars, the lateral walls present tubules that are almost parallel to the cut surface, while at the pulpal wall, the tubules are cut perpendicularly to the surface. In the case where the tubules are cut perpendicularly to the surface, acid etching opens the tubule orifices. These structural differences of the adhesive surface may influence the structure of the resin-dentin interface and the bond strength of resin to dentin. Therefore, it is desirable to investigate the influence of the direction of dentinal tubules on resin-dentin bond strength. To focus only on the direction of the tubules within the adhesive surface independent of the c-factor, we used a flat coronal dentin surface of human molars to simulate the walls of Class I cavities, and to minimize the effect of curing stresses of composite. Previously, in a study of regional bond strengths to cervical wedge-shaped cavity using self-etching/priming systems, we reported that the bond strengths of these systems were higher to the occlusal wall than to the gingival wall (Ogata et.al., 1999, see chapter 2). The orientation of the tubules within the occlusal wall was parallel to the interface, while that of the gingival wall was perpendicular to the interface. However, in our previous study using cervical wedge shaped cavities, the influence of tubule direction on bond strength to dentin was still

unclear.

Conventional testing methods for adhesion require relatively large surface areas for adhesion that makes it difficult to evaluate differences in regional bond strengths. A new bond-testing procedure, called the micro-tensile bond strength test, has been recently developed to permit the measurement of cross-sectional bonded areas as small as 0.5 mm² (Sano et.al., 1994a). It enables testing irregular surfaces such as class I, II, and V restorations. Since this method permits measurement of bond strengths of relatively small surface areas, it has been widely used to test different dentin substrates (Nakajima et.al., 1995, 1999; Yoshiyama et.al., 1996, 1998; Pereira et.al., 1997; Ogata et.al., 1999; Yoshikawa et.al., 1999). This method can reduce abnormal stress concentrations associated with conventional shear and tensile tests (Sudsangiam and Van Noort, 1999), therefore using the microtensile test, bond strengths tend to be higher than those of conventional tests. Using this testing method, it is possible to evaluate the influence of the dentinal tubule direction on bond strength to dentin.

The purpose of this study was to investigate the influence of the dentinal tubule direction on bond strength to dentin, as well as to observe the micromorphological appearance of the resin-dentin interface. The null hypothesis was that the direction of dentinal tubules would have no effect on resin-dentin bond strength.

Methods and materials

Thirty-six frozen extracted caries-free human upper third molars were thawed and used for micro tensile testing (Sano et.al., 1994a). The teeth were divided into two

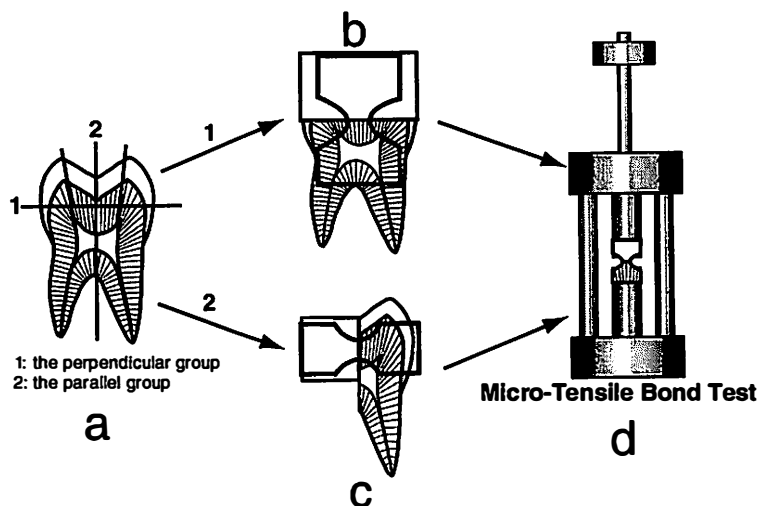**Figure 1.** Schematic indication of the methodology used for micro-tensile bond strength testing. a: The flat dentin surface was prepared for the perpendicular group (1), or the parallel group (2). b, c: The bonded specimens were sliced and trimmed for the perpendicular group (b) or the parallel group (c). d: The slabs were attached to the testing device for tensile testing at a cross-head speed of 1 mm / min.

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Table 1. Adhesive systems used for bonding

System	Ingredients	Code / Lot #	Procedures[a]	Manufacturer
Clearfil Liner Bond 2 (LB)				
LB-primer A	Phenyl-P[b] , 5-NMSA, CQ, ethanol	066	a; b (30s);	Kuraray Medical,
LB-Primer B	HEMA ,water	081	c; d (20s)	Tokyo,
LB-Bond	MDP, HEMA, Bis-GMA, microfiller	0099		Japan
Imperva Fluoro Bond (FB)				
FB-Primer A	water, acetone, initiator	089728	a; b (10s);	Shofu,
FB-Primer B	4-AET, HEMA, 4-AETA, initiator	089786	c; d (10s)	Kyoto,
FB-Bond	4-AET, HEMA, UDMA, glass ionomer filler, microfiller	089732		Japan
Single Bond (SB)				
Etchant	35% phosphoric acid gel	7423	e(15s); f; g;	3M Co.,
Adhesive	Bis-GMA, HEMA, polyalkenoic acid copolymer, ethanol, water, photoinitiater	7AF	h; d(10s)	St. Paul, MN, USA
One-step (OS)				
Uni-Etch	32% phosphoric acid gel	089077	e(15s); f; g;	Bisco,
Adhesive	BPDM, Bis-GMA, HEMA, acetone, photoinitiater	079107	h; d(10s)	Schaumberg, IL, USA

[a] Procedures : (a) mix primer; (b) apply primer; (c) apply adhesive; (d) light-cure; (e) acid-etching; (f) rinse; (g) blot-dry; (h) apply 2 coats of adhesive

[b] Abbreviations: Bis-GMA=bisphenol-glycidil methacrylate; BPDM=Bisphenyl-dimethacrylate; CQ=camphorquinone; HEMA=hydroxyetyl methacrylate; MDP=10-methacryloyloxy methacrylate; NMSA=N-methacryloyl-5-aminosalicylic acid; Phenyl-P=2-methacryloyloxyethyl-phenyl hydrogen phosphate; UDMA=urethane dimethacrylate; 4-AET=4-acryloxyethyltrimellitic acid; and 4-AETA=4-acryloxyethyltrimellitate anhydride

groups according to the direction of the dentinal tubule at the resin-dentin interface (Fig.1): a perpendicular group, in which the occlusal enamel was removed perpendicularly to the long axis of the tooth by means of a model trimmer under running water; and a parallel group, in which the mesial half of the tooth was removed parallel to the long axis of the tooth by means of a model trimmer under running water, and coronal part of the dentin surface was used for bonding (Fig.1).

The flat dentin surface was then polished with #600 silicon carbide paper to create a standard smear layer. Then the surface was treated with one of the four adhesive systems shown in Table 1, according to the manufacturers recommendation. After each adhesive resin was light-cured, a resin composite (Clearfil AP-X, Kuraray Medical Inc., Tokyo, Japan) was built up incrementally to a height of 3-5 mm to ensure sufficient bulk for the micro-tensile bond test (Sano et.al., 1994a). After light-curing, the specimens were stored in 37℃ water for 24 hours.

The resin-bonded teeth were then serially sectioned parallel to the long axis of the tooth into 5-6 slices approximately 0.7 mm thick, using a low speed diamond saw (Leitz 1600 Microtome, Leica Instruments GmbH, Heidelbelg, Germany) under water cooling. These sections were then trimmed and shaped to form a gentle curve with the narrowest portion at the adhesive interface using a superfine diamond point (c16ff, GC Ltd.,Tokyo,Japan) mounted in a high-speed turbine under copious water spray. The bonded surface area (ranging from 0.95 to 1.05 mm^2) was calculated before testing by measuring the width and thickness of each specimen, and the remaining dentin thickness (RDT) of each group was measured. For the perpendicular group, RDT-values were measured as the distance from the center of the bonded area to the closest region of the pulp chamber after trimming of the specimens. For the parallel group, RDT-values were measured as the distance from the pulp chamber to both DEJ-side and pulpal side of the trimmed specimens

(Fig. 1). The orientation of the dentinal tubules in the peripheral region of the coronal dentin was oblique to the long axis of the tooth. Therefore, the slices from the peripheral region were omitted, and the slices from center region of the coronal dentin (center area of the dentin between the pulp horns) were used for tensile bond testing. The trimmed specimens were then attached to the testing device (Bencor-Multi-T, Danville Engineering Co., San Ramon, CA, 94583) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, 91720) which, in turn, was placed in a universal testing machine (Autograph AG-500B, Shimadzu Co., Kyoto, Japan) for tensile testing at a cross-head speed of 1 mm / min (Sano et.al., 1994a) (Fig. 1). After the bondstrengths were measured, all of the specimens were both visually and microscopically inspected to determine the modes of fracture. In addition, representative samples were also observed using a scanning electron microscope (JXA-840, JEOL, Tokyo, Japan) to confirm the accuracy of the visual inspection.

Statistical analysis of the tensile bond strengths were performed using two- way analysis of variance (ANOVA) and Fisher's PLSD test at 95% level of confidence.

For the SEM observation of the resin-dentin interface, eight teeth (two for each material) were used. A flat dentin surface was produced on the each tooth in the same manner as when prepared for the perpendicular or parallel groups. Each surface was treated identically using the bonding procedures mentioned above. The resin-bonded samples were stored in 10% neutral buffered formalin for 24 hours (Inokoshi et.al., 1993), then washed in running tap water. Then the specimens were sectioned into two halves parallel to the longitudinal axis of the tooth, using a low-speed diamond saw (Leitz 1600 Microtome, Leica Instruments GmbH, Heidelbelg, Germany). Each specimen was embedded in epoxy resin (Epon 815, NISSIN EM Co., Ltd., Tokyo, Japan), then the cut surfaces were ground with a series of increasingly finer silicon carbide abrasive papers, and highly polished with diamond pastes (DP-Paste,P, Struers A/S, Copenhagen, Denmark) (6 μm, 3 μm, 1 μm). The samples were subjected to argon ion beam etching (EIS-1E, Elionix Ltd., Tokyo, Japan) for 5 minutes (Inokoshi et.al., 1993). The operating conditions for the ion source were the same as previously reported (Inokoshi et.al., 1993). The specimens were then gold-sputter-coated and observed with the SEM at an accelerating voltage of 10 keV. The thickness of the hybrid layers of resin-dentin interface of each group was measured on each photograph at a magnification of X5000.

Results

The micro-tensile bond strength values (μTBS) results and the thickness of the hybrid layer of each group are shown in Table 2. The remaining dentin thickness of each group are shown in Table 3. For the perpendicular group, there were no statistically significant differences among the bond strengths of any of the adhesive systems. When the dentin surfaces were treated with either of the self-etching priming systems (LB or FB), the parallel group tended to have higher tensile bond strengths (LB: 42.7 $\pm$ 13.2 MPa, FB:

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Table 2. Micro-tensile bond strength results (μTBS), and the thickness of the hybrid layer (HL) of each group (mean $\pm$ 1SD)

	μTBS (MPa)			HL(μm)		
	perpendicular		parallel	perpendicular		parallel
Liner Bond 2	39.3$\pm$13.3[a] (n=21)	NS	42.7$\pm$13.2[bc] (n=22)	1.0$\pm$0.1 (n=5)	p<0.05	1.3$\pm$0.1 (n=5)
Fluoro Bond	32.8$\pm$13.0[a] (n=22)	NS	39.8$\pm$14.8[bd] (n=20)	0.6$\pm$0.1 (n=5)	NS	0.8$\pm$0.1 (n=5)
Single Bond	32.2$\pm$10.4[a] (n=28)	p<0.05	44.5$\pm$15.4[cde] (n=27)	2.9$\pm$0.1 (n=5)	p<0.05	5.0$\pm$0.2 (n=5)
One-Step	36.4$\pm$12.3[a] (n=20)	p<0.05	51.2$\pm$12.5[e] (n=24)	2.5$\pm$0.3 (n=5)	p<0.05	4.3$\pm$0.4 (n=5)

n : μTBS ; number of specimens tested, HL; measured on the SEM photographs as the thickness between resintags.
NS: not significantly different (p>0.05). Groups that are not significantly different are marked with the same superscript letter (p>0.05).

Table 3. Remaining dentin thickness (RDT) of each group (mm), mean $\pm$ 1SD

	perpendicular	parallel-DEJ side	parallel-pulp side
Liner Bond 2	2.28±0.83	2.51±0.27	1.12±0.27
Fluoro Bond	2.08±0.75	1.95±0.29	0.53±0.23
Single Bond	2.38±0.70	2.43±0.52	1.03±0.42
One-Step	2.84±0.66	2.36±0.45	0.97±0.45

39.8 $\pm$ 14.8 MPa) than the perpendicular group (LB: 39.3 $\pm$ 13.3 MPa, FB: 32.8 $\pm$ 13.0 MPa). However, using these two adhesive systems, there was no statistical significant difference (p>0.05) between parallel and perpendicular groups. On the other hand, when the dentin surfaces were treated with the phosphoric acid etching systems (SB or OS), the bond strengths of the parallel group were significantly higher (SB: 44.5 $\pm$ 15.4 MPa, OS: 51.2 $\pm$ 12.5 MPa) than those of the perpendicular group (SB: 32.2 $\pm$ 10.4 MPa, OS: 36.4 $\pm$ 12.3 MPa) (p<0.05). Two-way analysis of variance (ANOVA) revealed that there was a statistically significant interaction between the type of bonding system (e.g., phosphoric acid etching system or self etching-priming system) and dentinal tubules direction (p = 0.0351). However, there was no statistically significant interaction between the particular bonding system (e.g., LB, FB, SB, or OS) and dentinal tubules direction (p = 0.1724).

When visually inspected, most specimens showed interfacial adhesive failure. This was confirmed by light microscopic examination (x20). The representative micromorphology of the failure pattern of the parallel group was classified as mixed failure within the bonding resin cohesive and composite resin cohesive (LB), or mixed failure within the adhesive interface and bonding resin cohesive (FB, SB, OS). In the
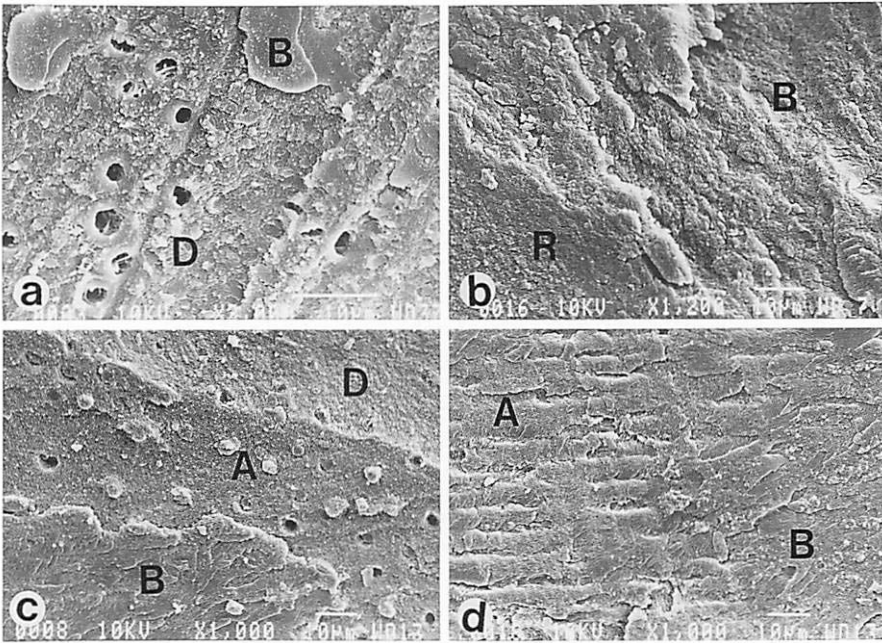
Figure 2. Representative SEM photographs of fractured specimens. For the perpendicular group, failure can be seen in the dentin (D) and bonding resin (B), and for the parallel group, failure can be seen in bonding resin (B) and composite (R) for Liner Bond 2, or the adhesive interface (A) and bonding resin (B) for Single Bond. Liner Bond 2: a; the perpendicular group, b; parallel group. Single bond: c; the perpendicular group, d; the parallel group. (Original magnification a:2000x, b: 1200x; c,d: 1000x, bar = 10 μm)
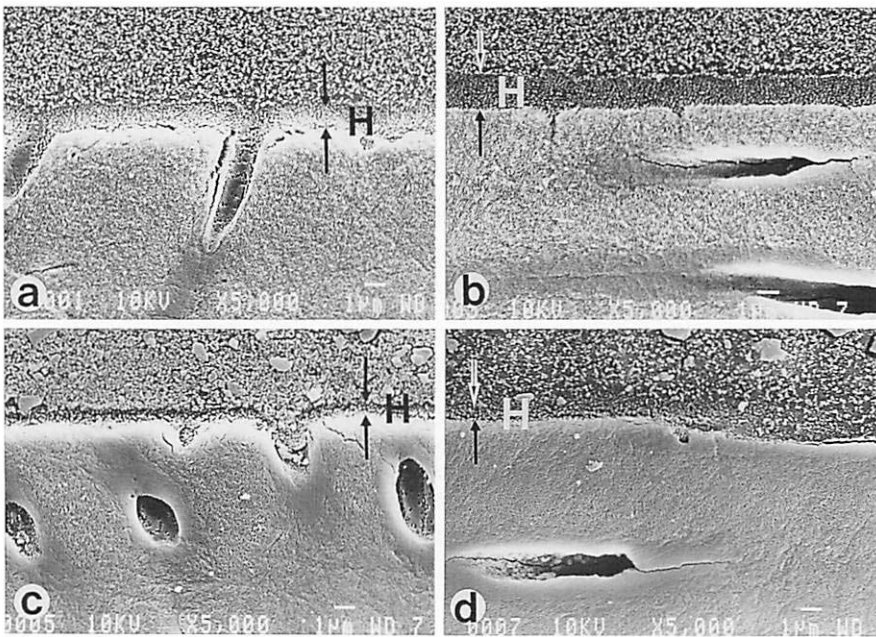
Figure 3. SEM of the resin-dentin interface after argon ion beam etching. a.b: the perpendicular and the parallel group of Liner Bond 2. c.d: the perpendicular and the parallel group of Fluoro Bond. Very thin hybrid layers were observed using Fluoro Bond. (Original magnification 5000x ; bar = 1 μm)
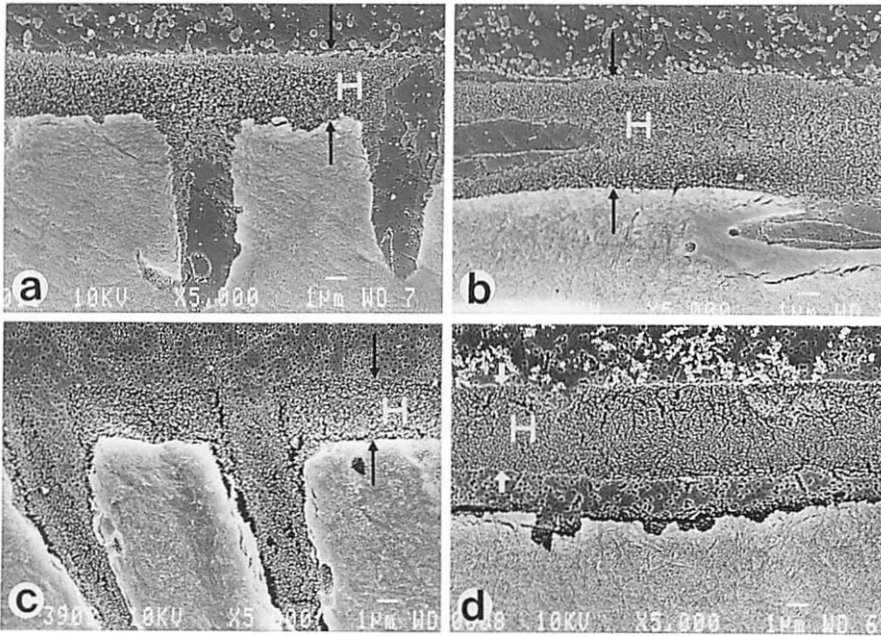
Figure 4. SEM of the resin-dentin interface after argon ion beam etching. a,b: the perpendicular and the parallel group bonded with Single Bond. c,d: the perpendicular and the parallel group bonded with One-Step. (Original magnification 5000x ; bar = 1 μm)

bonding resin   hybrid layer   dentin
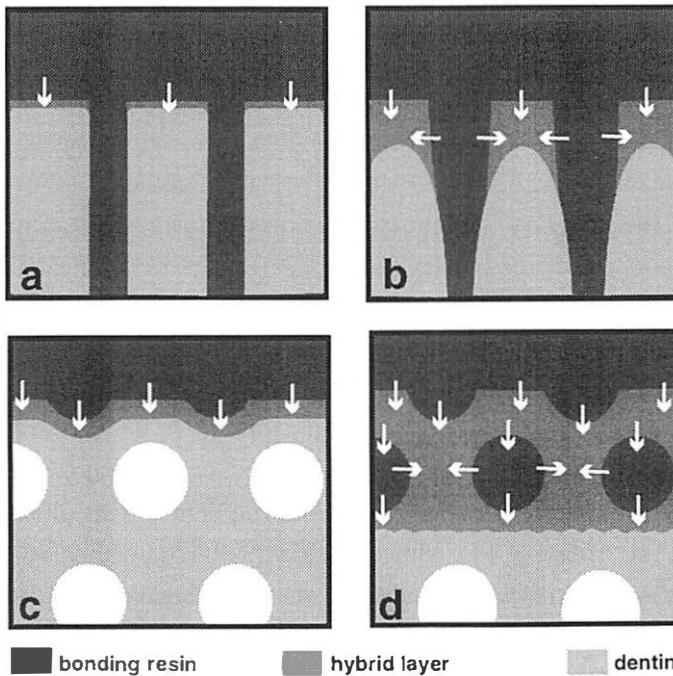
Figure 5. Schematic indication of the resin-dentin interfaces of the perpendicular group and the parallel group. The perpendicular group of a: self-etching priming system, and b: phosphoric acid etching system. The parallel group of c: the self-etching priming system, and d: the phosphoric acid etching system. The arrows indicate the penetration of the bonding resin into the demineralized dentin.

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perpendicular group, the modes of failure were classified as mixed failure within dentin cohesive and bonding resin cohesive (Fig. 2).

Scanning electron micrographs of the polished cross-sections of the bonded specimens of each adhesive system are shown in Figs. 3 and 4. There were statistically significant differences between the thickness of the hybrid layer of each adhesive system for the perpendicular group and the parallel group respectively (Table 2). For LB, SB, and OS, the hybrid layers created in the parallel group had greater thickness than those in the perpendicular group (p<0.05), and for FB, the parallel group tended to create thicker hybrid layer than the perpendicular group (p>0.05) (Table 2). For the perpendicular group of each of the bonding systems, the direction of the dentinal tubules and the resin tags with a characteristic funnel cone-shape was almost perpendicular to the interface (Figs. 3a, 3c, 4a, 4c). For the parallel group, the direction of the dentinal tubules and the resin tags were parallel to the interface (Figs. 3b, 3d, 4b, 4d). For LB and FB, the hybrid layer occupied a large area in the bonded interface, but there were few resin tags. For the parallel group bonded with SB and OS, the hybrid layer which also occupied a large area in the bonded interface, was created not only under the bonded interface but also beneath the resin tags. (Figs. 4b, 4d).

Discussion

In the present study, the hybrid layers created by the phosphoric acid etching systems (SB and OS) were thicker than those produced by the self etching-priming systems (LB and FB). This was true for both the perpendicular and the parallel groups (Table 2). For the perpendicular group, there were no statistically significant differences among the bond strengths of the adhesive systems. There were, however, statistically significant differences between the thicknesses of the hybrid layers of the adhesive systems. Previous investigators who examined resin bonded to dentin with perpendicular tubules, found no relationship between tensile bond strength and the thickness of the hybrid layer (Finger et.al., 1994; Nakabayashi and Saimi, 1996; Harada et.al., 1998). In the present study, the tensile bond strength of the parallel group tended to be higher than that of the perpendicular group for all the adhesive systems that were tested. This tendency was statistically significant for the systems which etch the dentin with phosphoric acid. Our results do not support those of Phrukkanon et.al. (1999) who compared the microtensile bond strengths of the Single Bond and an experimental adhesive to dentin as a function of tubule orientation. They found no consistent effect of tubule orientation on bond strength with either product. However, our sample size was over twice as large as theirs and the machining that was necessary to make their specimens into cylindrical hour-glass shapes may have created microcracks in the specimens.

For each adhesive system, the thickness of the hybrid layer of the parallel group was thicker than that of the perpendicular group. As shown in the Figs. 3 and 4, the resin-

dentin interfaces of the parallel group and perpendicular group showed different micromorphological appearances. Figure 5 illustrates the resin-dentin interfaces of the parallel group and the perpendicular group for each adhesive system. When the tubules have been cut perpendicularly, resin monomers can diffuse into acid etched intertubular dentin either directly from the surface or, indirectly by radial diffusion from the tubule lumen. However, the depth of removal of peritubular dentin was limited to the depth of the hybrid layer, a distance of only 4-5 μm. When resin was bonded to dentin surfaces cut parallel to the direction of the tubules, acid-etching removed peritubular dentin from long sections (ca. 20-30 μm) of tubules permitting much more radial diffusion into surrounding intertubular dentin (Fig. 5d).

The appearances of the resin-dentin interfaces that were treated with self etching-priming systems (LB and FB) were different from those were treated with phosphoric acid etching systems (SB and OS). The SEM photographs of the resin-dentin interfaces of LB and FB show very thin hybrid layers. In addition, resin tag formation was different in the parallel group and the perpendicular group (Fig 3). In the parallel groups of LB and FB, the hybrid layer was thin, but the interface consists predominantly of hybrid layer. That is, there are few resin tags (Figs. 3-b, d, 5-c). In the parallel groups of SB and OS, the hybrid layers were thicker than those of LB and FB, and resin tags were parallel to the bonded interface. A hybrid layer was observed not only at the interface but also beneath the resin tags (Figs 4-b, d, 5-d). For the perpendicular group of SB and OS, the dentinal tubules have been etched removing peritubular dentin and as a result, we observe funnel-shape resin tags (Figs. 4-a, c, 5-b). For the parallel group in all of the adhesive systems, the percent of the total interfacial area occupied by the hybrid layer was larger than that for the perpendicular group, because of the absence of resin tags. The hybrid layer was thicker than those of the perpendicular group because of the greater opportunity for radial diffusion of the adhesive from the entire length of the tubules into intertubular dentin (Fig. 5d). The bonded interface of the parallel group represents a unique condition where all of the bonded area is composed of a hybrid layer free of resin tags. Up to this time, such conditions had only been modeled theoretically (Pashley et.al., 1996).

Additionally, for the parallel group bonded with SB and OS, the phosphoric acid may have diffused into the dentin more deeply via tubules which were running almost parallel under the dentin surface, whereas the self-etching primers only slightly demineralized the superficial dentin surface. Deeper demineralization of the dentin makes it possible to form a thicker hybrid layer in the parallel group than in the perpendicular group. Deep demineralization in the parallel groups treated with SB or OS result in a more complex hybrid layer than is created by LB and FB. In the parallel group of SB and OS, the bonding resin could more easily penetrate into the collagen fibrils not only from the exposed dentin surface, but also through the peritubular dentin-free dentinal tubules which were surrounded by demineralized intertubular dentin (Fig.5-d). In the parallel group of specimens bonded with SB and OS, the larger area of the bonding resin which contacted

the hybrid layer, may have been the reason that the tendency of the bond strength difference between the parallel group and the perpendicular group reached statistical difference.

For the parallel group, the orientation of the tubules exposed to the adhesive surface was not completely parallel to the adhesive surface in all of the specimens. The schematic diagram for the parallel group of the Figure 5 represents an ideal. Actually, some of the tubules observed by SEM were oblique to the adhesive surface. Therefore, for the parallel group, the adhesive resin could penetrate not only from the exposed dentin surface, but also from the orifices of the tubules which were cut oblique to the adhesive surface, into the tubules surrounded by the demineralized dentin, and could diffuse into the demineralized dentin around the tubules.

The representative micromorphology of the failure pattern of the parallel group was classified as mixed failure within the bonding resin cohesive and composite resin cohesive (LB), or mixed failure within the adhesive interface and bonding resin cohesive (FB, SB, OS), and of the perpendicular group was classified as mixed failure within dentin cohesive and bonding resin cohesive (Fig 2). The morphological differences of the hybrid layer between the parallel group and the perpendicular group might have affected the stress concentrations at the resin-dentin interface, and might have caused these differences of the failure pattern of the debonded specimens. That is, the presence of resintags penetrating through the hybrid layers of the perpendicular group may provide more stress concentration foci at the bonded interface than occurs in hybrid layers free of resin tags at the surface.

The remaining dentin thickness (RDT) of the perpendicular group was similar to the distance from the pulp to the DEJ-side of the trimmed specimen of the parallel group (Table 3). This may have been caused by the method of specimen preparation in this study (Fig.1). For the perpendicular group, the bonded dentin surface was located just beneath the occlusal enamel of the center region. For the parallel group, the mesial half of the tooth was removed parallel to the long axis of the tooth to create a dentin surface for bonding in which of the tubules ran parallel to the bonded surface. Although relatively deeper dentin was used for the parallel group than for the perpendicular group, bond strengths of the parallel group were higher than those of the perpendicular group. In deep dentin, the amount of intertubular dentin matrix is decreased and the water content is increased due to the larger diameter and number of tubules, making it a difficult bonding substrate (Tagami et.al., 1990; Prati and Pashley, 1992) when the tubules are cut perpendicularly. However, by preparing the deep dentin surface in such a manner that the adhesive surface is parallel to the direction of the tubules, the influence of dentin wetness and limited intertubular dentin was avoided. This allowed for an evaluation of the intrinsic strength of bonded deep dentin.

Schüpbach et.al. (1997) measured the thickness of hybrid layers of class-V cavities bonded under *in vitro* pulpal pressure, using confocal scanning light microscopy to

evaluate the effect of tubule direction on hybrid layer formation. The wetness of dentin is important when testing adhesive materials *in vitro* with the intention of simulating the *in vivo* situation (Pashley et.al., 1991; Pereira et.al., 1999). In our study, there were more tubules connected to the dentin surface of the perpendicular group than were seen at the parallel group, and the wetness of the dentin surface would be different between the two groups. In Pereira's study (1999), which measured tensile bond strengths of Liner Bond II and One-Step to cross-sectioned tubules with or without hydrostatic pressure, they concluded that the intrinsic wetness of the dentin significantly affected regional bond strengths, whereas the application of a positive hydrostatic pressure had little influence. Thus, it is unlikely that application of pulpal pressure would be useful for evaluating the influence of the tubule direction on the bond strength to dentin, since few tubules in the parallel group were continuous with the bonded interface.

Conclusions

The tensile bond strength of the groups with tubules parallel to the bonded interface was higher than that of tubules cut perpendicularly, when the bond strength was measured on flat coronal dentin surfaces. This tendency reached statistical significance using Single Bond and One-Step. Thus, the direction of tubules appears to be an important variable determining bond strength. The tubule direction may determine the intrinsic wetness of the surface.

Chapter 4

Effects of different burs on dentin bond strengths of self-etching primer bonding systems

Introduction

High bond strengths of newly developed dentin bonding systems are regularly reported in many *in vitro* studies (Harada et.al., 2000; Ogata et.al., 2001). Most of these laboratory bonding studies are done using silicon carbide abrasive papers for preparing the dentin surfaces, whereas different cutting instruments such as diamond or steel burs are routinely used in the clinic. Therefore, information on the effect of cutting dentin with different burs on resin-dentin bond strength is essential for appropriate clinical use of dentin bonding systems. After the mechanical preparation of the cavity with any dental instrument like bur, an amorphous layer of organic and inorganic debris, the so-called smear layer is formed on the surface (Pashley, 1984). It is well known that the quantity and the quality of smear layer vary widely depending upon the manner in which they were created (Eick et.al., 1970; Gilboe et.al., 1980). Difference in smear layer prepared with bur cutting or abrasive paper have been reported to affect the bond strengths of resins to dentin (Tagami et.al., 1991; Watanabe et.al., 1994a). Sekimoto et.al. (1999) has also suggested that dentin bonding systems may have their effectiveness reduced when the dentin has been cut with burs.

Self-etching primers contain an acidic resin monomer. When the self-etching primers are applied to the smear layer-covered tooth surface, these acidic primers simultaneously modify or dissolve the smear layer and decalcify both enamel and dentin surfaces (Watanabe et.al., 1994b). It has been reported that adhesive systems using self-etching primer produce good adhesion to both enamel and dentin (Barkmeier et.al., 1995; Kanemura et.al., 1999; Harada et.al., 2000). These systems also have been reported to demonstrate excellent clinical performance and high retention rate in clinical situations (Latta et.al., 1997). On the other hand, Toida et.al.(1995) reported that the tensile bond strength of the self-etching primer bonding system to the dentin surfaces which were prepared by burs were lower than those of which were prepared by #600 silicon carbide abrasive paper. They concluded that the rough and thick smear layer created with burs should be removed with acid etching in order to obtain more reliable and higher bond strengths.

The purpose of this study was to evaluate the effect of cutting dentin with burs of different types and grits on tensile bond strength using three commercially available adhesive systems using self-etching primer. The null hypothesis was that the different surface preparation methods would have no effect on resin-dentin bond strength.

Methods and materials

The specimen preparation method used for tensile bond strength testing and SEM observation is illustrated in Figure 1. Thirty-six frozen extracted caries-free human third molars were thawed and used for micro tensile testing (Sano et.al., 1994a). The occlusal enamel was removed perpendicularly to the long axis of the tooth by means of a model trimmer under running water, and a flat dentin surface was polished with #600 SiC abrasive paper under running water. The teeth were then divided into four groups (nine teeth for each group) according to bur types and grits shown in Table 1, 1: fine cut 12 blade tapered fissure steel bur (SB600 group), 2: cross-cut tapered fissure steel bur (SB703 group), 3: regular grit diamond bur (the average diamond particle size: 100 μm) (DB group), 4: Control surface abraded with 600 grit SiC paper (AP#600 group). The dentin surfaces of SB600 and SB703 groups were cut with the respective steel burs which were mounted in a straight micromotor handpiece (INTRAmatic LUX2 10LN, Kavo, Germany) at 2000 rpm. The teeth in DB group were cut with a diamond bur which was mounted in a dental turbine (SUPER TORQUE LUX2 640B, Kavo, Germany) at 100,000 ～ 120,000 rpm. The teeth were prepared by bur for 30 passes across the dentin surface by the same operator under copious air water spray until the uniform scratches by each bur were made on the whole dentin surfaces. For the AP#600 group, teeth were prepared by use of 20 strokes of 15 cm length on #600-grit SiC paper under running water with hand pressure.
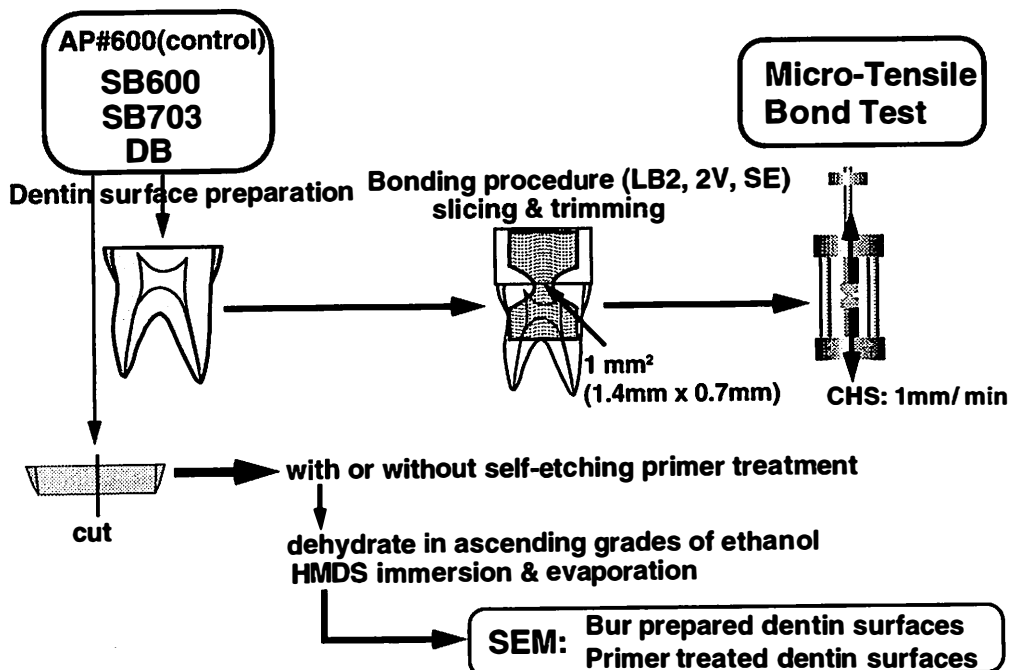

Figure 1. Schematic showing the specimen preparation method used for tensile bond strength testing, SEM observations of the dentin surfaces prepared with the burs or abrasive paper, and SEM observation of the dentin surfaces of each group treated with the self-etching primers

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Table 1. Identification of groups by dentin surface preparation

Group	method for preparation	Manufacturer	rpm
AP#600	#600 silicon carbide paper	Marumoto Struers Tokyo, Japan	-
DB	Diamond point, FG-REGULAR, #103 (average diamond particle size: 100 μm)	Shofu, Kyoto, Japan	100,000~120,000 rpm
SB600	Fine cut tapered fissure steel bur, #600 (12 blades)	Hager & Meisinger, Dusseldorf, Germany	2,000 rpm
SB703	Cross cut tapered fissure steel bur, #703 (6 blades)	Dentech, Tokyo, Japan	2, 000 rpm

Table 2. Adhesive systems used for bonding

System	Ingredients	primer pH	Procedures	Manufacturer
Clearfil Liner Bond 2 (LB2)				
LB-primer A	Phenyl-P, 5-NMSA, ethanol, photoinitiator, accelerators	1.51(A+B)	a; b (30s);	Kuraray Medical,
LB-Primer B	HEMA ,water		c; d (20s)	Tokyo,
LB-Bond	MDP, HEMA, Bis-GMA, microfiller, photoinitiator, accelerators			Japan
Clearfil Liner Bond 2V (2V)				
Primer Liquid A	MDP, HEMA, water, photoinitiator, accelerators	3.03(A+B)	a; b (30s);	Kuraray Medical,
Primer Liquid B	HEMA, water, initiator		c; d (20s)	Tokyo,
Bond Liquid A	MDP, HEMA, Bis-GMA, microfiller, photoinitiator, accelerators			Japan
Clearfil SE BOND (SE)				
PRIMER	MDP, HEMA, water, multifunctional methacrylate, photoinitiator	2.04	b (20s); c; d (10s)	Kuraray Medical, Tokyo,
BOND	MDP, HEMA, multifunctional methacrylate, microfiller, photoinitiator			Japan

Procedures : (a) mix primer; (b) apply primer; (c) apply adhesive; (d) light-cure

After preparation of the dentin surfaces, all teeth were treated with one of the three adhesive systems shown in Table 2 (three teeth for each bonding system), according to the manufacturers' recommendations. After each adhesive resin was light-cured, a resin composite (Clearfil AP-X, Kuraray Medical, Tokyo, Japan) was built up incrementally to a height of 5 mm to ensure sufficient bulk for the micro-tensile bond test (Sano et.al., 1994a). After light-curing, the specimens were stored in 37℃ water for 24 hours.

The resin-bonded teeth were then serially sectioned parallel to the long axis of the tooth into 7-8 slices, approximately 0.7 mm thick, using a low speed diamond saw (Leitz 1600 Microtome, Leica Instruments GmbH, Heidelberg, Germany) under water cooling. The bonded areas were isolated using a superfine diamond bur (c16ff, GC Ltd.,Tokyo,Japan) to create an hourglass configuration with a cross-sectional area of approximately 1 mm². The final width and thickness of the bonded area were measured by means of a digital caliper to adjust the raw bonding data to an equalized bond / 1 mm². The specimens were then attached to the testing device (Bencor-Multi-T, Danville Engineering Co., San Ramon, CA, 94583) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA,

91720) which, in turn, was placed in a table-top material tester (EZ-Test, Shimadzu Co., Kyoto, Japan) for tensile testing at a cross-head speed of 1 mm / min (Sano et.al., 1994a) (Fig. 1). After the bond strengths were measured, all of the specimens were both visually and microscopically (x20, DENTCRAFT DENT-OPTIC DX, Yoshida, Tokyo, Japan) inspected to determine the modes of fracture. In addition, representative samples were also observed using a scanning electron microscope (JXA-840, JEOL, Tokyo, Japan) to confirm the accuracy of the visual inspection.

Statistical analysis of the tensile bond strengths were performed using one-way and two-way ANOVA, and Fisher's PLSD test at 95% level of confidence.

Eight additional third molars were used for SEM observation of the dentin surfaces prepared with the burs or abrasive paper, before and after treatment with the self-etching primers. Flat dentin discs with thickness of approximately 1 to 1.5 mm were cut perpendicularly to the long axis of the tooth, by means of a low speed diamond saw (Leitz 1600 Microtome, Leica Instruments GmbH, Heidelberg, Germany) from the mid-coronal part of the teeth. Each disk was cut into halves. Four half-discs were used for each group (SB600, SB703, DB, or AP#600). Dentin surfaces were prepared with burs or silicon carbide paper as was done for dentin bond strength measurement described above. For the SEM observation of the degree of etching of these dentin surfaces, the surfaces of the three of the four half-disks were treated with one of the three self-etching primers. After each application time, the primer components were removed with 50% acetone/water solution (Harada et.al., 2000). The fourth half-disk was used for observation of the smeared surface. All specimens were then dehydrated in ascending grades of ethanol (50%, 75%, 95% and 100% for 30 min. each) followed by immersion in hexamethyldisilazane [(CH$_3$)$_3$SiNHSi(CH$_3$)$_3$, HMDS, Pierce, Rockford, Illinois 61105, USA]] for 10 min, placed on a filter paper inside a covered glass vial, and air-dried at room temperature (Perdigão et.al., 1995). The specimens were then gold sputter-coated and observed with a scanning electron microscope (JXA-840, JEOL, Tokyo, Japan) at an accelerating voltage of 10 keV.

Results

The micro-tensile bond strength (μTBS) results of each group are shown in Figure 2 and Table 3. All dentin surfaces which were prepared with #600 abrasive paper (AP#600 group) prior to the bonding procedure, produced the highest (although not statistically significant) tensile bond strengths (LB2: 40.4±9.7 MPa ; 2V: 54.4±11.3 MPa ; SE: 47.0±13.7 MPa). On the other hand, the DB group resulted in significantly lower bond strengths than those of the AP#600 group (LB2: 25.1±12.0 MPa ; 2V: 25.5±8.1 MPa ; SE: 30.2±7.9 MPa). For all adhesive systems, groups with bond strengths from highest to lowest were AP#600 > SB600 > SB703 > DB. There were statistically significant differences among the groups when the dentin surfaces were treated with 2V (p<0.05). Two-way ANOVA analysis revealed that there was a statistically significant interaction
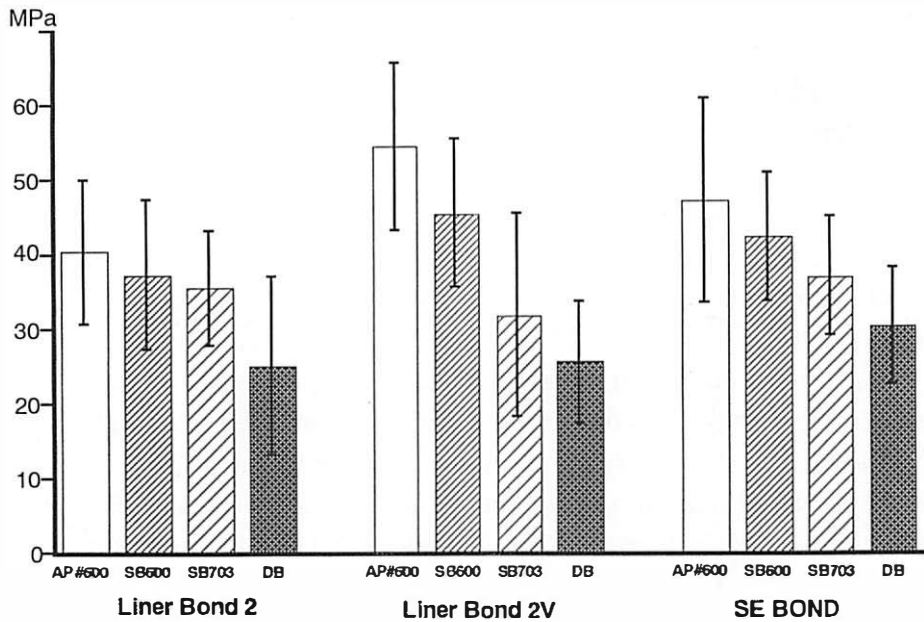
Figure 2. Results of micro tensile bond strengths for each group

between the bonding systems and the methods of dentin surface preparation ($p = 0.019$).

　　　　When visually inspected, most specimens showed interfacial adhesive failure. This was confirmed by light microscopic examination (x20). The representative micromorphology of the failure pattern was classified as mixed failures within dentin and bonding resin. There was no remarkable difference in the failure patterns among all the groups.

　　　　Scanning electron micrographs of each prepared dentin surface are shown in Figure 3, and micrographs of the prepared dentin surface treated with each self-etching primer are shown in Figures 4 - 6. For the groups AP#600, SB600 and SB703, the dentin surfaces revealed many scratches left by the abrasive paper or burs, and the surfaces were

Table 3. Results of micro tensile bond strengths for each group (mean $\pm$ SD) (MPa)

	Liner Bond 2	Liner Bond 2V	SE BOND
AP#600	40.4 $\pm$ 9.7[ab]	54.4 $\pm$ 11.3	47.0 $\pm$ 13.7[b]
	(n = 23)	(n = 19)	(n = 22)
SB600	37.3 $\pm$ 10.1[a]	45.5 $\pm$ 10.0[eh]	42.2 $\pm$ 8.6[bcfgh]
	(n = 23)	(n = 25)	(n = 24)
SB703	35.6 $\pm$ 7.7[ad]	31.8 $\pm$ 13.5[d]	36.9 $\pm$ 7.9[cd]
	(n = 22)	(n = 22)	(n = 24)
DB	25.1 $\pm$ 12.0[c]	25.5 $\pm$ 8.1[c]	30.2 $\pm$ 7.9[c]
	(n = 22)	(n = 22)	(n = 21)

(n) : number of the slabs tested.   Groups that are not significantly different are marked with the same superscript letter (p>0.05).

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Figure 3. SEM of the prepared dentin surfaces of each group. a: AP#600 group b: SB600 group c: SB703 group d: DB group (Original magnification 500x ; bar = 10 μm)

Figure 4. SEM of Clearfil Liner Bond 2 primer treated dentin surfaces. a: AP#600 group b: SB600 group c: SB703 group d: DB group (Original magnification 8000x ; bar = 1 μm)

Figure 5.   SEM of Clearfil Liner Bond 2V primer treated surfaces. a: AP#600 group b: SB600 group
c: SB703 group d: DB group (Original magnification 8000x ; bar = 1 μm
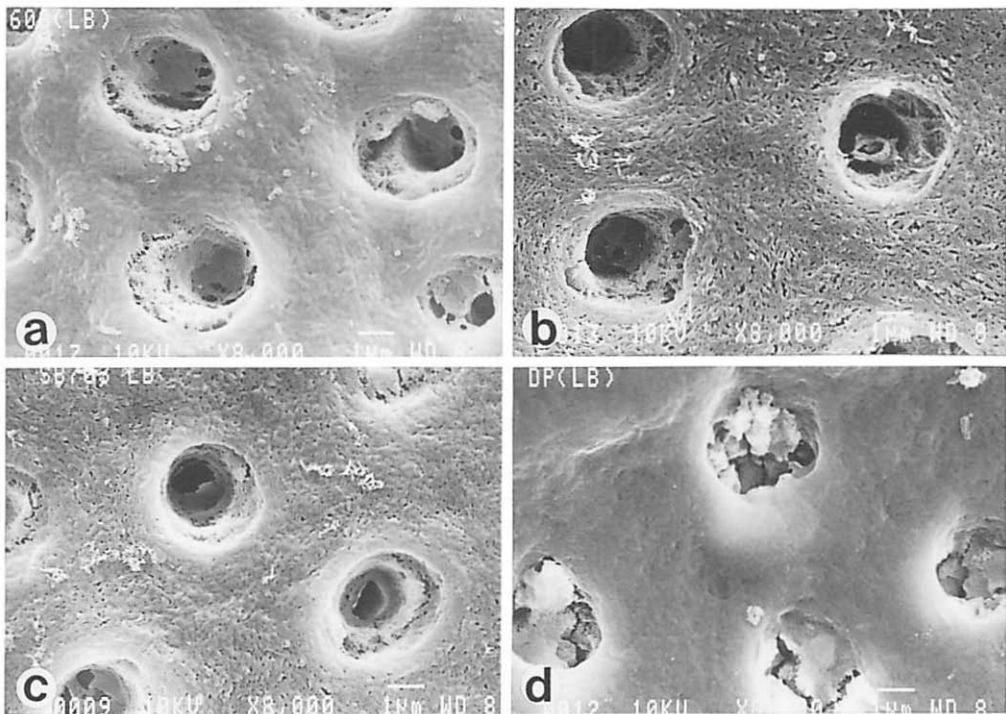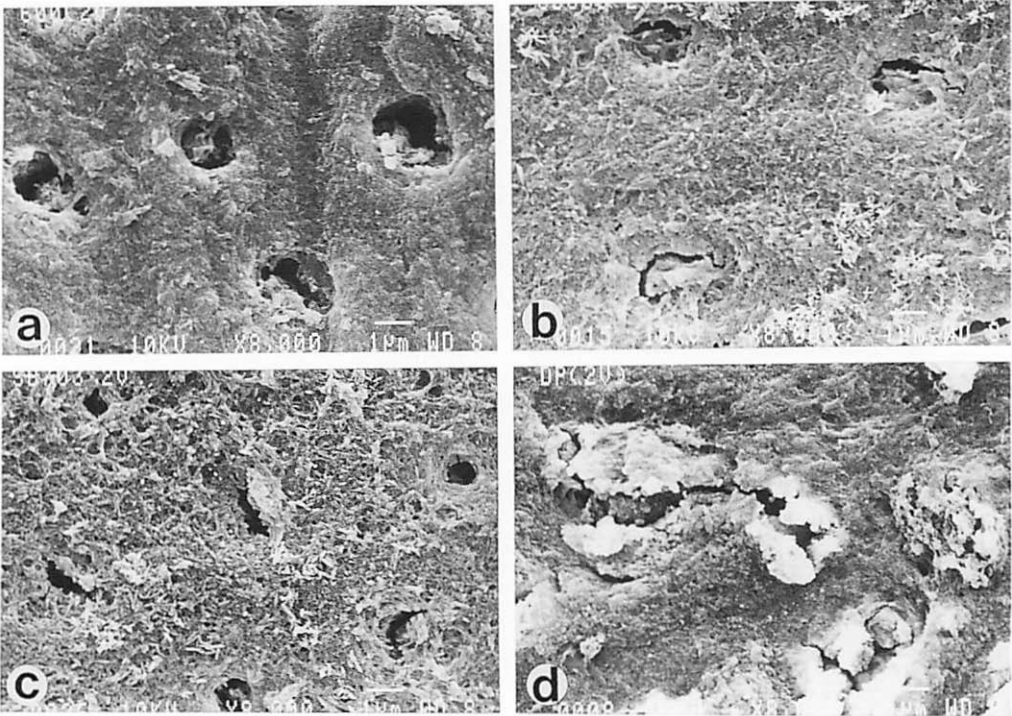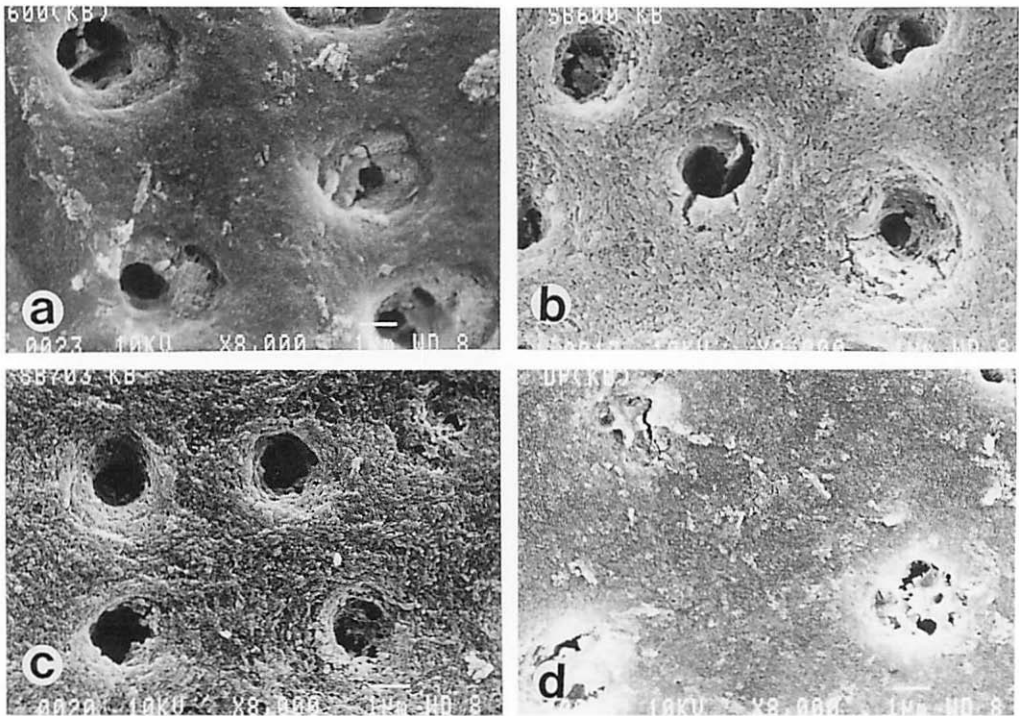

Figure 6.   SEM of Clearfil SE BOND primer treated surfaces. a: AP#600 group b: SB600 group c:
SB703 group d: DB group (Original magnification 8000x ; bar = 1 μm)

completely covered with smear layer. Dentinal tubules which were occluded by the smear plugs were also observed over the entire surface (Figs.3 a-c). The SEM observation of the dentin surface of the DB group demonstrated that grooves left by the bur were coarser than the other groups (Fig.3d). An irregular thick smear layer without any evidence of underlying dentinal tubules was seen on the top of the grooves while dentinal tubules occluded by the smear plugs could be observed at the bottom of the grooves (Fig. 3d). When the self-etching primers were rinsed from the prepared dentin surfaces using 50% acetone-water, the extent of etching was revealed. For the AP#600, SB600 and SB703 groups of the LB2 primer treated surface, and the AP#600, SB600 groups of the SE primer treated surface, the smear layer on the dentin surface and the smear plugs in the dentinal tubules were removed. For these groups, the intertubular dentin and the peritubular dentin of the tubule orifices were slightly etched, and the edge of the dentinal tubules were clearly observed (Figs 4 a-c, 6a,b). For the DB group treated with LB2 and SE, the smear layer on the dentin surface was removed, but the dentinal tubules remained occluded by residual smear plugs (Figs 4d, 6d). For the AP#600 group treated with 2V, the smear layer on the dentin surface was removed, but there was still residual smear plugs within the dentinal tubules, even though much of the peritubular dentin matrix was removed thereby enlarging the tubule orifices (Fig 5a). For the SB600 and the SB703 groups treated with 2V, the tubule orifices were evident but not enlarged and occluded with residual smear plugs (Figs 5b,c). For the DB group treated with 2V, the dentin surface remained covered with a great amount of smear layer (Fig 5d).

Discussion

Self-etching primers combine the etching and priming steps into one procedure. The self-etching primers are applied to smear layer-covered dentin, followed by brief air-drying and application of the bonding resin. The manufacturers' instructions specify that the primed surface should not be rinsed with water. Therefore, the self-etching primers' acidic component demineralize through the smear layer and diffuse a short distance into the underlying dentin, resulting in the creation of a thin hybrid layer but strong bonds to dentin (Watanabe et.al., 1994b; Chigira et.al., 1994). The self-etching primers disclose less etching ability because of their relatively high pH (Table 2) when compared with phosphoric acid etchants' pHs between (3M Scotchbond Etchant (35% phosphoric acid) = 0.6, information from the manufacturer). Therefore, it is believed that bond strengths of self-etching primer bonding systems to dentin could be affected by differences in the quantity and quality of the smear layer because of the weak acidity of self-etching primers. Watanabe et.al., (1994a) reported that the dentin bond strengths of an experimental self-etching primer bonding system (self-etching primer: aqueous solution of 20% Phenyl-P and 30% HEMA; bonding resin: 5% Phenyl-P in TEGDMA) were affected by the different smear layers that were created by the different grits of abrasive papers. Toida et.al., (1995)

reported that the tensile bond strengths of that same system used by Watanabe et.al., (1994a), to dentin prepared with diamond or steel burs were significantly lower than those to the dentin prepared with #600-grit abrasive paper. On the other hand, Akimoto et.al., (1999) reported that the micro-tensile bond strengths of the Liner Bond 2V and Clearfil SE Bond were not affected by dentin surface condition. They bonded to dentin surfaces prepared with #180 or #600-grit abrasive papers versus mirror-like surfaces of dentin. In the present study, the dentin surfaces were prepared with an abrasive paper or different types of burs, and the micro-tensile bond strengths of the LB2, 2V and SE were shown to be affected by dentin surface condition. The tensile bond strength of these adhesive systems to the bur-prepared dentin surfaces were lower than those to the #600-grit abrasive paper prepared dentin surfaces. For all adhesive systems, the DB group resulted in significantly (p<0.05) lower bond strengths than those of the AP#600 group. The quality and quantity of the smear layer created by the bur cutting should be different to that created by the #600 abrasive paper. Indeed, it was reported that the smear layer created by diamond or steel bur was coarser, and its mechanical property was weaker than that created by the #600-grit abrasive paper (Toida et.al., 1995). The SEM observations of the dentin surface of the DB group demonstrated that grooves left by the bur were coarser than were seen in the other groups. Bands of the dentin surface were completely covered with an irregular thick smear layer that alternated with bands in which the dentinal tubules occluded by the smear plugs could be observed (Fig 3d). This distinct structure of the smear layer of the DB group might be considered as the reason of the decrease of the bond strengths seen in this group when treated with all of the adhesive systems (Fig. 2, Table 3).

For the AP#600 group, 2V produced highest bond strength among the three adhesive systems. However, this high bond strength significantly decreased when the dentin surfaces were prepared with the burs. Ranking of bond strengths from highest to lowest yielded the following results: AP#600 > SB600 > SB703 > DB. This order was common to all adhesive systems, and between all groups treated with 2V, statistically significant differences existed (p<0.05). For the AP#600 group, SEM observation of primer-treated dentin surfaces demonstrated that the smear layer on the dentin surface was removed by the primers of all adhesive systems (Figs. 4a, 5a, 6a). For the LB2 and SE primed-surfaces of this group, the smear plugs were removed, the intertubular dentin and the peritubular dentin of the tubule orifices were slightly etched, and the edges of the dentinal tubules were clearly observed (Figs. 4a, 6a). On the other hand, for the DB group treated with Clearfil Liner Bond 2, the SB600, SB703, and DB groups treated with Clearfil Liner Bond 2V, and the DB group treated with Clearfil SE BOND, which revealed significantly lower bond strengths than the AP#600 group, the dentinal tubules remained occluded by residual smear plugs, and the intertubular dentin surface remained covered with smear layer (Figs. 4d, 5b-d, 6d). These structural differences of primer-treated dentin surfaces seem to reflect the difference of the demineralization effect which is due to the pH

of the self-etching primers. The pH of the primers were 1.51 for LB2 (primer A+B), 3.03 for 2V (primer A+B), and 2.04 for SE (Table 2, information from the manufacturer). Clearfil Liner Bond 2 primer contains the acidic monomer Phenyl-P (2-methacryloyloxyethyl phenyl phosphate). The manufacturer reformulated the Clearfil Liner Bond 2V primer by substituting MDP (10-methacryloxydecyl dihydrogen phosphate) for Phenyl-P. Since the pKa of MDP is higher than that of Phenyl-P, Clearfil Liner Bond 2V primer has a higher pH than Clearfil Liner Bond 2 primer (Nakajima et.al., 1999b). The difference of the quality and quantity of the smear layer created by the bur cutting may have strongly affected the bonding property of Clearfil Liner Bond 2V to dentin, since pH of this primer was milder than those of the other two primers. In spite of the smaller degree of demineralization, 2V provides good bond strength to AP#600 prepared dentin. One of the reasons for this might be that the 2V primer, which contains MDP as an acidic monomer and chemical polymerizing catalyst, successively penetrated into mildly demineralized collagen network. Then, the polymerized primer and bonding resin created a thin hybrid layer (1 μm), contributing to the improvement of the bond strength (Harada et.al., 2000).

When the dentin surfaces were cut by the different burs, some of the smear layers which could not be completely demineralized or removed by self-etching primers, remained on the dentin surface. Thus, demineralization of the underlying dentin, and further penetration of the bonding resin into the demineralized dentin could have been compromised. This may be the reason why bond strengths to dentin prepared by burs decreased, especially for the DB group. Therefore, selection of the bur for cutting of dentin surface for direct resin composite restoration is important to produce optimal bonding of self-etching primer bonding systems to dentin. Cutting the dentin surface with regular grit diamond burs should be avoided, or followed with finishing the cavity surface with steel burs should be done when bonding with self-etching primer bonding systems. Clinically, initial opening of a carious cavity is done with diamond or carbide burs, and generally followed by removal of carious dentin with round steel burs (Fusayama, 1980). In this study, the SB600 groups of all adhesive systems produced relatively high tensile bond strengths among the groups which used the bur (groups SB600, SB703, DB). Therefore, by using steel burs at low speed, relatively high bond strengths could be expected for the clinical use of these adhesive systems.

The high speed turning of the bur induces increase of thermal and mechanical stress. An abrading cutting instrument like a diamond bur creates more frictional stress increasing when compared to a cutting instrument like a steel bur. Actually, during cavity preparation for direct composite resin restoration, diamond burs are often used to open and rough out of the cavity at high speed, and low speed steel burs are used to excavate carious dentin. Since the steel burs were used at low speeds and the diamond bur was used at high speed, it is unclear that the lowest bond strengths were due to high speed or diamond bur or both. The final result is reduced bond strength when using self-etching primers. Further research is necessary in order to isolate the exact cause of that effect.

Previously, in a study of regional bond strengths to cervical wedge-shaped cavity using self-etching primer bonding systems, we reported that the bond strengths of Clearfil Liner Bond 2 was improved when LB-primer applied multiply to the cavity without expending the application period recommended by the manufacturer (Ogata et.al., 1999, see chapter 2). We used the multiple primer application method intending to supply adequate amount of primer into a wedge-shaped defect to replace primer that flows off the walls due to gravity. In that study, multiple layers of LB-primer could dissolve the smear layer completely and the bonding resin was able to penetrate more deeply into the demineralized dentin. Multiple primer application might overcome the resistance of bur-created smear layers to the etching effects of these primers leading to improvement of the bonding property of these systems.

Most clinically prepared cavities actually include regions of normal and sclerotic or caries-affected dentin. The chemical composition of smear layers may change due to the structure from which it is formed (Pashley, 1992). Kimochi et.al. (1999) suggested that the amorphous structure which was observed on the surface of the caries-affected dentin may inhibit hybrid layer formation by self-etching primer bonding systems. According to their study, the micro-tensile bond strengths of Clearfil SE Bond to caries-affected dentin showed significantly lower values than those of normal dentin. Nakajima et.al.(1999b) reported that the micro-tensile bond strengths of Clearfil Liner Bond 2 and Clearfil Liner Bond 2V to caries-affected dentin showed significantly lower values than those of normal dentin. This difference between caries-affected and normal dentin was not found when the substrates were acid-etched with 32-35% phosphoric acid and bonded with single bottle adhesives (Nakajima et.al., 2000a). Thus, development of bonding resins and procedures that produce high, uniform bond strengths to all types of dentin, whether normal or abnormal, as well as to the various types of smear layers prepared in various way has still not been achieved. More research needs to be done on clinically -relevant dentin substrates using clinically relevant surface preparations (i.e., high speed vs. low speed burs). The results of this study do not support the hypothesis that dentin preparation with different burs has no effect on resin-dentin bond strength using self-etching primers.

Conclusions

All self-etching primer bonding systems used in this study disclosed significantly highest tensile bond strengths for the AP#600 group, and significantly lowest bond strengths for the DB group. Groups with bond strengths from highest to lowest were AP#600 > SB600 > SB703 > DB, for all adhesive systems. There were statistically significant differences among all groups when the dentin surfaces were treated with 2V ($p<0.05$). Selection of the bur for cavity preparation is an important factor for improved bonding of adhesive systems using self-etching primer to dentin.

Chapter 5

Effect of self-etching primer vs. phosphoric acid etchant
on bonding to bur-prepared dentin.

Introduction

After the mechanical preparation of cavities with any dental instrument such as a bur, an amorphous layer of organic and inorganic debris, the so-called smear layer is created over the tooth surface (Pashley, 1984). This layer covers the dentin surface, adheres weakly to the underlying dentin, occludes the entrance of the dentinal tubules, and cannot be removed by ordinary water spray. It is well known that the quality and the quantity of the smear layer varies widely according to the way it is created (Eick et.al., 1970; Gilboe et.al., 1980). Although the smear layer diminishes the dentin permeability, it may impede the direct contact of the bonding material with the dentin (Pashley, 1984; Nakabayashi and Pashley, 1998). It has been reported that the bond strength to dentin depends on characteristics of the smear layer created by rotary cutting instrument on the dentin surface (Tagami et.al., 1991; Watanabe et.al., 1994a; Toida et.al., 1995; Sekimoto et.al., 1999). In order to obtain good adhesion to dentin, the smear layer should be removed or modified with conditioners such as acidic solutions (Toida et.al., 1995).

Previously, we have reported the effects of different types of burs on dentin bond strengths of three self-etching primer bonding systems, Clearfil Liner Bond 2, Clearfil Liner Bond 2V, and Clearfil SE Bond (Kuraray Medical) (Ogata et.al., 2001b, see chapter 4). High bond strengths produced by these bonding systems have been reported in in vitro studies which used #600-grit silicon carbide abrasive papers for dentin surface preparation (Harada et.al., 2000; Ogata et.al., 2001a). Most of laboratory bonding studies are done using silicon carbide abrasive papers for preparing the dentin surfaces, whereas different cutting instruments such as diamond or steel burs are routinely used in the clinic. In our previous study, however, the high bond strength obtaining using #600 grit SiC paper decreased when the dentin surfaces had been prepared with the burs, and particularly when it was cut using a regular-grit diamond bur (Ogata et.al., 2001b). The self-etching primers produced less etching because of their relatively high pH(1.5 - 3.0, information from the manufacturer), when compared with 32-37 % phosphoric acid pHs (-0.43 to 0.02, Perdigão et.al., 1996). When the dentin surfaces were prepared by burs, some of the smear layers could not be completely removed by self-etching primers due to their weak acidity. This may have compromised demineralization of the underlying dentin, and further penetration of the bonding resin into the demineralized dentin. We concluded that this may be the reason why bond strengths to dentin prepared with burs decreased, especially for the group prepared with diamond burs (Ogata et.al., 2001b). On the other hand, it has been reported that dentin bond strength was high and stable when the smear layer created by

various ways was removed with stronger etchants such as a phosphoric acid or a citric acid etchant (Tagami et.al., 1991; Toida et.al., 1995). Thus, information on the comparative effects of another self-etching primer vs. 35% phosphoric acid on bonding to bur-prepared dentin is desirable for determining appropriate clinical use of dentin bonding systems.

The goal of this study was to evaluate the effect of dentin conditioners on tensile bond strength to dentin which was prepared with different types of burs using a self-etching primer system and a phosphoric acid etching system.

Methods and materials

The specimen preparation method used for tensile bond strength testing and SEM observation is illustrated in Figure 1. The method was exactly what was done in our previous study (Ogata et.al., 2001b). Twenty-four frozen extracted caries-free human third molars were thawed and used for micro tensile testing (Sano et.al., 1994a). The occlusal enamel was removed perpendicularly to the long axis of the tooth by means of a model trimmer under running water, and a flat dentin surface was polished with #600 SiC abrasive paper under running water. The teeth were then divided into four groups (six teeth for each group) according to bur types and grits shown in Table 1, 1: fine cut 12 blade tapered fissure steel bur (SB600 group), 2: cross-cut tapered fissure steel bur (SB703 group), 3: regular grit diamond bur (the average diamond particle size: 100 μm) (DB group), 4:
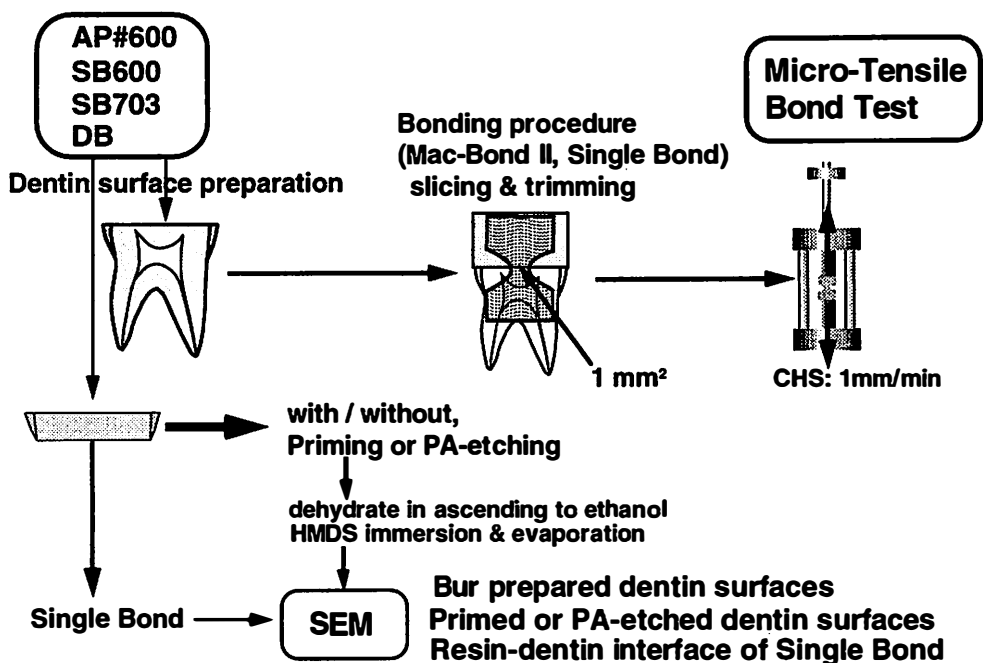

Figure 1. Schematic showing the specimen preparation method used for tensile bond strength testing, SEM observations of the dentin surfaces prepared with the burs or abrasive paper, and SEM observation of the dentin surfaces of each group treated with the self-etching primers. CHS=cross head speed.

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Table 1. Identification of groups by dentin surface preparation

Group	method for preparation	Manufacturer	rpm
AP#600	#600 silicon carbide paper	Marumoto Struers Tokyo, Japan	-
SB600	Fine cut tapered fissure steel bur, #600 (12 blades)	Hager & Meisinger, Dusseldorf, Germany	2,000 rpm
SB703	Cross cut tapered fissure steel bur, #703 (6 blades)	Dentech, Tokyo, Japan	2,000 rpm
DB	Diamond point, FG-REGULAR, #103 (average diamond particle size: 100 μm)	Shofu, Kyoto, Japan	100,000~120,000 rpm

Control surface abraded with 600 grit SiC paper (AP#600 group). The dentin surfaces of SB600 and SB703 groups were cut with the respective steel burs which were mounted in a straight micromotor handpiece (Intramatic Lux2 10LN, Kavo, Germany) at 2000 rpm. The teeth in DB group were cut with a diamond bur which was mounted in a dental turbine (Super Torque Lux2 640B, Kavo, Germany) at 100,000 - 120,000 rpm. The teeth were prepared by making 30 passes with the bur across the dentin surface, by the same operator, under copious air water spray until the uniform scratches by each bur covered the entire dentin surface. For the AP#600 group, teeth were prepared by use of 20 strokes of 15 cm length on #600-grit SiC paper under running water with hand pressure.

After preparation of the dentin surfaces, all teeth were treated with a self-etching primer system ; Mac-Bond II (Tokuyama Dental), or a one-bottle wet bonding system using 35% phosphoric acid etchant ; Single Bond (3M), according to the manufacturers' instructions (Table 2). After each adhesive resin was light-cured, a resin composite was built up using four layers of Clearfil AP-X (Kuraray Medical Co., Ltd., Tokyo, Japan) to a height of 5 mm to ensure sufficient bulk for the micro-tensile bond test (Sano et.al., 1994a). Each layer was light cured for 20 seconds. Specimens were then stored in 37°C water for 24 hours.

Table 2. Adhesive systems used for bonding

System	Ingredients	pH	Procedures	Manufacturer
Mac-Bond II				
Primer A	MAC-10, methacryloyloxyalkyl acid phosphate, isopropanol, acetone, water, accelerators	1.7(A+B)	a; b (20s); c; d (10s)	Tokuyama Dental, Tokyo, Japan
Primer B	isopropanol, water			
Bonding agent	MAC-10, Bis-GMA, TEGDMA, HEMA, photoinitiator			
Single Bond				
Etchant	35% phosphoric acid gel	0.6	e(15s); f; g; h; d(10s)	3M, St. Paul, MN, USA
Adhesive	Bis-GMA, HEMA, polyalkenoic acid copolymer, ethanol, water, photoinitiator			

Procedures : (a) mix primer; (b) apply primer; (c) apply adhesive; (d) light-cure; (e) acid-etching; (f) rinse; (g) blot-dry; (h) apply 2 coats of adhesive

The resin-bonded teeth were then serially sectioned parallel to the long axis of the tooth into 7-8 slices, approximately 0.7 mm thick, using a low speed diamond saw (Leitz 1600 Microtome, Leica Instruments GmbH, Heidelberg, Germany) under water cooling. The bonded areas were isolated using a superfine diamond bur (c16ff, GC Ltd.,Tokyo,Japan) to create an hourglass configuration with a cross-sectional area of approximately 1 mm^2. The final width and thickness of the bonded area were measured by means of a digital caliper to adjust the raw bonding data to an equalized bond / 1 mm^2. The specimens were then attached to a testing device (Bencor-Multi-T, Danville Engineering Co., San Ramon, CA, 94583) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, 91720) which, in turn, was placed in a table-top material tester (EZ-Test, Shimadzu Co., Kyoto, Japan) for tensile testing at a cross-head speed of 1 mm / min (Sano et.al., 1994a) (Fig. 1). After the bond strengths were measured, all of the specimens were inspected, both visually and microscopically (x20, Dentcraft Dent-Optics DX, Yoshida, Tokyo, Japan), to determine the modes of failure. In addition, representative samples were also observed using a scanning electron microscope (JXA-840, JEOL, Tokyo, Japan) to confirm the accuracy of the visual inspection.

Statistical analysis of the tensile bond strengths were performed using one-way and two- way ANOVA, and Fisher's PLSD test at 95% level of confidence.

Six additional third molars were used for SEM observation of the dentin surfaces prepared with the burs or abrasive paper, before and after treatment with the self-etching primer of Mac-Bond II or the 35% phosphoric acid of Single Bond. Flat dentin discs with thickness of approximately 1 to 1.5 mm were cut perpendicularly to the long axis of the tooth, by means of a low speed diamond saw (Leitz 1600 Microtome, Leica Instruments GmbH, Heidelberg, Germany) from the mid-coronal part of the teeth. Each disk was cut into halves, and three half-discs were used for each group (SB600, SB703, DB, or AP#600). Dentin surfaces were prepared with burs or silicon carbide paper as was done for dentin bond strength measurement described above. For the SEM observation of the degree of etching of these dentin surfaces, the surfaces of the two of the three half-disks were treated with the self-etching primer of Mac-Bond II or the phosphoric acid of Single Bond. After each application time, the primer components were removed with 50% acetone/water solution (Harada et.al., 2000), and the phosphoric acid gel was removed with water. The third half-disk was used for observation of the unetched smeared surface. All specimens were then dehydrated in ascending grades of ethanol (50%, 75%, 95% and 100% for 30 min. each) followed by immersion in hexamethyldisilazane [(CH$_3$)$_3$SiNHSi(CH$_3$)$_3$, HMDS, Pierce, Rockford, Illinois 61105, USA]] for 10 min, placed on a filter paper inside a covered glass vial, and air-dried at room temperature (Perdigão et.al., 1995). The specimens were then gold sputter-coated and observed with a scanning electron microscope (JXA-840, JEOL, Tokyo, Japan) at an accelerating voltage of 10 KV.

For Single Bond, the resin-dentin interface of each group was also observed by SEM. Four flat dentin disks were prepared with burs or abrasive paper, and treated with

Single Bond. The resin bonded samples were then sectioned into two halves, parallel to the longitudinal axis of the tooth. Each specimen was embedded in epoxy resin (Epon 815, Nissin EM Co., Ltd., Tokyo, Japan), then the cut surfaces were ground with a series of increasingly finer silicon carbide abrasive papers, and highly polished with a diamond pastes (DP-Paste,P, Struers A/S, Denmark) (6 μm, 3 μm, 1 μm). The samples were subjected to 10% phosphoric acid treatment for 3 to 5 seconds (Gwinett and Kanca 1992b; Sano et.al., 1995). The specimens were rinsed with water for 15 seconds and treated with 5% sodium hypochlorite solution for 5 minutes (Wang and Nakabayashi, 1991). After being extensively rinsed with water, the treated specimens were air dried, gold-sputter-coated and observed with the SEM at 10KV. This was not done for specimens bonded with Mac-Bond II because the hybrid layers were so thin that they could hardly be seen the differences between the groups by SEM.

Results

Figure 2 and Table 3 show the micro-tensile bond strength (μTBS) results of each group. For Mac-Bond II, there were no statistically significant differences among the
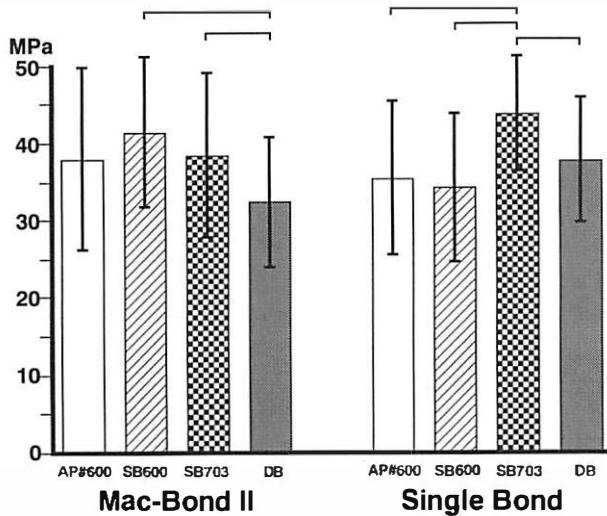

Figure 2. Results of micro tensile bond strengths for each group. Groups connected with horizontal lines are significantly different (p<0.05).

Table 3. Results of micro tensile bond strengths for each group (mean $\pm$ SD)(MPa)

	AP#600 (control)	SB600	SB703	DB
Mac-Bond II	37.9$\pm$11.8[ab]	41.3$\pm$9.7[bc]	38.4$\pm$10.6[bc]	32.3$\pm$8.4[a]
	(n = 23)	(n = 24)	(n = 21)	(n = 25)
Single Bond	35.4$\pm$ 9.9 [a]	34.1$\pm$9.7[a]	43.7$\pm$ 7.5[c]	37.6$\pm$8.1[a]
	(n = 22)	(n = 22)	(n = 21)	(n = 24)

(n): number of the slabs tested.   Groups that are not significantly different are marked with the same superscript letter (p>0.05).
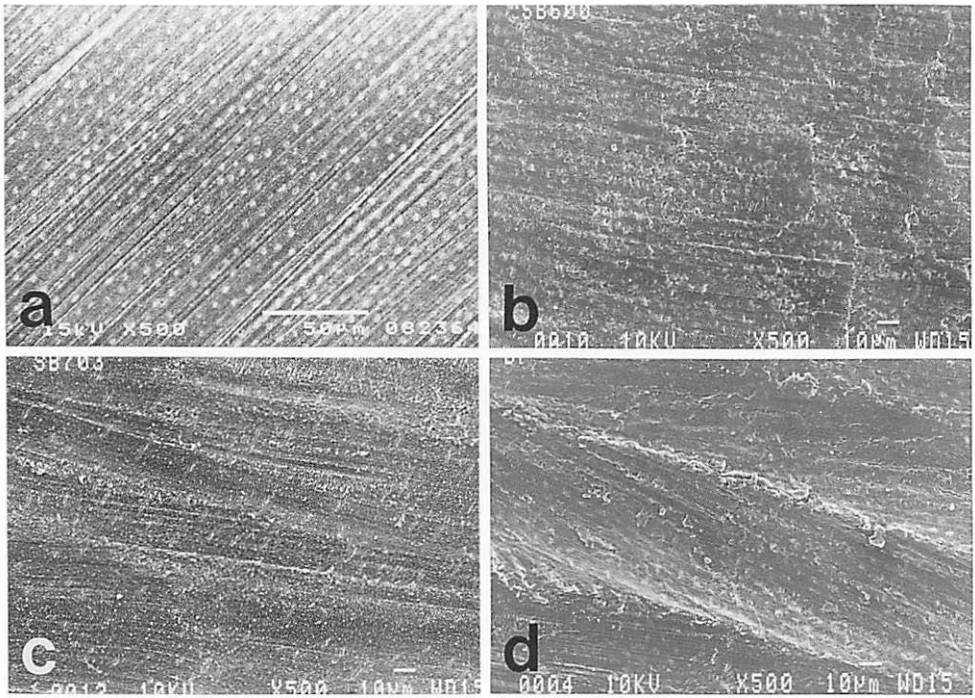
Figure 3. SEM of the prepared dentin surfaces of each group. a: AP#600 group b: SB600 group c: SB703 group d: DB group (Original magnification 500x ; bar : a = 50 μm, b-d = 10 μm)

Figure 4. SEM of Mac-Bond II primer treated dentin surfaces. a: AP#600 group b: SB600 group c: SB703 group d: DB group. On the dentin surface of the DB group, there were areas without smear layer (marked by the asterisk) and areas with remnants of smear layer (marked by the star). (Original magnification: a-c=8000x, d=1000x ; bar: a-c = 1 μm, d=10 μm)
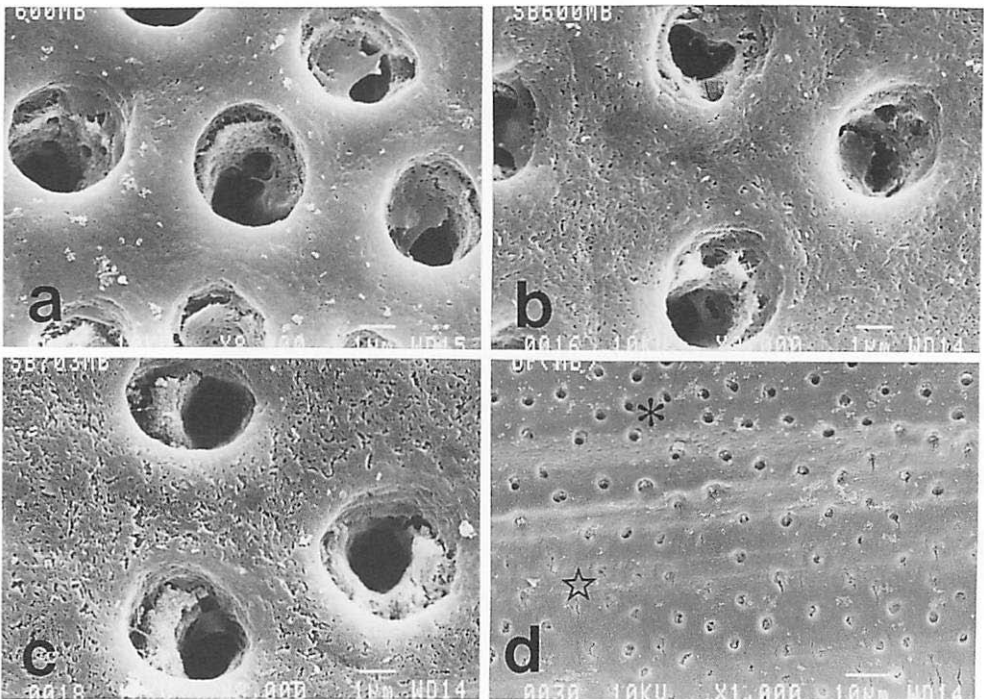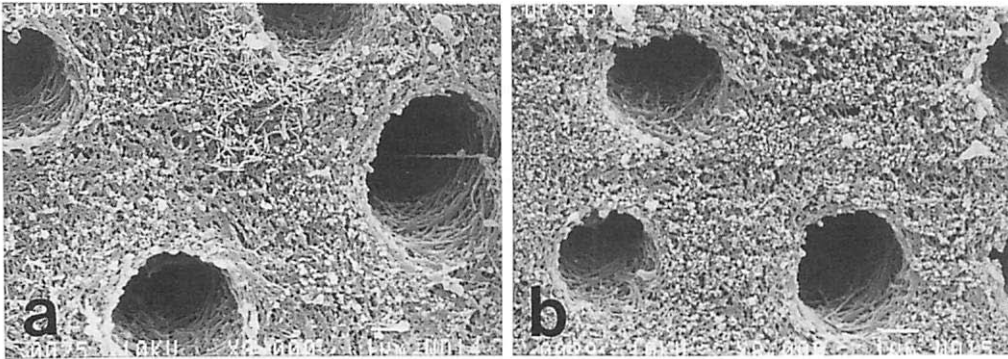
Figure 5.   SEM of 35% phosphoric acid etched dentin surface of Single Bond. a: AP#600 group b: DB group (Original magnification 8000x ; bar = 1 μm)

groups which were prepared with steel burs (SB600: 41.3±9.7 MPa ; SB703: 38.4±10.6 MPa) and the control (AP#600: 37.9±11.8 MPa). The DB group of Mac-Bond II produced lower tensile bond strength than the groups that received steel bur preparation (DB: 32.3± 8.4 MPa), and this group produced lower, although not significant, bond strength compared to the AP#600 group (p>0.05). For Single Bond, the SB703 group produced the highest tensile bond strength (43.7±7.5 MPa), but there were no statistically significant differences among the other groups and the control (AP#600: 35.4±9.9 MPa ; SB600: 34.1±9.7 MPa ; DB: 37.6±8.1 MPa). Two-way ANOVA analysis revealed that there was a statistically

Figure 6.   SEM of the resin-dentin interface of Single Bond. a: AP#600 group b: SB600 group c: SB703 group d: DB group R= bonding resin, H=hybrid layer, D=dentin. (Original magnification 8000x ; bar = 1 μm)

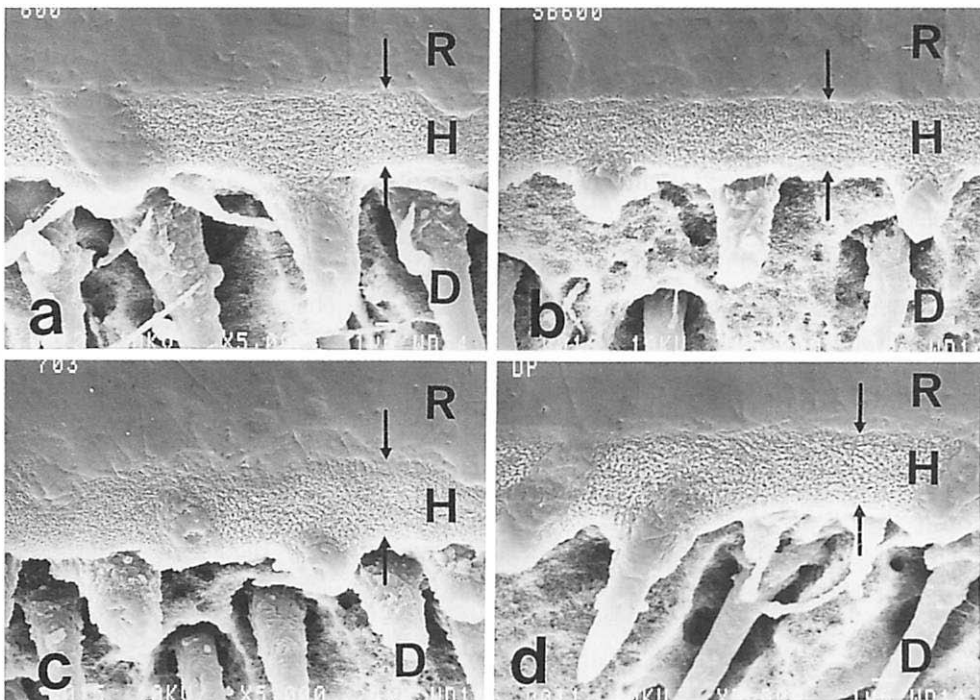significant interaction between the bonding systems and the methods of dentin surface preparation (p = 0.0032).

When visually inspected, most specimens showed interfacial adhesive failure. This was confirmed by light microscopic examination (x20). The representative micromorphology of the failure pattern was classified as mixed failures within dentin and bonding resin. There was no remarkable difference in the failure patterns among all the groups.

Scanning electron micrographs of each prepared dentin surface are shown in Figure 3. For Mac-Bond II, micrographs of the prepared dentin surface treated with the primer of each group are shown in Figure 4. For Single Bond, micrographs of the prepared dentin surface treated with the phosphoric acid are shown in Figure 5, and resin-dentin interface of each group are shown in Figure 6. For the groups AP#600, SB600 and SB703, the prepared dentin surfaces revealed many scratches left by the abrasive paper or burs, and the surfaces were completely covered with a smear layer. Dentinal tubules which were occluded by the smear plugs were also observed over the entire surface (Figs. 3 a-c). The SEM observation of the dentin surface of the DB group demonstrated that grooves left by the diamond bur were coarser than the other three groups (Fig. 3d). A thick irregular smear layer without any evidence of underlying dentinal tubules was seen on the top of the grooves while dentinal tubules occluded by smear plugs could be observed at the bottom of the grooves (Fig 3d). For the AP#600, SB600 and SB703 groups of the Mac-Bond II primer treated surface, the smear layer on the dentin surface and the smear plugs in the dentinal tubules were removed. For these groups, the intertubular dentin and the peritubular dentin of the tubule orifices were slightly etched, and the edge of the dentinal tubules were clearly observed (Figs 4 a-c). For the DB group treated with Mac-Bond II, two primed distinct zones which seemed to alternate were observed. In one zone, the smear layer and the smear plugs were removed, the intertubular dentin and the peritubular dentin were slightly etched, and the edges of the dentinal tubules were clearly observed. In the other zone, the residual smear layer and smear plugs could be observed (Fig. 4d). For all the groups of Single Bond, the smear layer on the dentin surface and the smear plugs in the dentinal tubules were completely removed, and the open tubules without peritubular dentin and a fine collagen fibril network on the surface was observed after the phosphoric acid etching (Figs. 5a,b). The resin-dentin interface of each group indicated no remarkable difference by altering the method of surface preparation (Figs. 6a-d). For all the groups, the thickness of the hybrid layer was about 3 μm, and the resin tags with a characteristic funnel shape could be observed (Figs. 6a-d).

Discussion

The self-etching primers' acidic components demineralize through the smear layer and diffuse a short distance into the underlying dentin, resulting in the creation of a

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thin hybrid layer but strong bonds to dentin (Watanabe et.al., 1994b; Chigira et.al., 1994). However, the self-etching primers do not etch as well as a 35% phosphoric acid etchant because of their relatively high pH (1.5 to 3.0 for self-etching primers, information from the manufacturer; -0.42 to 0.02 for phosphoric acid etchants, Perdigão et.al., 1996). Therefore, it is believed that bond strengths of self-etching primer bonding systems to dentin could be affected by differences in the quantity of residual smear layer left on the surface because of the weak acidity of self-etching primers. In our previous study which evaluated the effect of bur cutting using three self-etching primer bonding systems (Clearfil Liner Bond 2, Clearfil Liner Bond 2V, and Clearfil SE Bond), the bond strengths of these systems to dentin decreased when the dentin surface had been prepared using burs, and particularly when it was cut using a regular-grit diamond bur (Ogata et.al., 2001b). When the dentin surfaces were prepared by burs, some of the smear layer could not be completely removed by these self-etching primers due to their weak acidity. Thus, demineralization of the underlying dentin, and further penetration of the bonding resin into the demineralized dentin could have been insufficient for optimal bond strength.

The present experimental design was the same as that of the previous study. The pH of the Mac-Bond II primer of this system is 1.7 (primer A+B, information from the manufacturer), almost the same pH as Clearfil Liner Bond 2. Nakaoki et.al. (1996) reported that the demineralization effect of the Mac-Bond II primer was stronger than that of the primer of Clearfil Liner Bond 2. In this study, Mac-Bond II primer succeeded in removing the smear layer of the AP#600, SB#600, SB#703 groups (Figs. 4 a-c). For these groups, which had similar bond strengths, the smear layer on the dentin surface and the smear plugs in the dentinal tubules were removed. The intertubular dentin and the peritubular dentin of the tubule orifices were slightly etched, and the edge of the dentinal tubules were clearly observed. On the other hand, the DB group treated with Mac-Bond II primer produced the lowest tensile bond strength among the groups that received bur preparation (although not significantly lower than AP#600 group). Mac-Bond II primer could not completely remove the entire smear layer and the smear plugs created by regular-grit diamond bur. There were areas without smear layer and areas with remnants of smear layer on the dentin surface after primer treatment (Fig 4d). The SEM observations of the dentin surface of the DB group demonstrated that grooves left by the bur were coarser than were seen in the other groups. An irregular thick smear layer without any evidence of underlying dentinal tubules was seen on the top of the grooves while dentinal tubules occluded by the smear plugs could be observed at the bottom of the grooves (Fig 3d). The Mac-Bond primer partially could not remove all the irregular thick smear layer (Fig 4d). Thus, demineralization of the underlying dentin, and further penetration of the bonding resin into the demineralized dentin may have been limited. This peculiar structure of the DB created smear layer might be considered as the reason of the decrease of the bond strengths seen in this group.

Akimoto et.al. (1999) reported that the micro-tensile bond strengths of the Liner

Bond 2V and Clearfil SE Bond were not affected by dentin surface condition. They bonded to dentin surfaces prepared with #180 or #600-grit abrasive papers versus mirror-like surfaces of dentin. Tay et.al. (2000) also reported that the micro-tensile bond strength of the Clearfil SE Bond was not affected by various thickness of smear layers created by #60-, #180- or #600-grit abrasive papers or an absence of smear layer. These conflicting reports may be reconciled if the characteristics of the smear layers created by bur cutting are different from those created by abrasive paper. High speed burs may induce increases in thermal and mechanical stress. These stress could affect underlying dentin. An abrading cutting instrument like a diamond bur creates more frictional stress compared to a cutting instrument like a steel bur. Selection of the burs for cutting of dentin surface for direct resin composite restoration is important to produce optimal bonding of Mac-Bond II to dentin. Cutting the dentin surface with regular grit diamond burs should be avoided, or followed with finishing the cavity surface with steel burs. Clinically, access to a carious lesion is done with diamond or carbide burs, generally followed by removal of the carious dentin with round steel burs (Fusayama, 1980). Mac-Bond II showed the similar tensile bond strengths for the steel bur and abrasive paper control groups. Therefore, using steel burs, relatively high bond strengths could be expected for the clinical use of this system.

Tagami et.al. (1991) reported that the dentin bond strength of Clearfil Photobond was not affected by the different smear layer created by SiC paper or regular-grit diamond bur. Clearfil Photobond is a system that uses 37% phosphoric acid etchant and a light-curing bonding agent with the dry bonding technique. In the present study, the negative effect of dentin surface preparation by burs was not found for Single Bond, which used the wet bonding technique after 35% phosphoric acid etching. Due to the stronger demineralization effect of the phosphoric acid etchant (pH=0.6, Table 2), the smear layer and the smear plugs were completely removed regardless of how the surface had been prepared (Figs 5ab), and the resin-dentin interface of each group indicated no remarkable difference among the methods of surface preparation (Figs.6a-d). Toida et.al. (1995) evaluated the effect of different dentin smear layers created by various burs on the tensile bond strengths of two types of adhesive systems, using an experimental self-etching primer (aqueous solution of 20% Phenyl-P and 30% HEMA) or an acid etchant (3% ferric chloride in 10% citric acid). According to their study, the rough and thick smear layer created with burs should be removed with acid etching in order to obtain more reliable and higher bond strength. The result of the present study also support the efficacy of smear layer removal by strong acid etchants. For Single Bond, the selection of bur type for the dentin surface preparation is unimportant. On the other hand, a system that uses wet bonding technique has other problems. Although the wet bonding technique is an excellent idea, it is technique-sensitive in the clinical situations because it is difficult to produce a uniform wet state on all prepared surface (Tay et.al., 1996) especially in a large, complex shaped cavity restoration. Self-etching primer systems are less technique-sensitive but give lower bonds to diamond bur created smear layers. Thus, care must be taken during

placement of a resin restoration depending on which type of adhesive system is used. In this study, no attempt was made to deviate from the manufacturer's instructions. However, it is likely that higher or more consistent bond strengths could have been achieved using Mac-Bond II if multiple applications of primer had been used with continuous agitation (Ogata et.al., 1999).

Conclusions

When using Mac-Bond II, the DB group produced the lowest tensile bond strength among the groups which were prepared with a bur, and there were no statistically significant differences among SB600, SB703 and AP#600 groups. For Single Bond, the bond strength of the SB703 group was the highest, and there were no statistically significant differences among the other experimental groups and the control. The influence of the method used to prepare dentin on tensile bond strength depends upon the type of adhesive system used. For any adhesive system, the smear layer should be removed with a conditioner in order to obtain optimum adhesion to dentin.

Chapter 6

Rules to follow for best adhesive performance
of self-etching primer bonding systems.

Since the first self-etching primer bonding system, Clearfil Liner Bond 2 (Fujitani et.al., 1992, 1993; Hosoda et.al.,1993), was marketed in 1993, this type of adhesive system has become the most popular adhesive material for the clinical use. Harada et al. (2000) evaluated the in vitro bond strength of three self-etching primer bonding systems from Kuraray Medical (Clearfil Liner Bond 2, Clearfil Liner Bond 2V, Clearfil SE Bond), and reported that the adhesive properties of these systems are good to both ground enamel and dentin. In addition, Liner Bond 2V and SE Bond also produce high bond strengths to various dental substrates such as porcelain and metal by use of a special primer for each substrate. The self-etching primer systems have been considered to be less technique sensitive than the wet bonding systems. However, through several studies which evaluated clinical factors on bonding properties of the self-etching primer systems (Ogata et.al., 1998, 2001a, 2001b, 2002; Fujitani et.al., 1994, Nakajima et.al., 2000b; Abu-Kasim et.al., 2002), even these systems also require some rules to follow on the clinical applications to achieve their good adhesion.

When the clinicians use adhesive systems, it is logical to use them according to the manufacturer's instructions. Furthermore, self-etching primer systems have some rules to follow for their good adhesive performance. They are: 1) rules on storage condition of the self-etching primer, 2) multiple application of the self-etching primer, 3) surface condition after air-blowing of the primer, and 4) bur selection for cavity preparation. These points are trivial, but necessary where care needs to be taken during placement of a resin restoration.

Rule1: Storage condition of the self-etching primer

The first rule is regarding the storage condition of the self-etching primer. Self-etching primer, especially one-bottle type of self-etching primer, should be kept in a refrigerator at the clinic, and should be used as soon as possible within the expiration date.

As shown in Table 1, a self-etching primer generally contains an acidic monomer, HEMA, and water. Acidic monomers are hydrophobic, with an acidic radical such as a phosphoric or carboxyl radical at the end of their structure. For the acidity of the primer by the coexistence of the acidic monomer and water, HEMA is added to the primer solution. The acidic monomer demineralizes the underlying dentin and penetrates into the demineralized collagen network. HEMA also promotes the permeation of monomers and bonding resin into the demineralized collagen network to create a hybrid layer.

Nishiyama et. al. (2000) reported that HEMA is hydrolyzed to methacrylic acid and ethylene glycol in acidic conditions, and that this hydrolysis is influenced by the

Table 1. Ingredients of the self-etching primers

Systems	Ingredients	Manufacturer
Clearfil Liner Bond 2		
LB-primer A	Phenyl-P, 5-NMSA, ethanol, photoinitiator, accelerators	Kuraray Medical,
LB-Primer B	HEMA ,water	Tokyo, Japan
Clearfil Liner Bond 2V		
Primer Liquid A	MDP, HEMA, water, photoinitiator, accelerators	Kuraray Medical,
Primer Liquid B	HEMA, water, initiator	Tokyo, Japan
Clearfil SE BOND		
Primer	MDP, HEMA, water, multifunctional methacrylate, photoinitiator	Kuraray Medical,
		Tokyo, Japan
Mac-Bond II		
Primer A	MAC-10, methacryloyloxyalkyl acid phosphate, isopropanol, acetone, water, accelerators	Tokuyama Dental
Primer B	isopropanol, water	Tokyo, Japan
Imperva Fluoro Bond		
FB-Primer A	water, acetone, initiator	Shofu Co.
FB-Primer B	4-AET , HEMA, 4-AETA, initiator	Kyoto, Japan
UniFil Bond (GC)		
Primer	HEMA, water, 4-MET, ethanol	GC Co.
		Tokyo, Japan

storage temperature.　In demand for simplifying the bonding procedure, some of the recent self-etching primers are packaged as a single bottle, this means that HEMA is always in a acidic condition.　The one-bottle primer's components might be changed in quality depending on the storage conditions.　Nakajima et.al. (2000b) reported that the bond strength of the Clearfil SE Bond of the groups that the primer stored at 23 ℃, or 37℃ tended to decrease gradually.　Abu-Kasim et.al. (2002) also evaluated the effect of storage temperature (4℃, 23 ℃, or 40℃) of SE Bond primer on bond strength to dentin, by measuring the micro-tensile bond strengths after 6-months or 1-year storage of the primer (Table 2).　According to Abu-Kasim's report, only the 4℃ group could maintain almost same bond strength after 6 months storage. But after 1 year of storage, the bond strength of this group significantly decreased by a half. At 23 ℃ and 40 ℃, there was a significant gradual reduction in the bond strength after 6 months and 1 year storage. After one year storage, the color of the primer of the 40 ℃ group became quite dark, which indicated that chemical degradation of the primer component had taken place (unpublished data). In addition, the SEM image indicated that the hybrid layer of the 40 ℃ group appears much more porous

Table 2. Micro-tensile bond strength to dentin of Clearfil SE Bond at the each storage condition of the primer. (mean±SD (MPa))

Storage temperature	control	4℃	23℃	40℃
Base line	49.67±13.70[a]			
6 months		45.40±10.48[a]	33.57±9.67[b]	32.60±10.92[b]
1 year		22.38±10.62[c]	21.67±6.84[c]	7.59±2.99

Same letters indicate no significant difference

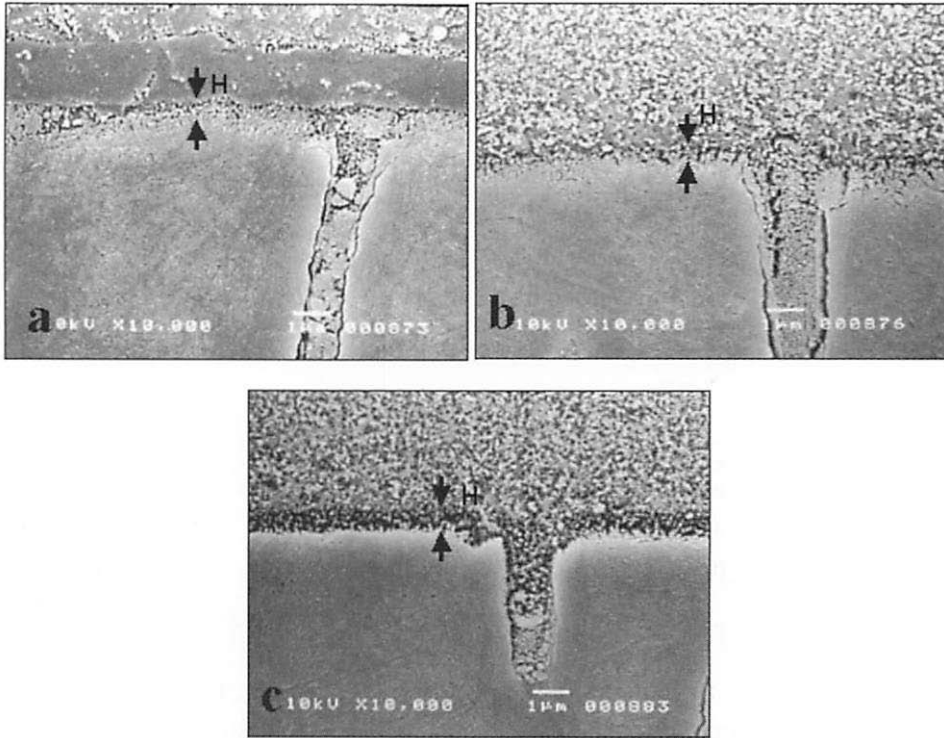(Abu-Kasim et.al., *Adhesive Dentistry* 19: 443, 2002.)

Figure 1. SEM of the argon-ion beam etched resin-dentin interface treated with 1-year stored SE primer. a: 4℃, b: 23 ℃, c: 40℃, H: hybrid layer

and concave than the other two groups by argon-ion beam etching (Figure 1, unpublished data). The chemical degradation of the primer due to the high temperature and long term storage could affect the quality of hybrid layer formation in the 40 ℃ group. From the ^{13}C NMR spectral analysis of SE Bond primer, Nishiyama (2000) reported that hydrolysis reaction of HEMA in the SE Bond primer is accelerated according to the storage temperature. Consequently, the demineralizing action is reduced because the water has been used for the hydrolysis reaction, and priming efficacy is lowered because of the reduction in HEMA in the primer. The apparent malformation of the hybrid layer in the 40 ℃ group observed in Fig. 1c may have been caused by this phenomenon. The situation of the 40 ℃ storage will be improbable because the temperature at the clinic is usually controlled by air conditioner in daytime. However, the room temperature soon may become very hot in mid summer, if clinics are closed, and the air conditioner is switched off. Furthermore, we can not confirm if temperature during transportation of materials is controlled or not. Therefore, it is suggested that the self-etching primer bonding system should be kept in a refrigerator at the clinic, and used as soon as possible within the expiration date.

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Rule 2: multiple primer application method

The second rule is regarding the application method of the self-etching primer. When restoring a relatively large flat surface or a big-size cavity, multiple application of the self-etching primer to the cavity without extending the application period will be recommended to enhance the bond strength to tooth surface. In chapter 2, the concept of this clinical rule was already mentioned and discussed using artificial wedge-shaped cavity.

The self-etching primer used on relatively large saucer shaped cavity near the cervical area, can easily flow off from the cavity or escape to the gingival sulcus. When restoring large sized, deep cavity of the molars, we also experience the same situation that the primer flows off the lateral walls due to gravity. This flow off of the primer is partly because of the low viscosity nature of the self-etching primers. In these cases, the dentin surfaces or marginal enamel may not be properly treated by the self-etching primer well enough to produce durable bonds. In chapter 2, bond strength of Liner Bond 2 to the each cavity wall of the wedge shaped cavity significantly increased by multiple primer application (Chapter 2, Table 3). The SEM of the interface treated one-time with LB-primer, showed that the thickness of the hybrid layer was about 1μm, with narrow and short resin tags which did not fill the tubular orifices completely (Chapter 2, Fig3-b). For the interface treated several times with LB-primer, the thickness of the hybrid layer was about $2\ \mu$m, with thick, long and funnel cone-shaped resin tags (Chapter 2, Fig3-d). When LB-primer was applied only one time to the cavity walls, the primer flowed-off from the cavity causing insufficient and non-effective treatment on the dentin surface. On the other hand, although the primer also flowed off from the cavity for the groups which received multiple primer application, fresh LB-primer was added continuously during the primer application time indicated by manufacturer's instructions. Therefore the dentin surfaces of these groups treated with multiple primer application were adequately etched and primed. In the case we need to fill relatively large saucer shaped cavities, large size, or deep cavities with composite resin, multiple primer application is recommended as an effective method to treat the dentin surface properly.

Rule 3: Sufficient air-blow of the primer

The third rule is regarding the air-blow of the primer. After conditioning the cavity, self-etching primers should be air-dried until the solvent has completely evaporated.

For restoration of a small cavity, it is easy to equally dry the entire cavity surface. However, to restore a large and deep, or complex shaped cavity, it can be possible that the primer remain pooled without evaporating its solvent at some part of the cavity, especially at a line angle or a point angle of deepest part, if the air-drying is not enough.

Fujitani et.al. (1994) evaluated the effect of various air-drying method of self-etching primer on the dentin bond strength of Liner Bond 2. They reported that tensile

bond strength to dentin was high for the groups which were air-dried enough with strong or mild air. Blot drying with cotton pellet was not so effective on bond strength to dentin. In addition, the groups which received insufficient air-drying or no air-drying produced significantly reduced bond strengths. As shown in Table 1, self-etching primers contain solvents such as water, alcohol, or acetone. According to Fujitani et.al. (1994), an explanation for the reduction in bond strengths is that the solvents in self-etching primers might act as inhibitors for the polymerization and adhesion of the bonding resin of the groups which received insufficient air-drying. Therefore, self-etching primers should be air-dried until the solvent has completely evaporated.

Rule 4 : Selection of the bur for cavity preparation

The fourth rule is regarding the selection of the bur for cavity preparation. For the resin restoration using self-etching primer, preparing the dentin surface with regular grit diamond burs should be avoided, or should be finished with steel burs. In chapters 4 and 5, we evaluated the influence of the dentin surface preparation with different types and grit of burs on bond strength of four self-etching primer bonding systems and one wet bonding system. The self-etching primer bonding systems that we evaluated were Clearfil Liner Bond 2, Clearfil Liner Bond 2V, Clearfil SE Bond and Mac-Bond II. All of these adhesive systems showed significantly lowest tensile bond strength when the dentin was prepared with regular grit diamond bur. On the other hand, the fine cut steel bur groups of all self-etching primer systems produced relatively high tensile bond strengths among the groups which received bur preparation. Thus, we concluded that cutting the dentin surface with regular grit diamond burs should be avoided, or followed with finishing the cavity surface with steel burs when we use self-etching primer systems. Generally, textbooks of Operative Dentistry in Japan instruct that, initial opening of a carious cavity by removal of enamel should be done with diamond or carbide burs, followed by removal of the carious dentin with round steel burs under the guidance of the caries detecting dye solution (Fusayama, 1980; Iwaku et.al., 2002). Therefore, this issue may not be so significant as long as carious dentin is excavated with steel burs according to the technique established by Fusayama (1980).

As mentioned before (Chapter 2, Chapter 6-Rule1), the multiple primer application method can supply adequate amount of primer into a cavity. Multiple primer application may overcome the resistance of bur-created smear layers to the etching effects of these primers leading to improvement of the bonding property of these systems.

Conclusion

Over the last 10 years, a number of advances have been made in adhesive dentistry that provided great benefit for patients. On the other hand, there are numerous clinical factors that influence bonding properties of adhesive systems. The ideal adhesive materials should be less technique sensitive, be able to provide reliable and durable bonds

regardless of the different operators, cavity form, region of the sites to be restored, or any clinical situations. Unfortunately the ideal adhesive materials have yet to be developed. Through the studies focused in the each clinical factor on bonding, it will be possible to control the clinical situations. Clinicians should know the clinical factors influencing the bonding and follow the rules on the clinical applications of the adhesive materials to overcome the negative affect of the clinical factors and to achieve good adhesion.

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